JULY–SEPTEMBER 2011 • VOLUME 65 NUMBER 3

California Agriculture

Food as medicine

Can what we eat help cure what ails us?

University of California | Peer-reviewed Research and News in Agricultural, Natural and Human Resources

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COVER: Biofactors in foods — such as isoflavones in soy, omega-3 fatty acids in fish, antioxidants in produce and hydroxytyrosol in olive oil — may play a critical role in reducing the risk and symptoms of chronic illnesses such as heart disease, diabetes and kidney disease. *Photo: Rita Maas, Getty Images*

Food as medicine:
Can what we eat help cure what ails us?

Research and review articles

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Editor's note:
California Agriculture gratefully acknowledges the faculty chair for this special issue, Sheri Zidenberg-Cherr, and the faculty co-chairs Carl Keen and Robert Rucker, all from the UC Davis Department of Nutrition. We also thank the Center for Health and Nutrition Research for providing funds to defray the costs of its expanded length.

E-Edition: Online

RSVP

WHAT DO YOU THINK?

The editorial staff of *California Agriculture* welcomes your letters, comments and suggestions. Please write to us at: 1301 S. 46th St., Building 478 - MC 3580, Richmond, CA 94804, or calag@ucdavis.edu. Include your full name and address. Letters may be edited for space and clarity.

TO OUR READERS:

California Agriculture kicks off E-Edition, allowing faster publication

Two electronic-only articles appear in this issue, launching *California Agriculture*'s first E-Edition, an expanded venue for rapid publication of time-sensitive findings.

New findings on a promising biofuel crop for California appear on the website only; read them at www.californiaagriculture.anr.org by clicking "Current Issue" and scrolling down to E-Edition. Similarly, research on why California rice growers protect their harvests from contamination by transgenic rice appears on the website alone.

"Initially, E-Edition is being offered to authors who have been waiting for publication due to our backlog," says Janet White, executive editor. "The statewide budget crisis has led to a 48-page cap on our journal. Some articles have waited a year or more for publication, an unacceptable delay."

E-Edition also means that, with this issue, the journal will change from print to electronic "version of record," the online version becoming the authoritative version to be indexed by databases and repositories. Readers can preview the abstracts and introductory comments of these articles at "E-Edition: Online" (page 159). Such "thumbnail" descriptions will appear in the print journal concurrent with each E-Edition. Space permitting, E-Edition articles may be printed in a future issue.

E-Edition articles will be laid out just like print articles, with tables, figures and photographs. Readers can download and print copies in HTML or PDF format. Authors will be able to print articles on demand for distribution to target audiences.

Like all published articles, E-Edition articles will benefit from *California Agriculture*'s augmented electronic presence (see "Indexing," page 100). The journal also appears in full on the California Digital Library and in the ANR Repository. It ranks high in Google and Google Scholar searches.

In addition, *California Agriculture* recently began accepting submissions via Thomson's ScholarOne peer-review management system. The new system allows authors and reviewers expanded access to Thomson's Web of Science (for ease of research and documentation) as well as other features. *California Agriculture* welcomes new research submissions. Go to: http://californiaagriculture.ucanr.org/submit.cfm.

Share your comments and suggestions: Janet White, jlwhite@ucdavis.edu or (510) 665-2201 or Janet Byron, jlbyron@ucdavis.edu or (510) 665-2194.

— Editors

Clarification: Olive fruit fly in Mariposa County

After our article "Understanding the seasonal and reproductive biology of olive fruit fly is critical to its management," was published in the January–March 2011 issue of *California Agriculture*, Cathi Boze, the agricultural commissioner in Mariposa County, noted that her county was not included as infested on the map of California on page 15. Mariposa County was not listed as trapping for olive fruit flies in the California Department of Food and Agriculture's list of counties that we used to make the map. However, Boze did indeed run traps in the county, and olive fruit flies were first detected there in 2003.

January–March 2011
California Agriculture

Frank Zalom
Professor, CE Specialist and Entomologist
UC Agricultural Experiment Station

Smart sprayers pay in Australia

I just read "Smart sprayer technology provides environmental and economic benefits in California orchards," April–June 2011. We retrofitted this system to our almond orchard sprayers for $5,000 Australian dollars ($5,218 U.S.) each, 4 years ago. If anything, the predicted estimates of savings are conservative. In addition to the tree sensors on our foliar sprayers, we have set up "Weed Seeker" heads on our herbicide sprayers. While these cost four times more than the tree sensors, the payback period has been similar.

Tim Orr
Lake Cullulleraine Almonds
Cullulleraine, Australia

April–June 2011
California Agriculture

Redwoods regenerate on 7,000-plus acres

The Mendocino Land Trust congratulates *California Agriculture* on the recent article about the remarkable regrowth of redwoods at Big River ("Scientists discover redwoods' resiliency in Fritz's Wonder Plot," April–June 2011). The Fritz Wonder Plot is part of 7,334 acres of former industrial timberland that now make up the Big River unit of the Mendocino Headlands

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About California Agriculture

University of California
Agriculture and Natural Resources

California Agriculture is a quarterly, peer-reviewed journal reporting research, reviews and news, published by the Division of Agriculture and Natural Resources (ANR) of the University of California. The first issue appeared in December 1946, making *California Agriculture* one of the oldest, continuously published, land-grant university research journals in the country. The print circulation is currently about 15,000 domestic and 1,800 international, with a strong online presence.

Mission and audience. *California Agriculture*'s mission is to publish scientifically sound research in a form that is accessible to a well-educated audience. In the last readership survey, 33% worked in agriculture, 31% were faculty members at universities or research scientists, and 19% worked in government agencies or were elected office holders.

Indexing. The journal is indexed by AGRICOLA; Current Contents (Thomson ISI's Agriculture, Biology and Environmental Sciences database, and the SCIE database); Commonwealth Agricultural Bureau (CAB) databases; EBSCO (Academic Search Complete); Gale, including Lexis-Nexis; Google Scholar; Proquest; and others, including open-access databases. It has high visibility on Google and Google Scholar searches. All peer-reviewed articles are posted to the California Digital Library's eScholarship Repository.

Authors and reviewers. Authors are primarily but not exclusively from ANR; in 2008 and 2009, 15% and 13% (respectively) were based at other UC campuses, or other universities and research institutions. In 2008 and 2009, 14% and 50% (respectively) of reviewers came from universities and research institutions or agencies outside ANR.

Rejection rate. The rejection rate has ranged between 20% and 25% in the last 3 years. In addition, associate editors and staff sent back 24% of manuscripts for revision prior to peer review.

Peer-review policies. All manuscripts submitted for publication in *California Agriculture* undergo double-blind, anonymous peer review. Each submission is forwarded to the appropriate associate editor for evaluation, who then nominates three qualified reviewers. If the first two reviews are affirmative, the article is accepted. If one is negative, the manuscript is sent to the third reviewer. The associate editor makes the final decision, in consultation with the managing and executive editors.

Editing. After peer review and acceptance, all manuscripts are extensively edited by the *California Agriculture* staff to ensure readability for an educated lay audience and multidisciplinary academics.

Submissions. *California Agriculture* manages the peer review of manuscripts online. Please read our Writing Guidelines before submitting an article; go to http://californiaagriculture.ucanr.org/submit.cfm.

Letters. The editorial staff welcomes your letters, comments and suggestions. Please write to us at the address below. Include your full name and address. Letters may be edited for space and clarity.

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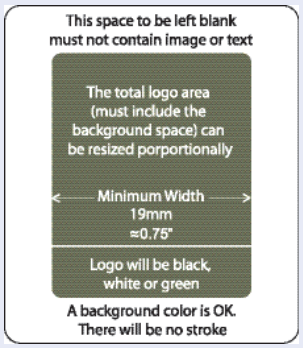

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State Park. The Mendocino Land Trust, with the support of individual and institutional donors, raised the $28 million needed to acquire the Big River Unit in 2002. Former Big River program manager Matt Gerhart (now with the state Coastal Conservancy), working with UC, organized the Fritz Plot resurvey and compiled and composed the historical and current data. To read the Fritz report or learn more about Big River, visit http://mendocinolandtrust.org.

Matt Coleman
Mendocino, CA

Greg Giusti, UC cooperative forest and wildlands ecology advisor, responds:

For more than 70 years, scores of people and organizations worked to keep the Fritz Wonder Plot operational. Scientists and researchers, many unnamed in the article, spent large portions of their careers protecting and collecting data from the site. The focus of the article was not on all the players involved, but rather on the unique characteristics, history and story the plot tells. Thanks to the Mendocino Land Trust, the Conservation Fund and the Save the Redwoods League, the activities started in 1923 continue today. The property was transferred from private to public ownership in 2002, and California State Parks is the official land steward.

Long-term care and financial-planning data

Editor's note: The following letter and response have been edited substantially for space. To read the entire exchange, with references, go to: http://ucanr.org/u.cfm?id=8.

I greatly enjoy reading *California Agriculture*, and I especially liked the October–December 2010 issue, since I was born in 1941 and am experiencing the privilege of aging. However, the brief article "Long-term care is an important consideration in financial planning for later life" (page 206) lacks an original source reference regarding the claim, "While not all Californians will need expensive long-term care, 70% of those over age 65 will need some during their lifetimes." The reference given is: California HealthCare Foundation, *Survey: Many Californians Not Ready for Health Care Costs*, California Healthline (April 22, 2010).

The California HealthCare Foundation published a more definitive report in November 2009. In 2007, there were 1,391,281 California residents in some type of long-term care. I used the higher 2010 U.S. census estimate of 4,640,000 residents to divide into the 2007 total of 1,391,281 residents in some type of long-term care. The result was 30%.

Three of the seven references in this article were from a long-term care insurance company, Genworth Financial. I am not a health services research professional, but I would like a more rigorous peer review from objective scientific references using original sources, not newsletters that reference a government summary that has no sources.

Bob Whitney
Willits, CA

Patti C. Wooten Swanson, Nutrition, Family and Consumer Science Advisor, UC Cooperative Extension, San Diego County, and Karen P. Varcoe, Consumer Economics Specialist, UC Riverside, respond:

The widely quoted and generally accepted projection that at least 70% of U.S. residents ages 65 and older will need long-term care services can be traced back to analysis of data from the 1986 National Mortality Followback Survey (NMFS) conducted by the Centers for Disease Control's National Center for Health Statistics. The figure appears to have originated in a 2001 Journal of Risk and Insurance *article. Included from the NMFS data was the finding that persons age 65 and older in 1995 had a 68.5% probability of needing assistance with two or more activities of daily living (ADLs) during their lifetimes, including eating, toileting and dressing. "Needing assistance with two or more ADLs" is generally the criteria for receiving benefits from a long-term care insurance policy sold in California.*

October–December 2010
California Agriculture

The California HealthCare Foundation data cited have limitations. For example, it includes only those who utilize long-term care "services" and not the estimated 70% of elders who get most or all of their care from (unpaid) family members and friends.

Genworth Financial's annual cost-of-care surveys are commonly quoted in educational materials to help consumers estimate and plan for potential future costs of long-term care. The surveys collect data from a random sample of nursing homes, assisted living and adult day health facilities, and home care providers. Genworth's 2011 survey contacted more than 53,000 long-term care providers to complete nearly 15,500 surveys. Results cover the four major categories of long-term care in all 50 states, the District of Columbia and Puerto Rico.

Private long-term care insurance is only one of several ways families afford the long-term care they may need. In addition to unpaid care from family and friends, seniors may qualify for Medi-Cal by spending down their assets. Seniors with more resources may choose to access their home equity. Others pay for care from personal savings and assets, funds generated from cash-value life insurance or viatical settlements from companies that buy insurance policies from terminally ill patients. For more information, see the UC ANR publication "Planning and Paying for Long-Term Care" (http://anrcatalog.ucdavis.edu/Items/8383.aspx).

Diet factors could ease disease, build healthier California

Sheri Zidenberg-Cherr
UC Cooperative Extension Nutrition Specialist, Davis
Co-Director, Center for Nutrition in Schools, UC Davis

Dave Campbell
UC Cooperative Extension Community Development Specialist, Davis
Strategic Initiative Leader, Healthy Families and Communities

Health professionals have long recognized that what we eat can foster wellness or disease. This idea is the focus of the new *2010 Dietary Guidelines for Americans*, the federal guidelines to promote health, reduce chronic disease and diminish overweight and obesity through better nutrition and physical activity.

Today, more than one-third of children and more than two-thirds of adults in the United States are overweight or obese. Investigating the causes and consequences of this health crisis and finding effective responses are key priorities for land-grant universities. As the articles in this special issue demonstrate, UC scientists are pioneering this vital public work, integrating research, education and public service to improve health outcomes.

The 2010 guidelines emphasize the need to balance caloric intake with physical activity. The guidelines encourage Americans to consume more vegetables, fruits, whole grains, fat-free and low-fat dairy products, and seafood, and they recommend an overall diet low in sodium, saturated and trans fats, added sugars and refined grains. Secretary of Agriculture Tom Vilsack stated: "These new and improved dietary recommendations give individuals the information to make thoughtful choices of healthier foods in the right portions and to complement those choices with physical activity. The bottom line is that most Americans need to trim our waistlines to reduce the risk of developing diet-related chronic disease. Improving our eating habits is not only good for every individual and family, but also for our country."

Adopting healthier diets

Few question the guidelines, but many encounter obstacles to their implementation. Healthier foods are often unavailable to those who most need them due to high prices, limited access, confusing food labels or insufficient understanding of food preparation options. Trends toward decreased physical activity are reinforced by the nature of many jobs, the physical design of communities and the ubiquity of electronic gadgets.

Robyn Mackenzie/iStockphoto

Research is elucidating how antioxidants in many foods can improve human health and prevent chronic disease.

Building on historic expertise in nutrition education, UC is poised not only to recommend healthy food options but also to devise consumer-friendly messages identifying what these foods are and how they can be incorporated into a typical diet. Increasingly, UC Cooperative Extension (UCCE) faculty and staff find themselves leading or supporting coalitions to address these issues at the community scale through public policy and planning.

Over the past century, our expectations of food have moved beyond reducing well-recognized nutritional-deficiency diseases (such as scurvy, rickets and pellagra). The research advances of the past decade have enabled us to relate nutrition to chronic disease and aspects of the aging process. Evidence indicates that chronic diseases such as osteoporosis, dementia and cardiovascular disease are also "deficiency diseases" that develop over a long period of time — years or decades. The progression of these deficiency diseases may be modulated by newly recognized dietary factors distinct from the previously characterized essential nutrients. The identification and characterization of such health-promoting dietary factors hold promise for preventing or treating a range of debilitating afflictions.

Research and outreach strategies

We also need to change our focus from an ever-shifting list of "bad diets" and "bad foods," to identifying dietary components that confer health benefits. For example, basic research on several UC campuses is investigating the role of specific nutrients and phytochemicals in human metabolism and disease prevention. The Center for Health and Nutrition Research (CHNR) at UC Davis has investigated the roles of fruits, vegetables and nuts in providing vitamins and other phytochemicals that can lower the risk of chronic diseases such as cancer and heart disease. Examples of research that has been facilitated by the Center include the impact of diets that are rich in plant-based flavonoids during pregnancy, and the effects of fish and almond consumption on cardiovascular disease prevention.

Research supported by CHNR is highlighted in this special issue. UC scientists review the role of nutrition in optimizing health outcomes in female athletes (page 124), report recent research on the role of omega-3 fatty acids in moderating disease progression (pages 106 and 112), review the role of soy in the prevention of cardiovascular disease (page 118) and describe the characteristics of mitochondria, suggesting

mechanisms by which biofactors may enhance mitochondrial function and reduce disease signs or symptoms (pages 136 and 141). Other research explores the use of tangerines to prevent vitamin A deficiency (page 130).

But simply knowing what foods are healthy is not enough. We need to increase access to healthy food and physical activity for all segments of society. The UC Berkeley Robert C. and Veronica Atkins Center for Weight and Health, a national leader in obesity prevention, has long worked with community partners to develop and evaluate obesity prevention strategies. Campus-based scientists at UC Berkeley and UC Davis have joined with county-based UCCE advisors to investigate and evaluate obesity issues in a variety of environments including childcare, school, after-school, community, work site and health care settings. Research suggests that the solution to this health care crisis is a combination of educational and environmental interventions to promote healthy eating and active lifestyles.

For more information

UC Berkeley Robert C. and Veronica Atkins Center for Weight and Health
http://cwh.berkeley.edu

UC Davis Center for Health and Nutrition Research
http://chnr.ucdavis.edu

UC Healthy Families and Communities Strategic Initiative
http://ucanr.org/sites/HFC

USDA dietary guidelines
http://dietaryguidelines.gov

Healthy Families and Communities Initiative

UCCE, the statewide extension arm of UC Agriculture and Natural Resources (UC ANR), has launched the Healthy Families and Communities Initiative. The initiative addresses critical challenges faced by children, teens and young adults, including childhood obesity and science literacy. Childhood and adolescence are critical periods to develop healthy behaviors that can be sustained into adulthood (see box).

Known for their effective collaboration with diverse public and private partners, UCCE faculty and staff are in a favorable position to create and evaluate school and community interventions in counties across California.

These include school, nonformal educational settings and youth programs, such as 4-H. Interventions will serve as learning laboratories through which UC research and education can strengthen efforts to reduce obesity and chronic disease, improving the health of all Californians.

UC Cooperative Extension focuses on youth health and science literacy

UC Cooperative Extension is launching a new effort to promote the physical, intellectual and emotional health of California's young people.

Called Healthy Families and Communities (HFC), this initiative addresses three critical challenges faced by children, teens and young adults in our state: childhood obesity, lack of science literacy and the need for positive development.

"Rather than concentrating on risky behaviors, the focus is on nurturing youth to help them reach their potential and strengthen their connections with the community," says Dave Campbell, initiative leader.

Nearly a third of California's school-aged children are overweight or obese, and the state has the unfortunate distinction of leading the nation in obesity-related health care costs. "To effectively address obesity, we need to wed traditional nutrition education outreach with youth and community development," Campbell says. "It's not enough to just educate individuals, you also need to address the social and built environment to see who has and who needs opportunities."

California's science literacy ranking is also dismal, with only Mississippi scoring worse. "Addressing this is absolutely critical," Campbell notes. "We need new scientists to retain our economic competitiveness, and the ability to recognize good science and think through problems systematically is also part of being a good citizen who can participate in the political discourse."

Alarming statistics jumped out during the panel's research. High school dropout rates are high, and about one-sixth of 16 to 24 year olds are out of school and out of work. This has high social costs and is a missed opportunity to train a skilled workforce to replace people who are about to retire.

HFC has solicited proposals for studies on the initiative's three strands. Funded projects will be carried out through campus-county research and extension partnerships that assess the effectiveness of individual and community change strategies. These projects will build on existing UC research on school wellness policies, garden-based learning, farm-to-school programs, after school and nonformal education, and youth development. "We're connecting our work to key issues in California," Campbell says.

— Robin Meadows

ilbusca/iStockphoto

Finding solutions to the epidemic of obesity is a key goal of land-grant universities.

Biofactors in food linked to health benefits

Can what we eat help fix what ails us? Research increasingly suggests that the answer could be "yes." Many foods contain biofactors — biologically active compounds — that may prevent and treat illnesses including asthma, diabetes and heart disease, according to new studies from the Center for Health and Nutrition Research (CHNR) at UC Davis.

Established in 2006, CHNR was initially supported by the Vitamin Case Consumer Settlement fund. In this antitrust case, vitamin manufacturers controlling 80% of the world market were found guilty of international price fixing. CHNR received $6.4 million from the resulting settlement fund over 4 years and allocated most of it to innovative pilot research on the role of California's fruits, vegetables and nuts in preventing chronic diseases. More than one-third of the 33 pilot projects have already been awarded additional grants.

"Our goal was to leverage the settlement funding to make a much larger impact on improving health in California," says CHNR co-director Carl Keen, a UC Davis nutrition professor. "There's been tremendous research under the vitamin settlement." One key to CHNR's success is that the projects were multidisciplinary, fostering collaboration across academic departments.

"The research went from the cellular level all the way to applications to human health, to the great benefit of the Center," says CHNR administrator Sheri Zidenberg-Cherr, a UC Davis Cooperative Extension nutrition specialist. This comprehensive approach is critical to identifying the health benefits of particular nutrients, which can be hard to pin down.

Findings from CHNR projects have been incorporated into the research-based nutrition information and guidelines that are disseminated through the CHNR website. These include Nutrition Bites, which provide targeted information on selected foods and phytochemicals, and Nutrition Fact Sheets, which provide up-to-date information on controversial research on health and phytochemicals (biofactors in plant-based foods) to the general public as well as health professionals.

Juanmonino/iStockphoto

There is evidence that the isoflavones in soybeans help to protect against heart disease, while pyrroloquinoline quinone, also found in soy, is a potent antioxidant.

Micronutrients and biofactors

The CHNR pilot projects focused on how micronutrients, biofactors and phytochemicals can help reduce the risk of chronic diseases. Micronutrients include vitamins and minerals, and one CHNR project shows that slender female athletes are particularly at risk for low bone mass and so require diets higher in calcium and other nutrients (page 124).

Biofactors are compounds in our food that affect us at the biochemical level and may ultimately benefit our health. For example, the omega-3 fatty acids in foods such as walnuts, flax seeds and salmon may protect against a range of diseases associated with inflammation, including asthma and the hypertension-related inflammation that can damage kidneys. CHNR research suggests that omega-3 fatty acids could reduce asthma symptoms (page 112) as well as kidney damage (page 106).

Other research at the Western Human Nutrition Research Center, at UC Davis, shows that tangerines can lessen vitamin A deficiencies common in developing countries, which can cause blindness (page 130).

Phytochemicals and health

Epidemiological studies link particular diets to less risk of chronic diseases. Notably, the traditional Mediterranean diet — mostly vegetables, fruits and whole grains, with moderate amounts of nuts, olive oil and red wine — is associated with lower rates of heart disease, cancer, and Parkinson's and Alzheimer's diseases.

However, it has yet to be firmly established that specific phytochemicals in our diets can protect against diseases. Nutritionists therefore advise eating a wide variety of plant-based foods rather than taking phytochemical supplements.

That said, a number of phytochemicals do show promise in protecting against and even treating chronic diseases. For example, CHNR research shows that soybeans contain estrogenlike compounds called isoflavones that may protect against heart disease (page 118), and that phytochemicals in olive oil and red wine may protect against heart disease and diabetes (pages 136 and 141).

Mitochondrial nutrients and aging

The Mediterranean diet is rich in phytochemicals that boost mitochondria (organelles in our cells that convert glucose and other nutrients into energy) and so are known as mitochondrial nutrients. When mitochondria are scarce or have genetic defects that keep them from working properly, this can generate toxic metabolites and damaging free radicals.

"Mitochondria are central to aging," says UC Irvine biochemist Edward Sharman. "Improving their function may modulate or delay the onset of diseases related to aging, such as type 2 diabetes and age-related macular degeneration [vision decline]." Mitochondrial dysfunction also plays a key role in chronic illnesses such as heart disease and the inflammation contributing to arthritis, type 2 diabetes and other diseases.

One of the most promising mitochondrial nutrients is hydroxytyrosol, which is abundant in the

extra-virgin olive oil that provides most of the fat in the traditional Mediterranean diet. Moreover, the red wine that is integral to the Mediterranean diet also increases hydroxytyrosol levels, even though it contains relatively small amounts of this phytochemical. "Alcohol induces the body to produce more hydroxytyrosol," Sharman says.

Using cultured fat cells as a model of age-related macular degeneration, Sharman and his collaborators found that hydroxytyrosol increases the number of mitochondria, thereby enhancing the function of these energy-producing organelles. "Hydroxytyrosol could ameliorate many age-related diseases," Sharman says. The team also found that a combination of four mitochondrial nutrients improves liver and immune function in diabetic rats.

A new essential nutrient?

Another promising mitochondrial nutrient is pyrroloquinoline quinone (PQQ), which was first found in nitrogen-fixing soil bacteria and is now known to be ubiquitous (page 141). "We're exposed to PQQ all the time at low levels," says CHNR co-director Robert Rucker, UC Davis nutrition professor. "It can be derived from amino acids found in stellar dust, and stellar dust is what the earth is made of." While *Escherichia coli* and other common gut bacteria do not make PQQ, soil bacteria provide it to plants in our diet. Good sources include fermented soybeans, wine, tea and cocoa.

Animal studies show that PQQ affects health markedly. Rucker and his colleagues found that depriving rats of PQQ compromised their immune systems and retarded their growth and reproductive rates. In contrast, restoring PQQ to their diets reversed these effects and returned them to good health. Moreover, PQQ stimulated nerve growth and counteracted aging in cultured cells.

How does this biofactor work? Rucker and his colleagues found that, like hydroxytyrosol, PQQ increases the number of mitochondria in cells. "It's also an extremely good antioxidant and anti-inflammatory agent," he says. "It decreases C-reactive protein, which is an anti-inflammatory marker that is high in, for example, rheumatoid arthritis."

For all that, it is not clear whether or not PQQ is an essential nutrient — those that our bodies require but do not make, including vitamins, minerals, and some fatty acids and amino acids. "It's hard to say if PQQ is essential," Rucker says. This is because even though it is clearly beneficial, other nutrients may play the same roles.

"There are dozens of compounds identified in the diet that have biological impacts, and many of them do multiple things," Rucker says. "The question becomes relative: is PQQ more important than other biofactors with similar actions?"

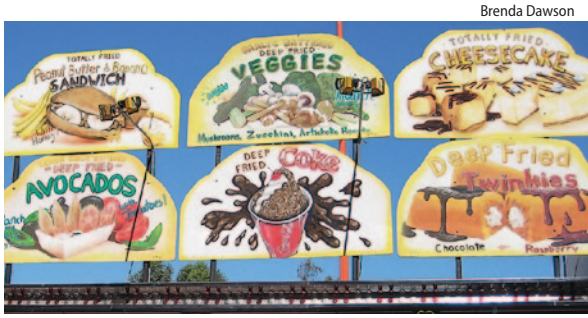
Personalized medicine

Understanding what biofactors do in our bodies could ultimately lead to personalized medicine, where nutrition-based treatments are tailored to the particulars of each person's biochemistry. This individual variation at the biochemical level may help explain the inconsistent outcomes of research on omega-3 fatty acids and inflammation.

"The studies are mixed," says UC Davis pulmonologist Nicolas Kenyon. "Some have shown little effect, and others have shown that omega-3 fatty acids can reduce arthritis and blood vessel inflammation."

Another factor is that different omega-3 fatty acids have different effects in the body. In a study of a mouse model of asthma led by Gertrud Schuster, UC Davis nutrition assistant project scientist, airway inflammation was reduced by one omega-3 fatty acid (eicosapentaenoic acid, or EPA) but not by another (docosahexaenoic acid, or DHA).

Brenda Dawson



Research-based nutritional information distributed by the Center for Health and Nutrition Research highlights healthy food choices. *Above,* fried foods are offered at the California State Fair.

Asthma can be caused by multiple biochemical pathways, which are series of chemical reactions in our cells that metabolize compounds into other products. One pathway leading to asthma may be counteracted by the omega-3 fatty acids in fish oil, and this pathway may be more active in some patients than in others. To identify those likely to benefit from treatment with omega-3 fatty acid, Kenyon and his collaborators are genotyping asthma patients (page 106). "We're looking for patients with high inflammatory mediators in this pathway," he says. "There are going to be subgroups that do really well with fish oil and others that do not."

This genotyping is targeted to DNA sequences associated with asthma and so is not comprehensive. "Some people are nervous about genome-wide analysis, which is scary because none of us is perfect," Kenyon says. "But people are more interested when the focus is specific screening that could increase their chances of treatment."

While all the vitamin settlement funds have now been disbursed, the advances and additional funding resulting from these pilot grants motivate the CHNR administrators to continue their work. "Our intent is to keep things running. We have the infrastructure to facilitate pilot projects that can attract larger grants," CHNR co-director Keen says. "This represents what we need to do for agriculture in California: we need to be adventurous about getting new resources into research and out into the community."

— Robin Meadows

More information

Center for Health and Nutrition Research
http://chnr.ucdavis.edu

RESEARCH ARTICLE

Dietary omega-3 fatty acids aid in the modulation of inflammation and metabolic health

by Angela M. Zivkovic, Natalie Telis, J. Bruce German *and* Bruce D. Hammock

This article focuses on the role of omega-3 fatty acids as precursors for lipid signaling molecules known as oxylipins. Although omega-3 fatty acids are beneficial in autoimmune disorders, inflammatory diseases and heart disease, they are generally underrepresented in the American diet. A literature review confirms that the consumption of omega-3 fatty acids — whether in food sources such as walnuts, flax seeds and fatty fish (including salmon and sardines), or in supplements — is associated with decreased morbidity and mortality. This growing body of evidence, including the results of a recent study of patients with kidney disease, highlights the need to measure omega-3 fatty acids and their oxylipin products as markers of metabolic health and biomarkers of disease. In addition, there is substantial evidence of the need to increase the omega-3 fatty acid content of American diets to optimize metabolic health.

Walnuts, flax seeds and salmon are good sources of omega-3 fatty acids, important nutrients that are generally deficient in American diets.

Many of the most significant U.S. health concerns today are modulated by omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Omega-3 fatty acids are associated with the prevention or reduction in severity of a multitude of diseases, from metabolic diseases such as heart disease, diabetes and kidney disease to neurodegenerative diseases such as Alzheimer's to an array of other inflammatory diseases including osteoarthritis.

EPA and DHA attenuate the development of atherosclerosis, or arterial plaques, by reducing concentrations of inflammatory signaling molecules called cytokines and adhesion molecules at the arterial wall where plaque forms (De Caterina et al. 2004). EPA and DHA have also been shown to stabilize atherosclerotic plaques, thereby reducing the likelihood of fatal and nonfatal cardiovascular events (Thies et al. 2003). EPA and DHA additionally reduce the synthesis of triglycerides (fat molecules) and secretion from the liver, and increase the size of low-density lipoproteins, which contribute to the reduction of cardiovascular disease risk (Griffin et al. 2006). EPA and DHA improve liver health by reducing steatosis (accumulation of fat in the liver) in patients with nonalcoholic fatty liver disease (Capanni et al. 2006). They also improve kidney health by attenuating or even reversing the loss of kidney function and reducing hypertension in kidney diseases involving the glomerulus, the main filtering part of the kidney (Donadio et al. 1994). Omega-3 fatty acids affect the joints and are used as analgesics or pain reducers in rheumatoid arthritis (Goldberg and Katz 2007).

The omega-3s even play a role in brain health: high blood plasma levels of omega-3 fatty acids are associated with a reduced risk of neurodegenerative diseases such as Alzheimer's disease (Schaefer et al. 2006) and mental disorders such as schizophrenia (McNamara et al. 2007) and depression (Sanchez-Villegas et al. 2007). Taken in supplement or food form, omega-3 fatty acids have been found to reverse the progression of a number of inflammatory diseases, from inflammatory bowel disease to diseases of the skin and joints, to other autoimmune diseases such as lupus and multiple sclerosis (Simopoulos 2002). This review focuses on the basic biology of omega-3 fatty acids as nutritional modulators of inflammation and presents preliminary results of a study of oxylipin biomarkers in kidney disease patients.

Intake, food sources and metabolism

Saturated and monounsaturated fatty acids, which have no double bonds or a single double bond, respectively, can be synthesized in the liver. In contrast,

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polyunsaturated omega-3 and omega-6 fatty acids, which have multiple double bonds, are considered essential in the human diet: they cannot be synthesized and must be consumed. Terrestrial plants contain the omega-6 fatty acid linoleic acid and the omega-3 fatty acid alpha-linolenic acid but not the long-chain metabolites arachidonic acid, or EPA and DHA. Both linoleic acid and alpha-linolenic acid can be converted to their respective long-chain metabolites in the liver through a series of enzymatic conversions (fig. 1).

However, since omega-3 and omega-6 fatty acids compete for the same enzymes, the relative dietary proportions of precursor fatty acids determine the net rate of conversion to their respective long-chain derivatives (Goyens et al. 2006; Liou et al. 2007). Linoleic acid is readily converted to its long-chain metabolite arachidonic acid, and this conversion is driven by the amount of linoleic acid ingested. However, most humans convert a smaller proportion of ingested alpha-linolenic acid into EPA and even less into DHA (Pawlosky et al. 2003). Most studies agree on estimates of about 5% conversion to EPA and less than 1% conversion to DHA (Burdge and Calder 2006). Due to this low rate of conversion of alpha-linolenic acid to EPA and DHA, and a generally low consumption of preformed dietary long-chain omega-3 fatty acids, many Americans are likely deficient in EPA or DHA.

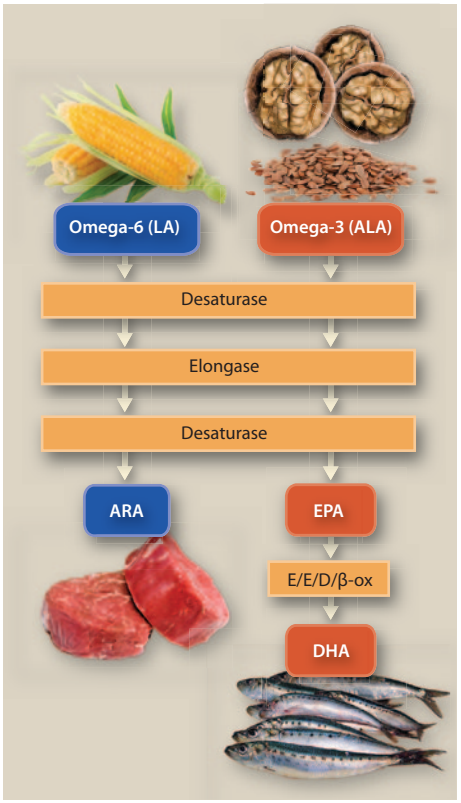
Omega-6 fatty acids are abundant in the Western diet and are found in high proportions in most cooking oils, grains and grain-fed animal products. On the other hand, omega-3 fatty acids are generally deficient in the foods typically consumed by Americans. Certain foods — including leafy greens, walnuts, canola oil, flax-fed chicken eggs and fatty fish (e.g., wild salmon, anchovies, mackerel and tuna) — have relatively large concentrations of omega-3. Historically, humans consumed diets with much higher relative proportions of omega-3, with ratios of omega-6 to omega-3 of 1-to-1 or 2-to-1, in contrast to modern diets with ratios as high as 15-to-1 to 25-to-1 (Simopoulos 2008).

The disproportionate consumption of omega-6 as compared to omega-3 fatty acids is the result of common U.S. dietary patterns. Foods such as salmon, walnuts, kale and eggs from chickens fed flax have higher omega-3 fatty acid content than do more common American foods, like corn oil, beef and potatoes (USDA 2011). For example, raw pink salmon has 419 milligrams of EPA and 586 milligrams of DHA per 100 grams, whereas raw ground beef contains none. Walnuts contain 3,800 milligrams of linoleic acid and 9,080 milligrams of alpha-linolenic acid per 100 grams. On the other hand, peanuts contain 15,600 milligrams of linoleic and only 3 milligrams of alpha-linolenic acid per 100 grams. These ratios mean that the typical American diet is deficient in omega-3 fatty acids, especially when considered in relation to omega-6 fatty acids (Kris-Etherton et al. 2000).

Because of the increasing levels of contaminants, like mercury and dioxins, in both domestic and imported fish (Stahl et al. 2009; Sunderland 2007), the consumption of fish as a main source of long-chain omega-3 fatty acids is problematic, and some types of fish are not recommended for consumption by pregnant women and small children (Jedrychowski et al. 2007). Since terrestrial plants contain only the short-chain omega-3 precursor alpha-linolenic acid, and since alpha-linolenic acid is poorly converted into its long-chain metabolites EPA and DHA, it is likely that the American diet will continue to be relatively deficient in these important molecules unless significant dietary changes are made.

Dennis S. Hurd

Eggs from chickens that have been fed flax have higher concentrations of omega-3 fatty acids, which have been shown in a number of studies to be beneficial to human health.



Corn, Yasonya/iStockphoto; walnuts, Jack Kelly Clark; flaxseed, Zbigniew Ratajczak/iStockphoto; red meat, Stuart Monk/iStockphoto; sardines, Kathleen Nolan

Fig. 1. Dietary sources and enzymatic conversion pathways of omega-3 and omega-6 fatty acid precursors. The omega-3 precursor alpha-linolenic acid (ALA) is found in walnuts and flax seeds, and the omega-6 precursor linoleic acid (LA) is found in corn and vegetable oils. Both are converted by desaturase and elongase enzymes into their long-chain derivatives: ALA is converted to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); and LA is converted to arachidonic acid (ARA). ARA is found in animal products, such as beef, and EPA and DHA are found in fatty fish, such as sardines.

Reducing disease risks

Cardiovascular disease. Omega-3 fatty acids play an important role in reducing cardiovascular disease, a leading cause of death in the United States (Harris et al. 2009). They reduce events that lead to heart attack by stabilizing the heart muscles against arrhythmia (irregular heartbeat or abnormal heart rhythm) (Ballantyne 1999). For example, 200 milligrams DHA per day decreases the chance of death from cardiac arrest by 50% (Horrocks and Yeo 1999).

EPA and DHA metabolites stimulate the dilation of arterioles (small arteries) (Ye et al. 2002), which decreases blood pressure and inhibit the expression of inflammatory genes (Bouwens et al. 2009). A literature review covering 2002 to 2004 — including a pooled analysis of 48 randomized, controlled trials and 41 cohort trials — found no significant benefit of supplementation with omega-3 fatty acids for the reduction of total mortality, cardiovascular events or cancer (Hooper et al. 2004). However, as the authors point out, there was "significant statistical

heterogeneity" among the results given that the dosages and formulations of omega-3 fatty acids and the outcomes evaluated varied substantially, with some studies showing benefits and others not. This suggests that further studies are needed to evaluate the specific effects of different dosages and formulations of omega-3 fatty acids in different population groups and at different supplementation levels.

Kidney disease. Animal trials show that omega-3 fatty acid treatment decreases blood pressure, has anti-inflammatory effects, slows renal failure and moderates the side effects of hypertension (Imig et al. 2005; Zhao et al. 2004). This has been the basis for the study of the effects of omega-3 fatty acids on kidney disease. A recent literature review found that no definitive conclusions can be made about the effectiveness of omega-3 fatty acids for the prevention or treatment of kidney disease (Fassett et al. 2010).

However, just as in the review of effects on cardiovascular disease, the authors point out that there was substantial variability from study to study in the dosages, proportions of specific omega-3 fatty acids administered (i.e., formulations), duration of supplementation, sample sizes and specific outcomes assessed. This again suggests that further studies are needed to better understand the specific formulations, dosages and markers of effectiveness for omega-3 fatty acid supplementation.

Neurological disease. There is increasing evidence that DHA specifically plays a significant role in neurological development and disease prevention. DHA is an important component of brain development, and DHA deficiencies have been found in patients with neurological conditions. For example, there is a strong correlation between depression and DHA deficiency (Horrocks and Yeo 1999). Deficiency in DHA has also been strongly correlated with neurodegenerative diseases common in the elderly population, particularly Alzheimer's disease (Freemantle et al. 2006).

Arthritis. Omega-3 fatty acid intake is associated with the improvement of rheumatoid arthritis, as omega-3 metabolites inhibit the production of inflammatory cytokines responsible for arthritic pain (Zainal et al. 2009). Supplementation with EPA and DHA is effective against arthritic pain as well as other symptoms, including joint stiffness (Goldberg and Katz 2007).

Oxylipins and inflammation

Oxylipins are fatty acids that have been converted into lipid signaling molecules by enzymes in the body. The omega-6 fatty acid arachidonic acid, and the omega-3 fatty acids EPA and DHA, are converted into a wide variety of oxylipins with diverse and important signaling pathways that mediate a number of biological functions (Funk 2001).

Most of the functions of oxylipins derived from arachidonic acid, also called eicosanoids, are well known and primarily associated with immuno-stimulatory events (which stimulate the immune system) and pro-coagulant actions (which promote blood coagulation). However, the actions of these oxylipins are typically oversimplified as "pro-inflammatory," when, in fact, over 90 bioactive omega-3 and omega-6 oxylipins with both pro- and anti-inflammatory effects are produced by enzymes — cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP).

Pro-inflammatory, pro-coagulant and immuno-stimulatory oxylipins are produced acutely in response to a diverse array of stimuli, such as injury to a blood vessel wall in order to initiate repair and immune cell recruitment, or to initiate blood clotting in response to injury to prevent hemorrhage (fig. 2). An injury to the vessel wall leads to an influx of calcium ions into the cell, which in turn stimulates the action of an intracellular enzyme that cleaves fatty acids from phospholipids (complex lipids that make up the cell membrane). This is followed by conversion of the released fatty acid into oxylipins, which initiate the recruitment of immune cells and stimulate signaling pathways to repair the injury via paracrine (signaling between neighboring cells) and autocrine (signaling within the same cell) pathways. Finally, after the injury has been repaired, the released fatty acids are converted into oxylipins that terminate the immuno-stimulatory and repair mechanisms.

The interplay of the various oxylipins produces results in nuanced responses to specific events over the course of an injury such that the net physiological effect

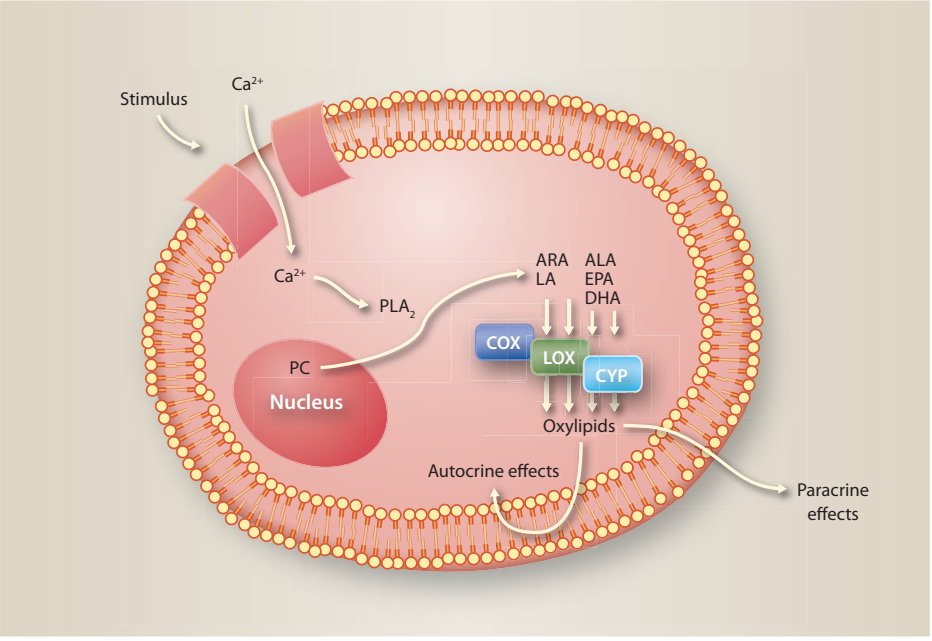


Fig. 2. Cellular mechanisms involved in producing oxylipin derivatives of omega-3 and omega-6 fatty acids. A stimulus such as cell wall injury activates transporter proteins, moving Ca^{2+} ions into the cell. This stimulates the release of fatty acids from nuclear membrane phospholipids (phosphatidycholine [PC]) by the enzyme phospholipase A2 (PLA_2), and the conversion of omega-3 (eicosapentaenoic [EPA], docosahexaenoic [DHA] and alpha-linolenic [ALA] acid) and omega-6 (arachidonic [ARA] and linoleic [LA] acid) fatty acids into signaling oxylipin products via the COX (cyclooxygenase), LOX (lipoxygenase) and CYP 450 (cytochrome P450) enzymes. These oxylipins are signaling molecules that help regulate inflammation, increasing and decreasing this important immune response within the cell (autocrine) and in neighboring cells (paracrine).

of the complex combination of oxylipins is key, rather than the absolute concentration of any one of the oxylipins alone (Serhan and Savill 2005). Because oxylipins initiate and terminate various immune responses in different body tissues, they are important players in diseases such as heart disease, chronic inflammation, autoimmune disorders and cancer.

The dietary imbalance between omega-6 and omega-3 fatty acids can result in inappropriately elevated or sustained immuno-stimulatory responses to injury or inappropriately diminished immuno-suppressing responses after an injury has been repaired. It has been suggested that the increasing incidence of hypertensive and inflammatory conditions corresponds to the proportional increase in omega-6 fatty acids and decrease in omega-3 fatty acids in the American diet (Simopoulos 2002).

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Evidence is mounting that omega-3 fatty acids can moderate inflammation in the body and help reduce the risk and symptoms of kidney disease, heart disease and arthritis. *Above,* a patient undergoes dialysis for kidney disease.

Assessment of omega-3 status

Despite growing evidence supporting the beneficial effects of omega-3 fatty acids, there is little basic science about its metabolism under different lifestyle conditions and its variation across different populations and genotypes (genetic backgrounds). An adequate daily intake for adults of alpha-linolenic acid has been established by the U.S. Department of Agriculture at 2.22 grams per day based on consensus among field experts, and recommended EPA and DHA intake totals are 0.65 gram per day (Simopoulos et al. 1999). However, the biological endpoints for which omega-3 fatty acids are effective have not been established. For example, although omega-3s are known to prevent cardiac arrhythmia and heart attacks (Horrocks and Yeo 1999), a plasma level of omega-3 considered adequate for this effect has not been determined. Clearly, the measurement of omega-3 status would be beneficial in determining which individuals may be at increased risk of heart attack, as well as a number of other disorders that they mediate.

Even less is known about the optimal plasma concentrations of oxylipins. The normal ranges of omega-3-derived oxylipins were recently reported before versus after supplementation in a small sample of healthy men (Shearer et al. 2009). This is the first such investigation, and while little is known about the concentrations of omega-3 fatty acids themselves across populations, even less is known about concentrations of their oxylipin metabolites. The accurate measurement in plasma and red blood cells of omega-3 and omega-6 fatty acids, and the oxylipins derived from them, would enable the investigation of how these concentrations are affected by different disease states. Much like the measurement of cholesterol concentrations in plasma has led to reductions in the incidence of heart disease (EAS 1987), and the measurement of arachidonic acid–derived oxylipins has enabled the estimation of inflammation (Funk 2001), the measurement of omega-3 fatty acids and their oxylipin products will be valuable as a clinical toolset for detecting, monitoring and reversing an array of diseases. However, oxylipin measurement is currently limited to the research laboratory and is not available to the medical community or the general public as a diagnostic.

Kidney disease study

One example of the type of information obtainable by measuring oxylipin levels in humans was illustrated by a recent study of patients with immunoglobulin A nephropathy (IgAN), a type of inflammatory kidney disease. Because inflammation is one of the underlying causal mechanisms in IgAN, and because omega-3 fatty acids and their oxylipin products tend to be anti-inflammatory, a study was conducted to evaluate the effectiveness of omega-3 fatty acids in modulating kidney function in a set of IgAN patients.

In the current study, we identified a unique opportunity to measure oxylipins in a set of stored samples from a previously conducted trial of IgAN patients and to compare those to oxylipins in healthy control subjects. This was unique because the former patient trial involved 37 nephrology centers and was the culmination of over 8 years of work and millions of dollars in funding, which would have been nearly impossible to replicate.

For the stored samples from IgAN patients, the study design has been described in detail (Hogg et al. 2006). Briefly, 96 patients were recruited and randomly assigned to receive one of three daily treatments over 2 years: (1) a steroidal anti-inflammatory drug, (2) fish oil containing 2 grams EPA plus 1.5 grams DHA or (3) a corn oil placebo. Subjects had biopsy-confirmed IgAN, and kidney function was assessed as changes in glomerular filtration rate and urine protein to creatinine (UP/C) ratios.

The healthy control subjects were supplemented for 6 weeks with 2 grams EPA plus 1.5 grams DHA daily to match the concentrations of active ingredients (EPA and DHA) given to the IgAN patients. Controls were supplemented for 6 weeks instead of 2 years because as healthy subjects their kidney function was not assessed and because preliminary trials in our labs showed that 6 weeks was needed to achieve maximal plasma levels of omega-3 fatty acids at this supplementation dose.

Over 80 different individual oxylipin molecules were analyzed by mass

spectrometry and grouped into categories based on their synthetic pathways. Oxylipins that are metabolized by the CYP enzymes are called epoxides, which can be further metabolized by the soluble epoxide hydrolase enzyme into diols. Oxylipins that are metabolized by the COX enzymes and those metabolized by the LOX (5-LOX and 12/15-LOX) enzymes were added together. Total oxylipins were also calculated by summing all 87 individual oxylipins together.

Oxylipin responses. The IgAN patients had significantly higher total oxylipins as well as each of the categories of oxylipins — including epoxides, diols, COX and LOX metabolites — relative to healthy controls (table 1). These results are consistent with the systemic inflammation in IgAN patients that is associated with overproduction of oxylipins. These oxylipins are likely mediators of a vast array of inflammatory signaling events.

When the response to supplementation with omega-3 fatty acids was compared among IgAN patients and controls, there were clear differences (table 2). Whereas diols increased in controls by 27%, they decreased by 17% in IgAN patients supplemented with fish oil. In controls, the COX metabolites decreased by 4% in response to fish oil, whereas in IgAN patients they did not change. Total oxylipins decreased by 15% in IgAN patients on fish oil but did not change in the controls.

These data indicate that IgAN patients responded differently to omega-3 fatty acid supplementation than did healthy controls. Since the IgAN patients had almost 200-fold higher total oxylipins than the controls at baseline, the 15% decrease in response to fish oil suggests that although fish oil was able to reduce inflammation in these patients, it was not enough to reduce their oxylipin levels to those of controls. As expected, oxylipin levels did not change in IgAN patients on the corn oil placebo.

Oxylipins may be a potential marker of improvement in kidney function and response to treatment.

Kidney function. The patients were also examined as two separate groups: those whose kidney function improved (greater than 25% decrease in UP/C) over 2 years versus those whose kidney function did not improve (less than a 25% decrease in UP/C). In patients whose kidney function improved, total LOX metabolites were lower at baseline, and total COX metabolites were lower at the post time point (table 3). These data indicate that oxylipins may be a potential marker of improvement in kidney function and response to treatment.

Targeted nutrition recommendations

Once the normal ranges of omega-3 fatty acids and their oxylipin metabolites have been established, metabolite databases necessary for the discovery of specific plasma-based biomarkers will be used both to assess initial metabolic status and monitor treatment progress. Plasma oxylipin profiles could eventually be used to determine both inflammatory status and responsiveness to dietary changes in the ratio of omega-6

TABLE 1. Differences in serum oxylipins between immunoglobulin A nephropathy (IgAN) patients and controls at baseline*

	IgAN patients		Controls		
Oxylipin†	Mean	SE	Mean	SE	*P*‡
	nmol/l				
Epoxides	313.6	27.20	9.61	0.93	< 0.001
Diols	114.0	6.88	24.74	2.69	< 0.001
COX	238.8	20.14	22.45	2.09	< 0.001
5-LOX	47,560	4,372	144.8	18.81	< 0.001
12/15-LOX	52,210	5,998	342.6	45.99	< 0.001
Total oxylipins	103,600	11,190	566.8	66.38	< 0.001

* Data are the mean and SE for each significantly different oxylipin in patient and control groups at baseline (presupplementation).
† COX = cyclooxygenase; LOX = lipoxygenase.
‡ *P* values are for unpaired *t*-tests comparing patients with controls.

TABLE 2. Changes in serum oxylipins pre- and post-supplementation in IgAN patients given fish oil or corn oil placebo, and controls given fish oil*

		IgAN patients	
Oxylipin	Controls†	Corn oil placebo	Fish oil
	%		
Diols	27	0	–17
COX‡	–4	0	0
Total oxylipins	0	0	–15

* Data are percent change from pre- to postsupplementation, calculated as mean values of total oxylipins of each category for each group ((Post-Pre)/Pre) × 100.
† Controls = supplemented with omega-3 fatty acids from fish oil; Fish oil = immunoglobulin A nephropathy (IgAN) patients supplemented with omega-3 fatty acids from fish oil; Corn oil = IgAN patients supplemented with corn oil placebo.
‡ COX = cyclooxygenase.

TABLE 3. Differences in serum oxylipin categories between patients whose kidney function was either nonimproved or improved*

	Nonimproved		Improved		
Oxylipin†	Mean	SE	Mean	SE	*P*‡
	nmol/l				
5-LOX§	54,790	6,346	39,330	5,752	0.06
COX¶	358.0	43.99	243.5	33.18	0.02

* Data are means and SE for oxylipin categories significantly different between patients whose kidney function was either nonimproved (> –25% change in UP/C ratio) or improved (≤ –25% change in UP/C ratio) from baseline to 2 years.
† LOX = lipoxygenase; COX = cyclooxygenase.
‡ Student's *t*-tests comparing two groups.
§ Total 5-LOX values measured at baseline (presupplementation).
¶ Total COX values measured postsupplementation.

to omega-3 fatty acids. More studies are needed to determine the normal ranges of oxylipins as well as the deviations from these normal ranges in different disease states, so that dietary interventions can be evaluated. The long-term goal is the ability to design targeted nutritional recommendations for health optimization and disease prevention in individuals and groups.

Omega-3 fatty acids are generally deficient in Western diets. This deficiency is due in part to the disproportionately high consumption of omega-6 fatty acids, which are more common in commercial products like fast food, as compared to omega-3 fatty acids, which are less common. Dietary deficiencies in omega-3 fatty acids may be contributing to the preponderance of inflammatory diseases in the United States and elsewhere.

The effects of omega-3 fatty acids on modulating inflammation are poorly understood, in part due to the lack of information regarding the plasma concentrations of omega-3 fatty acids and their oxylipin products in both healthy and diseased states. However, omega-3 fatty acids have well-supported broad effects on human health; they improve cardiovascular health, improve the condition of patients suffering from autoimmune and neurological diseases, and promote renal health.

Though in general we can conclude that omega-3 fatty acids are beneficial to health, a better understanding of the mechanisms and individual variations in the metabolism of the omega-3 fatty acids and their oxylipin products is needed. With this understanding, nutritional recommendations regarding the intakes of these anti-inflammatory dietary compounds could be improved to better treat and prevent inflammatory diseases.

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Asthma patients with specific genotypes identified for fish oil treatment trial

by Olga Fortenko, Amir Zeki, Gertrud Schuster, Cristina Davis, Hooman Allayee, Charles Stephensen *and* Nicholas J. Kenyon

The lifetime prevalence of asthma in California is nearly 20%, and better therapies are needed to manage this common chronic disease. Fish oils containing omega-3 fatty acids are considered a potential therapy for asthma and other inflammatory diseases. Fish oil inhibits the production of arachidonic acid 5-lipoxygenase (ALOX5), an enzyme that exacerbates the lung inflammation that causes asthma. We discuss the genetics of asthma and our preliminary results using a strategy to identify the subgroup of patients who may respond well to treatment with fish oil. These findings, and others, suggest that certain gene polymorphisms of the ALOX5 gene predispose patients to the increased production of inflammatory leukotrienes. Our clinical trials will test the hypothesis that patients with moderate to severe asthma, and with specific high-risk ALOX5 gene sequence variations, will have fewer asthma symptoms when treated with fish oil. The strategy is to decrease the total burden of leukotriene production by supplementing with omega-3 polyunsaturated fatty acids. These studies will also help determine whether genotyping or metabolic profiling (for example, with exhaled breath condensate) can help establish "personalized medicine" for asthma.

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Clockwise from top left, **sardines, fish oil pills, flax seeds and soybeans contain omega-3 fatty acids. The omega 3s inhibit the activity of ALOX5, an enzyme that exacerbates lung inflammation and causes asthma.**

Asthma is a chronic inflammatory disorder of the body's lungs that is associated with airway reactivity (or "twitchy" airways) and obstruction. It affects an estimated 25 million people in the United States, including 6 million children. Among adults, asthma accounts for approximately 14 million doctor visits and 2 million emergency room visits annually. The annual cost of asthma nationwide is nearly $15 billion, mostly from hospitalizations and emergency room visits but also the cumulative time lost from school absences and missed work (CDC 2002). Sixty to 80% of the health care dollars dedicated to asthma are spent on caring for patients with difficult-to-control asthma, a subgroup that comprises about 10% of all asthmatics (McFadden and Hejal 2000).

In California, as in much of the country, the prevalence of asthma in children and adults has increased dramatically over the past three decades, straining health system resources. Asthma is a classic disease with "gene-environment" interactions, meaning that there is both a host of genes that are associated with the risk of developing asthma but environmental triggers affect this risk. Asthma triggers in the Central Valley of California, for example, include trees, grasses, pollens, air pollution, ozone and possibly insecticides. Scientists do not know all asthma triggers, and it is still difficult to fully explain the increase in asthma in recent decades.

Approximately 13% of Californians have been diagnosed with asthma, and the chance of developing asthma anytime in life is close to 20% (Meng et al. 2003). There are remarkable disparities in asthma prevalence among different ethnic groups in California: among 5 to 17 year olds, the rates are 27.2% for black, 15.6% for Latino and 18.7% for white children. Emergency room visits for asthma in California are four times higher for blacks than whites (144.5 compared to 35.7 visits per 10,000 residents, respectively). The risk of death is highest for adult black women over age 65 (CDPH 2008).

In 2008, the California Department of Public Health (CDPH) published the *Strategic Plan for Asthma in California: 2008–2012* (CDPH 2008). The document identified the following focus areas: eliminating asthma disparities among ethnic groups, providing asthma education to all

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Californians, improving asthma care for all ages and promoting effective asthma management programs. One such program is the UC Davis Asthma Network (UCAN), which includes referral clinics for over 700 patients with difficult-to-control asthma (more than 70% women) (Mealey et al. 2007). Central Valley patients enrolled in this program undergo a rigorous evaluation for environmental and air pollution triggers of their asthma attacks. Other asthma triggers — including indoor house dust mite, cockroach and food allergies — are also determined; the basis of asthma treatment is appropriate avoidance of predictable asthma attacks, making the process of trigger identification critical.

In addition to the CDPH plan, several national and international societies have published asthma guidelines for managing mild to severe asthma, including the British Thoracic Society, Global Initiative for Asthma, and the National Institutes of Health National Asthma Education and Prevention Program (NAEPP). The most recent NAEPP expert panel report (NIH 2007) emphasizes four essential components of asthma care: assessment and monitoring, patient education, control of environmental triggers that affect a patient's asthma, and medications. It provides a detailed approach to achieving asthma control.

Treating asthma

There are two major classes of medications used to treat asthma: inhaled corticosteroids and bronchodilators. Daily inhaled corticosteroids (ICS), such as fluticasone and budesonide, are the preferred long-term asthma control therapy for all ages. Albuterol and other short-acting bronchodilators are used for sudden symptoms of wheezing and shortness of breath, while long-acting bronchodilating beta-2 agonists (LABA), including salmeterol and formoterol, are preferred as add-on maintenance therapy to ICS. Both short- and long-acting beta-2 agonists cause bronchodilation and smooth muscle relaxation, and are commonly prescribed to asthmatic patients for symptom relief. However, LABAs have received black-box warnings from the U.S. Food and Drug Administration (FDA) because a few clinical studies have suggested an increased risk of asthma deaths in patients on these medications. Discussions are ongoing about their use. High-dose ICS and LABA with or without oral, pill-form corticosteroids are also used to treat severe asthma. In addition to medications, patient education is encouraged at all points of asthma care, including clinics, emergency departments and hospitals, pharmacies, schools, community centers and patients' homes.

Despite the availability of the excellent NAEPP guidelines for physicians, all patients respond differently to treatment, necessitating individualized treatment plans. Some patients have greater control of asthma symptoms with ICS than others; this may be due to differences in the inflammatory cells present in the lungs of asthma patients. Alternatives to inhaled and oral steroids are sometimes needed to help certain patients with severe asthma. While a stepwise approach enables doctors to refine treatments based on individual patient responses and asthma severity, scientists are now developing powerful technologies that may someday enable doctors to identify much more precisely which treatment plans are most likely to yield good results based on a patient's genetic profile.

Personalized medicine for asthma

Disease heterogeneity and highly variable patient responses to drugs make prescribing effective treatments difficult for some asthmatics. One approach or set

Glossary

Allele: One of two or more alternative forms of a gene at a specific location on a chromosome.

Eosinophil: A type of white blood cell involved in asthma, allergic responses and parasitic infections.

Genotype: Genetic composition of an organism; set of genes inherited from each parent that determines specific traits.

Leukotriene: Fatty molecule derived from arachidonic acid and involved in mediating inflammation and bronchoconstriction in asthma.

Nutraceutical: A food or dietary supplement that provides medicinal benefits as well as nutritional value.

Phenotype: Trait of an organism that is observable, the result of gene expression and environment.

Polymorphism: Occurrence of two or more phenotypes within a population.

Single-nucleotide polymorphism: A variation of a single nucleotide (A, T, C or G) within a DNA sequence that results in alternative forms of a gene in different individuals.

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Asthma affects about 25 million people each year, including 6 million children. The prevalence of asthma is higher among black children and youths (27.2%) than among Latino (15.6%) or white (18.7%) children and youths.

of guidelines does not fit all. The concept of personalized medicine has centered on pharmacogenomics, which recognizes that the variability of patient responses to medical therapy is attributable to genetic variations. Ultimately, physicians hope to prescribe asthma medications based on a clear understanding of which drug each patient may benefit from. However, despite stepwise guidelines designed to tailor asthma therapy to patients based on their disease severity, a personalized approach to asthma management has not been achieved.

Genomics is the study of genes and their effects on a particular disease state. More than 100 genes have been associated with the development of asthma, but fewer than 10 have been identified in more than 10 studies. Most of these genes confer an increased susceptibility to allergic inflammation or hyperresponsiveness of airways.

Genotyping is the analysis of a person's specific gene sequence. Differences in the gene sequences that code for certain proteins offer possible insights into subtypes of asthma; this is a promising approach to personalizing asthma care. From a therapeutic perspective, there is also interest in understanding how diet and nutraceutical supplements influence the development of asthma. Epidemiological data has shown that a Western diet rich in fats and depleted of whole grains correlates with high incidences of asthma, and also that populations that eat large amounts of fish have low incidence rates (Hodge et al. 1996).

We used a genomic approach to test whether fish oil supplements could improve clinical outcomes for people with difficult-to-control asthma. Fish oil concentrate contains omega-3 polyunsaturated fatty acids (n-3 PUFAs) — comprised of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) — which inhibit the activity of arachidonic acid 5-lipoxygenase (ALOX5). ALOX5 is an enzyme present in many inflammatory cells that makes inflammatory proteins called leukotrienes. Leukotriene levels are very high in asthma patients and contribute to the inflammation and airway reactivity that are the hallmarks of the disease. ALOX5 is an important target for newer drugs being developed for asthma. Our clinical trial is studying whether patients with moderate to severe asthma who have specific high-risk ALOX5 gene polymorphisms have fewer asthma attacks or exacerbations when using a fish oil supplement.

Genetic variation and asthma responses

Single nucleotide polymorphisms (SNPs) are variations in the coding of a DNA sequence that occur as a result of a single nucleotide change. They are the most frequent type of genetic variation, with about 10 million identified in the human genome. One such SNP encodes for the receptor ADRB2. This receptor is important in human health, and its different forms, point mutations or down-regulation are associated with nocturnal asthma, obesity and type 2 diabetes.

For asthma sufferers, this genetic variation (a switch from guanine to adenine at one position) may be associated with a worse response to asthma inhalers such as albuterol or salmeterol beta-2 agonists (substances that initiate a physiological response when combined with a receptor). Because human cells have two sets of chromosomes, there are three possible genotypes that result from this SNP (A/A, A/G and G/G). Asthmatics with the A/A type have shown poorer improvements in expiratory flow rates after receiving albuterol than asthmatics with either G/G or A/G genotypes.

Asthma genomics

Genetic studies in asthma are difficult to interpret because of the sheer number of gene abnormalities that correlate positively with the presence of asthma. However, of more than 100 genes of potential interest, ADRB2 and ALOX5 are perhaps the most clinically relevant.

ADRB2. ADRB2 is the gene for the beta-2 adrenergic receptor (Warrier and Khurana Hershey 2008). It is of interest to clinicians because there is good evidence that the variability in patient responses to beta-2 agonists such as albuterol can be attributed to single nucleotide polymorphisms (SNPs) on the ADRB2 gene (see box) (Moffatt 2008). An SNP is a DNA sequence variation of a single nucleotide that can lead to very different protein structure or function. It is important that doctors and scientists understand these SNP variations in order to determine whether certain medications will affect patients differently. Certain ADRB2 SNPs predict poor response to beta-2 agonist medications. This new knowledge contributed to the FDA's decision to place a black-box warning on LABAs. Because there are risk factors with medical treatment, utilizing SNP information could eliminate trial and error in treatment. This is an example of how pharmacogenomics may provide doctors and scientists with the information to more appropriately target medications to certain patients.

ALOX5. ALOX5 encodes the enzyme that produces leukotrienes, which play a role in the predisposition to severe asthma. It may be the second most clinically important gene discovery in asthma. Polymorphisms in ALOX5 and also in 5-lipoxygenase-activating-protein (FLAP) are associated with the excessive production of leukotrienes (Koshino et al. 1999). The levels of ALOX5 and FLAP messenger RNA — the RNA molecule that is the blueprint for the protein — are increased in peripheral blood leukocytes from asthmatics (Koshino et al. 1998). In addition, the concentrations of leukotrienes in sputum were greater in patients with asthma than in nonasthmatic subjects (Ogawa and Calhoun 2006).

A study by Klotsman et al. (2007) found that a subset of asthmatics with ALOX5 polymorphisms were predisposed to having higher cysteinyl-leukotriene (cys-LT) concentrations, and they constituted an asthma phenotype more likely to respond to a leukotriene-inhibiting drug such as montelukast. Another study examined the association of ALOX5 promoter genotypes with responses to montelukast therapy. While on therapy, patients with the ALOX5 SNP variant had fewer asthma symptoms than controls (Lima et al. 2006).

Fish oil and the leukotriene pathway

Some asthma patients benefit greatly from treatment with anti-leukotriene drugs, which can attenuate the effects of leukotrienes not blocked by steroids. Overall, however, the anti-leukotriene drugs are only moderately anti-inflammatory and are considered second-line

asthma therapies, after inhaled corticosteroids (Tantisira and Drazen 2009). The leukotriene inhibitors (montelukast and zafirlukast) and 5-lipoxygenase inhibitor (zileuton) improve airway function primarily by blocking the inflammatory effects of various leukotrienes (fig. 1).

Nutraceuticals such as fish oil may play a role in the treatment of asthma by inhibiting 5-lipoxygenase, reducing the need for medicines. Components in fish oil (omega-3 fatty acids, composed of EPA and DHA) suppress the production of arachidonic acid–derived leukotrienes via the ALOX5 pathway. Arachidonic acid is an omega-6 fatty acid present in the body's cell membranes; it is a key substrate for the synthesis of eicosanoids, potent mediators of the inflammatory response that include cys-LTs, prostaglandins and thromboxanes (Calder 2006).

Byproducts of these mediators (LTC4 and related cyst-LTs) are directly involved in the bronchoconstriction and increased mucus production seen in the airways of asthmatics. EPA-enriched fish oils may competitively inhibit the production of LTC4 by competing with arachidonic acid as a substrate for ALOX5 (fig. 2). EPA may also suppress an allergic response in asthma by competing with arachidonic acid as a substrate for the production of leukotrienes.

One leukotriene, prostaglandin E2, mediates the formulation of immunoglobulin E (IgE), an antibody responsible for initiating allergic responses. It is usually elevated in patients with asthma. EPA and DHA are precursors to potent bioactive mediators that possess both anti-inflammatory and protective properties.

The World Health Organization recommends a dietary ratio of omega-6 to omega-3 fatty acids of four to one. Studies have shown that after just 2 weeks of dietary supplementation with EPA, the composition of cell membranes can change to include higher levels of omega-3s (Wong 2005). Changing the membrane composition of fatty acids via daily fish oil intake may modulate the production of inflammatory leukotrienes and have a significant impact on curbing asthma symptoms.

We are performing a clinical study to determine whether dietary supplementation with EPA- and DHA-enriched omega-3 fatty acids can ameliorate the production of inflammatory leukotrienes and decrease the number of acute exacerbations in patients with moderate to severe asthma. The hypothesis is that fish oil supplementation can impede the production of inflammatory leukotrienes in asthmatics with SNPs in the promoter of ALOX5. Furthermore, we believe that this subset of asthmatics, which we term high risk, is likely to have one or more polymorphisms of specific genes in the 5-lipoxygenase pathway.

This new approach — using genotyping to identify a subset of patients with asthma who are likely to respond to a specific therapy — can have far-reaching implications in terms of asthma outcomes. Self-administration and ingestion of fish oils as anti-inflammatory or high-cholesterol medications is common in the general population; several people considered for our study were unwilling to discontinue their home supplements and were therefore ineligible. Subjects interested in enrolling had a 2-month fish oil–free "washout" period. Fish intake was limited to less than three servings per week, and a dietary questionnaire was given to assess recent fish consumption. In this manner, we hoped to standardize extraneous fish oil intake among the study subjects.

TABLE 1. Prevalence of ALOX5 genotypes in asthma cohort (n = 35)

ALOX5-genotype*	Total	Gender M/F	Age	Cohort
			years	*%*
35	2	1/1	63, 68	5.7
36	1	0/1	48	2.8
44	2	1/1	43, 49	5.7
45	11	3/8	19–56	31.4
55	13	6/7	27–76	37.1
56	4	3/1	39–65	11.4
57	2	1/1	19, 68	5.7

* The number of gene sequence repeats for ALOX5 for each allele.

Genotyping of asthmatics

We have performed genotyping of the ALOX5 gene in a full cohort of 35 subjects, 22 of whom are female. The mean age is 46.7 years with a range of 19 to 76 years, reflecting the diversity of moderate

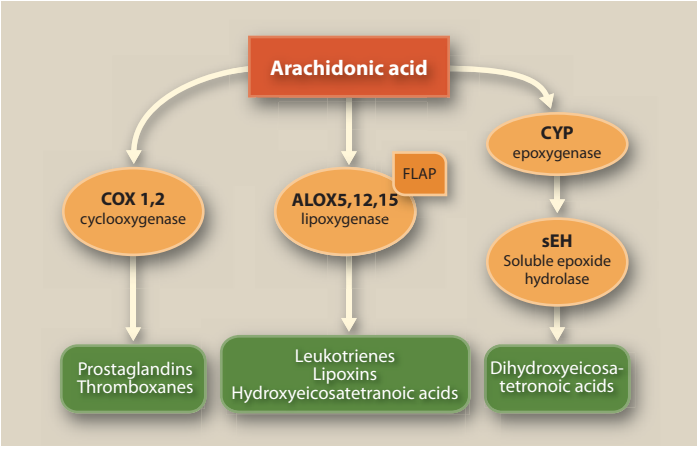


Fig. 1. Arachidonic acid is metabolized by multiple enzymatic and nonenzymatic pathways. Leukotriene receptor antagonists and 5-lipoxygenase inhibitors are used to treat asthma patients, about 15% of whom are sensitive to cyclooxygenase inhibitors. Soluble epoxide hydrolase inhibitors are presently being investigated in clinical trials and are of therapeutic interest.

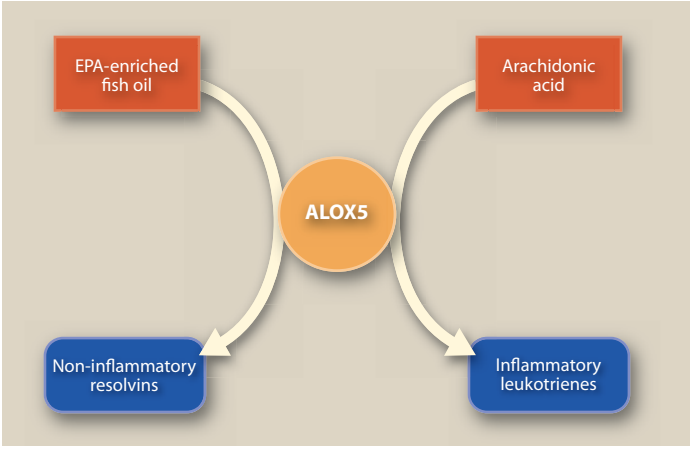


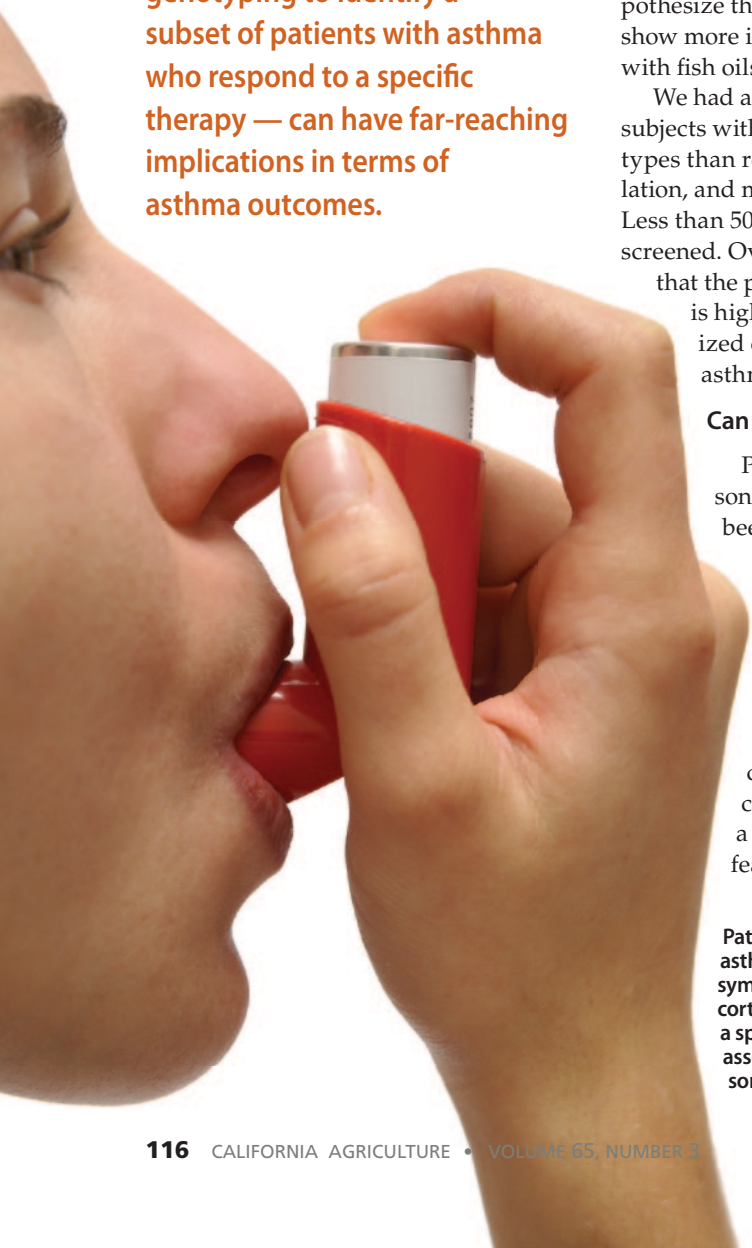
Fig. 2. Specifically enriched fish oils compete with arachidonic acid for the ALOX5 enzyme, potentially decreasing the production of inflammatory leukotrienes. We hypothesize that asthma patients with specific high-risk ALOX5 gene polymorphisms will derive greater clinical benefit from fish oils enriched with eicosapentaenoic acid (EPA), an omega-3 fatty acid.

TABLE 2. Classification of high-risk and control ALOX5 genotypes

Genotype*	Recruitment category
55	Control
56	Control
57	Control
53	High risk
54	High risk
33	High risk
34	High risk
44	High risk
36	High risk
46	High risk
Other	Not eligible

* The number of gene sequence repeats of ALOX5 for each allele.

This new approach — using genotyping to identify a subset of patients with asthma who respond to a specific therapy — can have far-reaching implications in terms of asthma outcomes.



Patients with moderate to severe asthma often have persistent symptoms, even with use of inhaled corticosteroids. There is evidence that a specific genetic variation may be associated with poor responses to some medications.

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to severe asthma in adults (table 1, page 109). Subjects were identified as having a low-risk (control) genotype, or a high-risk genotype based on the number of gene sequence repeats (three to seven) for the binding site of the transcription factor Sp1, which regulates the expression of ALOX5. The most common DNA sequence, or allele, has five such repeats, and a 55 genotype reflects the presence of five repeats on each of the two DNA strands (table 2).

Our preliminary studies suggest that subjects with non-five repeat alleles have more severe asthma and may respond better to omega-3 fatty acids. We classified the people with 55, 56 and 57 sequences as the control and subjects with either three or four repeats on at least one DNA sequence as high risk and more likely to respond to fish oil. Among our 35 subjects, 16 (45.7%) were high risk. We hypothesize that this group will ultimately show more improvement in their asthma with fish oils than the control group.

We had a much higher percentage of subjects with the high-risk ALOX5 genotypes than reported in the general population, and more than we had predicted. Less than 50 subjects were therefore screened. Overall, this finding suggests that the prevalence of these genotypes is higher in carefully characterized cohorts of severe, persistent asthma patients.

Can exhaled breath help?

Pharmacogenomics and personalized medicine have already been applied in the field of oncology; several anticancer drugs have been shown to be beneficial to patients with certain gene mutations. One problem with this approach is that numerous genes (more than 100 in the case of asthma) are potential candidates for targeting in a single disease, and it is not feasible to test all new drugs in prospective studies that focus on single genes, such as ours. Instead, determining responses to therapy may be best served by analyzing proteins and their metabolic byproducts as biomarkers in blood serum and other body fluids.

Matt Allworth

The lung is easily sampled, which is an advantage for studying lung diseases such as asthma. For example, Brasier et al. (2008) were able to sample lavage fluid from the lungs of patients with severe and mild asthma. They identified 10 cytokines, molecules involved in cell signaling, that could be used to characterize several asthma subtypes. These studies offer potential new diagnostic tools that could be used to identify subtypes of asthmatics, monitor disease severity and target therapies to specific groups of patients who will respond to them.

However, obtaining lung lavage fluid is fairly invasive. Less invasive methods include the measurement of eosinophil numbers in sputum and, more recently, the concentration of exhaled nitric oxide in breath (Ueno et al. 2008). The fractional excretion of nitric oxide has a reported specificity of up to 90% for the diagnosis of asthma (Kharitonov and Barnes 2006). In addition to nitric oxide, there are more than 20 biomarkers that are increased in the liquid condensate of exhaled breath of asthmatics, including leukotrienes and other inflammatory proteins.

Having a broader profile of measured biomarkers would provide a more accurate assessment of a patient's disease severity. Exhaled breath condensate (EBC) analysis has emerged as a promising method to noninvasively measure the concentration of inflammatory mediators. EBC consists of volatile and nonvolatile components, which are evaporated from fluid in the airway epithelial lining. The collection of EBC is performed by cooling exhaled air during spontaneous respiration (Kostikas et al. 2008). Several studies have shown that patients with moderate to severe asthma have significantly

higher concentrations of leukotrienes in EBC than mild asthmatics and controls (Samitas et al. 2009).

Metabolomic analysis identifies potential biomarkers for a disease by analyzing the products of cell metabolism using mass spectrometry or high-resolution proton nuclear magnetic resonance spectroscopy (^{1}H-NMR). All the molecules that produce a signal in the mass or NMR spectrum are detected, creating a "metabolic fingerprint" of the analyzed sample. Metabolomics have been used to study inborn errors of metabolism. This method can be applied to EBC, enabling the detection of known metabolites and the prediction of novel biomarkers that would allow further characterization of asthma phenotypes and the prediction of drug efficacy. For example, Baraldi et al. (2009) used metabolomic analysis of EBC to demonstrate a difference between biomarkers found in the airways of asthmatic and healthy children.

Toward effective asthma treatment

This clinical study addresses the need to focus on patients with more severe asthma, as well as patients with specific genotypes or metabolomic phenotypes that may identify them as responsive or nonresponsive to therapy. Preliminary data from ours and other studies suggests that patients with certain polymorphisms of the ALOX5 gene are more susceptible to the production of inflammatory leukotrienes. This minority of asthmatics likely represents the fraction of asthmatics that would improve significantly from anti-leukotriene drugs, including a 5-lipoxygenase inhibitor or leukotriene receptor antagonist. These drugs are not completely efficacious in blocking this pathway, however, and a strategy of decreasing the total burden of leukotriene production further by supplementing with omega-3-rich fish oil is rational.

Prior clinical trials focused primarily on subjects with milder asthma. Patients with moderate to severe asthma are likely to notice a significant benefit from an intervention such as fish oil. These patients often have persistent airflow obstruction, despite therapy with inhaled corticosteroids. Our trials involving such patients will conclude this year. Further trials are needed to assess whether fish oil supplements will be effective in asthma patients receiving standard medications.

Many genes are associated with the risk of developing asthma; environmental triggers such as air pollution compound the risk.

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REVIEW ARTICLE

Soy may help protect against cardiovascular disease

by Emily R. Cena *and* Francene M. Steinberg

Diet and lifestyle choices are major factors contributing to the risk of cardiovascular disease, which is responsible for more deaths in the United States than any other cause. One dietary component that has received considerable attention for its potential cardioprotective effects is soybeans, which contain lean vegetable protein, dietary fiber and bioactive compounds known as isoflavones. Recent research investigating the relationship between soy and cardiovascular disease has identified several potential mechanisms for the observed protective effects, including cholesterol-lowering properties, antioxidant activity and gene regulation. This review highlights current understanding of the complex relationship between soy and the risk of cardiovascular disease.

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Soybeans and foods made from soy are the major source of isoflavones, which serve as antioxidants, scavenging and neutralizing free radicals that might otherwise cause inflammation and increase the risk of heart disease.

Cardiovascular disease — comprised of heart disease, heart failure and stroke — is the leading cause of death in the United States. The estimated combined costs of health care services, medications and lost productivity attributed to cardiovascular disease were more than $475 billion in 2009 (Lloyd-Jones et al. 2009).

Although this chronic inflammatory disease affects the lives of millions of Americans, the development of cardiovascular disease is somewhat preventable. Since diet is arguably the most modifiable risk factor, scientists have devoted a great deal of research to the relationship between dietary choices and cardiovascular disease. A healthy diet is generally high in fruits, vegetables, whole grains and legumes. These plant foods tend to be rich in bioactive compounds, or "extra-nutritional" constituents, that are associated with reducing the risk of cardiovascular disease.

One dietary component of considerable research interest is soybeans, a legume linked to the decreased risk of cardiovascular disease. Epidemiological studies suggest that Asian populations consuming large amounts of soy have lower rates of cardiovascular disease than Western populations (Zhang et al. 2003). In the mid-1990s, a meta-analysis of 29 clinical trials found that compared to animal protein, soy protein significantly reduced blood levels of several lipids (total cholesterol, LDL cholesterol and triglycerides) (Anderson et al. 1995). This prompted the U.S. Food and Drug Administration to approve the current health claim that 25 grams of soy protein per day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease.

While an American Heart Association statement issued in 2006 supported the inclusion of soy in the diet, it was not definitive about the protective mechanism of action (Lichtenstein et al. 2006). Research over the past 10 years has reported mixed results due to varying study designs and soy preparations, but the literature overall still supports the conclusion that soy protein with isoflavones can decrease blood levels of LDL cholesterol (Zhan and Ho 2005). Moreover, recent studies suggest that the relationship between soy and cardiovascular disease is more complex than just lowering cholesterol to protect against atherosclerosis.

How soy isoflavones work

Effects. Soybeans provide high-quality vegetable protein, dietary fiber and bioactive compounds called isoflavones, a class of phytochemicals (or plant compounds) that provide health benefits. Many different phytochemicals have been identified in nature and studied for their health effects. For example, anthocyanins found in red, blue and purple fruits and vegetables (such as berries and grapes) have been associated with reduced inflammation and lower risk of heart disease. Sulforaphane, a phytochemical found in broccoli and

Isoflavones are found almost exclusively in soybeans and foods made from soy.

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other cruciferous vegetables, may reduce the risk of certain types of cancer.

Isoflavones are found almost exclusively in soybeans and foods made from soy. The cardiovascular health benefits associated with isoflavones appear to be due to the fact that in the body they serve as antioxidants, which neutralize the free radicals that cause oxidative damage to cells. A free radical is a molecule containing an unpaired electron, which makes it highly reactive. Within blood vessels, free radicals can oxidize circulating LDL cholesterol, starting a cascade of inflammatory events that ultimately increase the risk of developing heart disease. Antioxidants interfere with this process by neutralizing, or scavenging, the free radicals.

Likewise, soy acts like compounds similar to the hormone estrogen, affecting processes including blood vessel dilation and gene regulation (fig. 1, page 120). Some health concerns have been raised related to soy's estrogenlike properties, particularly with regard to breast cancer risk, but these concerns are beyond the scope of the current article. Soy protein has also been shown to provide health benefits, such as helping to reduce LDL cholesterol levels. In addition, eating intact soy protein and isoflavones may improve vascular function better than either component by itself (Steinberg et al. 2003).

Bioavailability. The amount of isoflavones in a person's diet does not directly determine their bioavailability, the amount of isoflavone metabolites that reach the body's tissues and so are available to exert physiological effects. Rather, bioavailability depends on absorption, metabolism, distribution and excretion (fig. 1) (Lampe and Chang 2007). The isoflavones in soy are not biologically active until after they are digested and absorbed in the small intestine. Enzymes in the small intestine remove glycoside, or sugar, residues from the isoflavones, turning them into bioactive forms that can be absorbed.

Metabolites. In humans, the primary forms of bioactive isoflavone are daidzein and genistein, which are similar to the hormone estrogen. Daidzein and genistein are absorbed along the length of the small intestine, where they undergo further metabolism. The resulting variety of isoflavone metabolites, such as glucuronides and sulphates, then leave the liver and enter the systemic circulation. These metabolites travel to various tissues of the body, where they exert their cellular effects.

Isoflavone metabolism

Some isoflavone metabolites are secreted by the liver into bile, which is then secreted into the small intestine. There, the isoflavone metabolites in the bile once again undergo digestion and absorption. This recycling process is known as enterohepatic (or intestine-liver) circulation. Another type of recycling, known as enteric recycling, occurs in the small intestine, where isoflavones are metabolized into glucuronidated and sulphated forms that are then secreted back into the intestine for further reabsorption and metabolism (Chen et al. 2003).

Enterohepatic circulation and enteric recycling of isoflavones are important because they allow body tissues to be exposed to these phytochemicals for longer amounts of time following soy intake. After absorption, metabolism and distribution, isoflavones are ultimately

Glossary

Bioactive compounds: Any chemical compounds, organic or inorganic, that exert physiological effects on a living organism. Bioactive compounds generally refer to those found in foods or metabolites formed in the body that affect human health, such as soy isoflavones.

Cholesterol (HDL, LDL, total): A type of lipid that is used by the body for cell membrane structure, cell signaling and the synthesis of hormones, bile and vitamin D. Sources include synthesis by the liver and the dietary intake of animal products. Cholesterol is transported through the bloodstream bound to lipoproteins that are classified according to their lipid-to-protein ratio. Low-density lipoproteins (LDL) contain relatively large amounts of fat and cholesterol compared to protein, whereas high-density lipoproteins (HDL) contain more protein and less cholesterol. LDL cholesterol is often referred to as "bad" cholesterol because it can become oxidized by free radicals, resulting in inflammation and atherosclerosis. High levels of HDL cholesterol, sometimes called "good" cholesterol, are associated with lower risk of heart disease and other chronic diseases, possibly because HDL molecules transport cholesterol to the liver for excretion.

Cytokines: Intercellular signaling molecules involved in physiological processes such as inflammation. Cytokines consist of many different small molecules that enable cells to communicate. Some examples include tumor necrosis factor alpha (TNF-α) and interleukins such as interleukin-6 (IL-6), both of which are involved in the inflammatory process.

Endothelial cells, function: Cells that line blood vessels throughout the body, providing a barrier between the bloodstream and body tissues. They are actively involved in blood clotting, controlling white blood cell migration in and out of the bloodstream during the inflammatory response, and constriction and dilation of blood vessels.

Estrogen: A primary sex hormone that performs a number of important functions related to human health. Although present at higher levels in women, estrogen helps regulate reproductive development and function in men and women. Estrogen reduces the risk of cardiovascular disease by decreasing LDL cholesterol and triglyceride levels, and increasing HDL cholesterol. Estrogen also plays a key role in bone health.

Triglyceride: The predominant form of fat in food and in the body, consisting of a glycerol backbone with three fatty acids bound to it. Most circulating fat and most fat stored in adipose cells is in the form of triglycerides. Elevated triglyceride levels are a marker of increased risk for heart disease, diabetes and other chronic diseases, and high triglycerides are one of the defining components of the metabolic syndrome.

excreted in urine, and most excretion occurs within 24 hours of consuming a soy-containing meal (Setchell et al. 2003). Isoflavones that pass through the small intestine without being bioactivated then pass into the colon, where precursors to the bioactive form of daidzein are instead metabolized by bacteria into another bioactive compound called equol. The equol is then absorbed into the bloodstream and transported to various body tissues, where it appears to exhibit greater antioxidant and estrogenic effects than daidzein (Arora et al. 1998; Mitchell et al. 1998). Although there are other bacterial byproducts of isoflavone metabolism in the colon, equol is the primary one with significant bioactivity in humans.

Not all humans have colonic bacteria capable of producing equol. Equol is produced by an estimated 30% to 40% of white persons (Lampe et al. 1998) and up to 60% of Asians (Morton et al. 2002). Factors associated with equol production are not fully understood but are believed to include genetics, diet and lifestyle, in addition to the composition of gut microflora (Atkinson et al. 2008). It is unclear whether equol producers have increased protection against cardiovascular disease compared to nonproducers, and this remains a topic of great research interest (Atkinson et al. 2005).

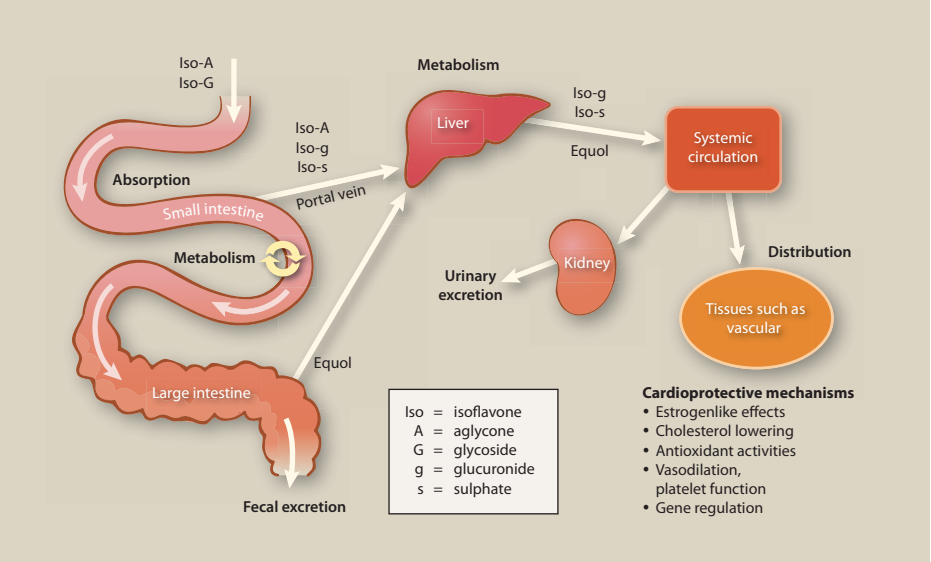


Fig. 1. Overview of the absorption, metabolism, distribution and excretion of soy isoflavone metabolites.

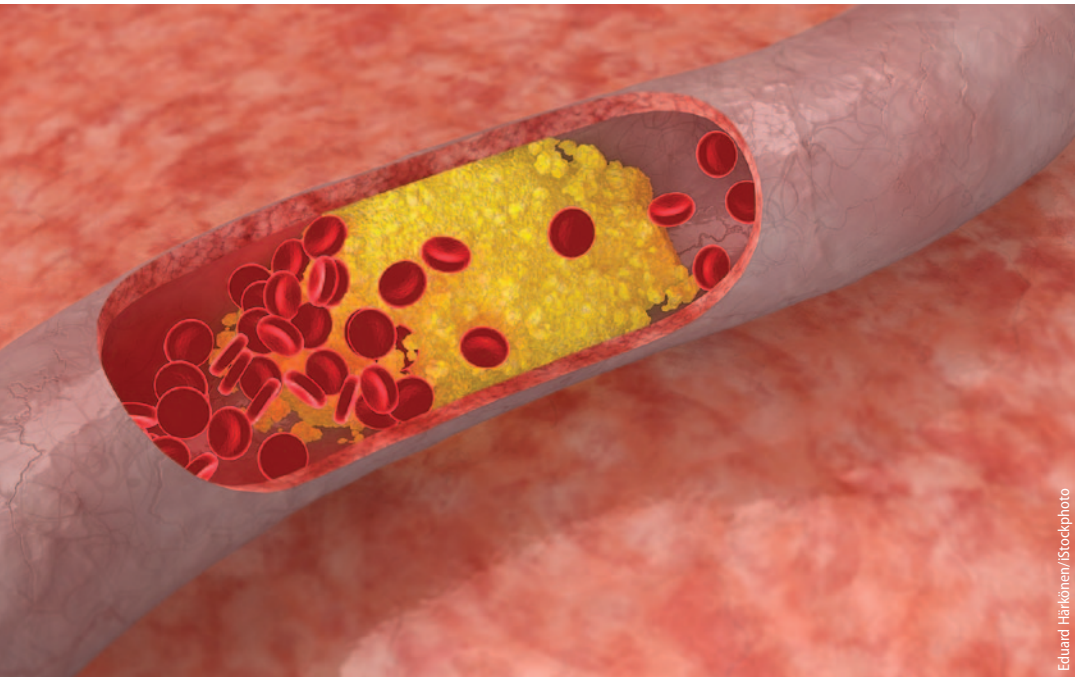
Cardiovascular disease protection

Atherosclerosis, which hardens and narrows the arteries, is a primary cause of cardiovascular disease (see box). The development of atherosclerosis is quite complex, involving chronic inflammation, the generation of reactive oxygen species, abnormal endothelial cell function, lipid-laden foam cell formation and clot formation within blood vessels. The observed protective effect of soy and soy isoflavones on atherosclerosis and cardiovascular disease risk appears to be related to a number of different mechanisms.

Estrogenlike effects. In cardiovascular disease, the blood vessels become constricted or blocked, forcing the heart to work harder to push blood through the body. One factor that promotes blood vessel dilation (vasodilation) is the production of nitric oxide by endothelial cells in the blood vessel wall. Estrogen can rapidly increase endothelial nitric oxide production (Rimbach et al. 2008), providing anti-atherosclerotic effects. Because isoflavones are so similar to estrogens, they can also rapidly increase endothelial nitric oxide production, enhancing vasodilation and improving blood flow.

Another factor contributing to the development of cardiovascular disease is inflammation in blood vessels. In animal models, estrogen decreases the levels of two small proteins (cytokines TNF-α and IL-6) that increase inflammation, reducing the risk of cardiovascular disease (Ito et al. 2001). It is unclear whether isoflavones similarly inhibit the production of pro-inflammatory cytokines in humans (Beavers et al. 2009), but this remains a current area of research interest.

Cholesterol-lowering properties. In addition to the widely recognized cholesterol-lowering effects of soy protein, soy isoflavones also help lower both total and LDL cholesterol levels, while increasing cardioprotective HDL cholesterol (Taku et al. 2007; Zhan and Ho 2005). In fact,



Soy can reduce LDL cholesterol circulating in the blood, thereby decreasing the formation of plaque in blood vessel walls.

the combination of soy protein and isoflavones appears to exhibit the strongest hypocholesterolemic effects compared to isolated soy protein or soy isoflavones alone (Mortensen et al. 2009). Whole soy can promote a 3% to 5% reduction in blood cholesterol (Lichtenstein et al. 2006; Zhan and Ho 2005). Although soy's effects are not as large as the 20% or greater reductions observed with statin drug therapy (Rosenson 2006), even a 3% to 5% reduction can have clinical significance. Reduced circulating LDL cholesterol means less is available to become oxidized and retained in lipid plaques in the blood vessel wall. Less oxidized LDL cholesterol results in a lower risk of atherosclerosis and therefore cardiovascular disease.

Antioxidant activity. Another way to reduce LDL cholesterol oxidation, aside from decreasing the amount that is circulating, is to prevent or slow oxidation through the action of antioxidants. Soy isoflavones and their metabolites, particularly genistein and equol, have antioxidant properties.

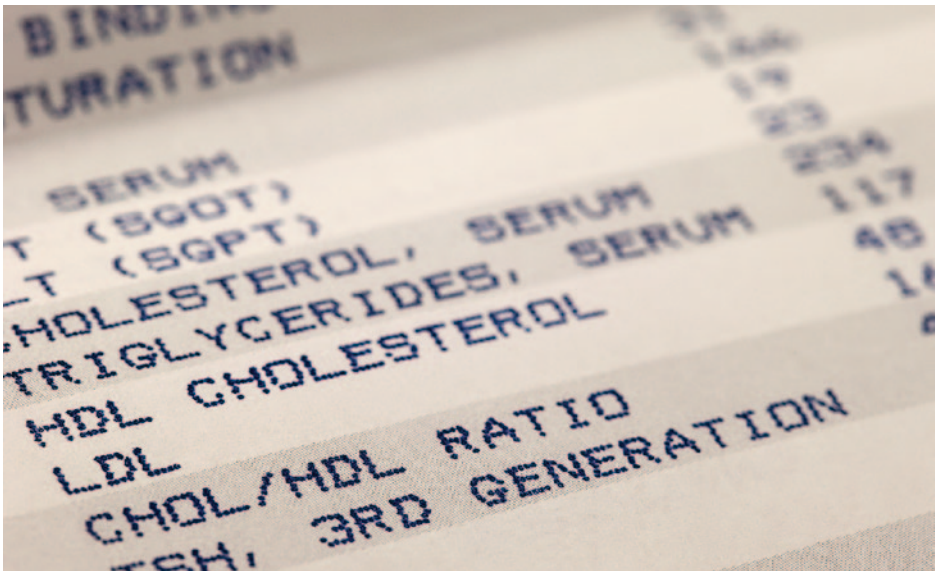
Two studies in humans demonstrated that daily consumption of soy protein with isoflavones reduced levels of LDL oxidation (Tikkanen et al. 1998; Wiseman et al. 2000). In vitro studies have revealed potential mechanisms for the antioxidant activity of isoflavones, including: (1) their ability to scavenge free radicals and chelate metals (or bind to metals, making them less chemically reactive); (2) their role in inhibiting the production of hydrogen peroxide, a naturally occurring cellular byproduct that oxidizes compounds and damages blood vessel walls, stressing the body by inflammation; and (3) their capacity to stimulate the production of antioxidant enzymes such as superoxide dismutase (Mortensen et al. 2009).

Reactive oxygen species are molecules, ions or free radicals that contain oxygen and are highly reactive because of unpaired electrons. Cellular processes produce them naturally, but large amounts cause oxidative damage. A key partner in cellular defenses against oxidative damage is the transcription factor Nrf2, which promotes the transcription of genes for various antioxidant and detoxifying enzymes. The activation of antioxidant genes via the Nrf2 pathway is a potential role for isoflavones (Mann et al. 2009).

Soy isoflavones help promote vasodilation by stimulating nitric oxide production in the endothelial cells lining blood vessels. Interestingly, macrophages (a type of white blood cell) also produce nitric oxide during the inflammatory response, in an effort to destroy foreign particles such as bacteria. In cases of chronic inflammation, however, increased nitric oxide production by macrophages causes oxidative stress that contributes to the formation of atherosclerotic plaques, thereby increasing the risk of cardiovascular disease. Soy isoflavones are able to inhibit inducible nitric oxide production in macrophages, reducing excessive oxidative stress (Rimbach et al. 2008).

Improved vasodilation. Soy isoflavones can promote vasodilation by stimulating the production of nitric oxide. A recent study showed that genistein, one of the estrogenlike forms of isoflavones, modified the expression of genes that encode for a number of proteins related to vasodilation and blood pressure (Ambra et al. 2006). While in vitro and animal studies seem to support the vasodilatory effects of isoflavones, a review of human intervention studies found conflicting results, with some studies reporting improved vasodilation and others reporting no change (Mortensen et al. 2009). In addition, isoflavones appear to promote vasodilation in ways that do not involve estrogenic effects. More research is necessary to better elucidate the relationship between isoflavones and vasodilation in humans.

Inhibition of platelet aggregation. One of the greatest threats of cardiovascular disease is the formation of clots within blood vessels. If a blood clot forms and breaks loose, it can travel to a coronary artery (which supplies the heart with blood), block the artery and cause a life-threatening heart attack. Similarly, if a



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Soy isoflavones increase the levels of cardioprotective HDL ("good") cholesterol and decrease levels of LDL ("bad") cholesterol, lowering the risk of heart disease.

The risks of cardiovascular disease

In 2006, cardiovascular disease was responsible for 26% of all deaths in the United States (Heron et al. 2009). In California alone, it claimed the lives of more than 73,000 people in 2004 — more than cancer, diabetes, chronic liver disease, homicide, suicide and HIV/AIDS combined (Reynen et al. 2007). The costs associated with the treatment of cardiovascular disease are staggering, particularly at a time when health care costs in the United States have never been higher.

Several risk factors for cardiovascular disease are at least partially modifiable by lifestyle choices, including obesity, high blood pressure, diabetes, chronic inflammation, dyslipidemia (abnormally high blood cholesterol and/or triglycerides), smoking, physical inactivity and dietary choices.

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High blood pressure is one of the factors of metabolic syndrome, which results in higher risks of cardiovascular disease and type 2 diabetes. The consumption of soy lowered blood pressure in a study of postmenopausal women.

clot obstructs the blood supply to the brain, it can cause a stroke. A key step in the formation of blood clots is platelet aggregation. Although the mechanism is not yet fully understood, in vitro and animal studies have demonstrated that soy isoflavones inhibit platelet aggregation, reducing the risk of blood clots (Gottstein et al. 2003; Peluso et al. 2000). It remains to be demonstrated whether this effect occurs in humans with typical levels of circulating isoflavones.

Gene regulation. A growing body of evidence supports the gene regulatory effects of soy isoflavones, both through their estrogenic and antioxidant properties. Genistein has been shown to inhibit the DNA binding of a transcription factor called NF-κB, resulting in the reduced transcription of several vascular cell adhesion molecules that are crucial to the development of atherosclerotic plaques. Inhibition of NF-κB or other such transcription factors could slow the progression of atherosclerosis (Nagarajan 2010). In addition, in cultures of the endothelial cells that line blood vessels, physiologically relevant levels of genistein upregulated the transcription factor peroxisome proliferator–activated receptor gamma (PPAR-gamma), which resulted in reduced adhesion of monocytes (a type of white blood cell) to vessel walls (Chacko et al. 2007).

Similarly, both genistein and daidzein reduce the transcription of a protein called MCP-1 that attracts monocytes (Mortensen et al. 2009). MCP-1 is involved in atherogenesis because it is known to recruit monocytes and other immune system cells into the arterial wall, contributing to plaque formation. By reducing the secretion of MCP-1 and so inhibiting monocyte infiltration into the blood vessel wall, soy isoflavones may help prevent the formation of plaque.

As researchers continue to learn more about the nutrigenomics of soy, or the effects of soy on gene expression, it is likely they will find wide variation in the way individuals digest, absorb, metabolize, distribute and eliminate isoflavones and their metabolites. As with equol producers versus nonproducers, genetic and environmental factors appear to mediate the relationship between soy intake and cardiovascular disease risk. These individual variations may help explain why human intervention studies have given mixed results.

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Abdominal obesity is another factor of metabolic syndrome. Eating soy may help people improve glycemic control.

Soy and metabolic syndrome

Metabolic syndrome is a constellation of risk factors including abdominal obesity, high blood pressure, insulin resistance and alterations in blood lipids that results in increased risk of cardiovascular disease and type 2 diabetes. The prevalence of metabolic syndrome is rising at an alarming rate in the United States (Ford et al. 2004). Dietary and lifestyle modifications are central to managing the syndrome.

The role of soy in ameliorating metabolic syndrome and its associated risk factors is an emerging area of nutrition research. Rodent models have demonstrated that soy diets can reduce adiposity and circulating cholesterol levels and improve insulin sensitivity (Davis et al. 2007; Ronis et al. 2009). Human studies have also supported the protective effects of soy against risk factors associated with metabolic syndrome. Postmenopausal women with the syndrome who consumed soy nuts showed improvements in glycemic control and blood lipids as well as a marker of inflammation (Azadbakht et al. 2007). In contrast, adults with type 2 diabetes who consumed soy protein had beneficial changes in blood lipid profiles but not glycemic control (Pipe et al. 2009). Hypertensive, postmenopausal women who consumed soy nuts had improvements in blood pressure that were associated with markers of endothelial function, suggesting a decrease in vascular inflammation (Welty et al. 2007).

To date, little is known about potential fetal or prepubertal exposure to soy and its influence on cardiovascular risk later in life, but it is possible that these unexplored questions might help explain some of the beneficial effects of soy observed in Asian populations (Bonacasa et al. 2011).

Health and nutrition research

Research strategies have previously focused on consumption of soy protein and isoflavones in relatively healthy individuals, examining effects on vascular outcomes and safety (Heneman et al. 2007; Steinberg et al. 2003; Steinberg et al. 2011). Currently, a research project funded by the Center for Health and Nutrition Research is under way at UC Davis to investigate the effects of consuming whole

soy foods on biomarkers of cardiovascular risk in individuals with metabolic syndrome; it may be completed in 2012.

The cardioprotective effects of soy appear to be mediated by several mechanisms related to the inflammatory processes of atherosclerosis, involving both genomic and nongenomic activities. Soy isoflavones have a broad variety of biological actions. The beneficial effects of soy are modest compared to pharmacological treatment, such as statin drugs. However, the cumulative effects over a lifetime are likely to be significant. Soy and its bioactive isoflavone components are an important part of a diet rich in foods for health promotion.

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The beneficial effects of soy have been observed in Asian populations, which have lower heart disease rates than Western populations.

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REVIEW ARTICLE

Proper nutrition can prevent negative health outcomes in young female athletes

by Michelle T. Barrack *and* Marta D. Van Loan

Since the onset of Title IX, opportunities have dramatically increased for female athletes, largely to their benefit. However, some negative health outcomes such as disordered eating, chronic menstrual disturbances and low bone mass have been associated with high-level competition among some female athletes, particularly in sports such as gymnastics and cross-country running, where a slender physique or lean body build is important. Adolescent female athletes, in a rapid growth and development phase, may be at greatest risk. We sought to identify athletes at risk, understand the origin of possible negative outcomes and recommend behavioral modifications that promote participation in competitive sports while supporting lifetime health. This review discusses the development and impact of disordered eating and menstrual dysfunction on bone mass in young, competitive, female athletes and provides nutrition recommendations for their energy, carbohydrate, protein, vitamin and mineral intake.

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Girls and young women who participate in endurance sports such as long-distance running may suffer negative health outcomes as well as positive ones. They may have a higher risk of low bone mass, menstrual disturbances and disordered eating.

The arena of competitive sports for girls and young women was transformed by Title IX of the Education Amendments of 1972, which states, "No person in the United States shall, on the basis of sex, be excluded from participation in, be denied the benefits of, or be subjected to discrimination under any education program or activity receiving Federal financial assistance."

In 1971, male competitors outnumbered females by more than five-to-one in college sports and more than twelve-to-one in high school sports (NCWGE 2008). Due to regulations set forth by Title IX, the number of female competitors in the National Collegiate Athletic Association (NCAA) increased more than 4.5-fold (from fewer than 30,000 to nearly 167,000) between 1971 and the 2004-2005 academic year (NCWGE 2008). Female high school athletic participation in the United States increased to a greater extent, from 300,000 in 1972 to over 2,950,000 in 2006 (NCWGE 2008). In 2009, there were nearly 300,000 female competitors in the California Interscholastic Federation alone (CIF 2009).

Athletic involvement by girls and young women has resulted in many positive health, cognitive, psychological and behavioral benefits. Research shows that regular exercise increases overall strength and aerobic fitness, improves cardiovascular health and lowers the risk of chronic degenerative diseases such as atherosclerosis and diabetes (Haskell et al. 2007; Hasselstrom et al. 2002; Kemper et al. 1999). For girls, sports participation enhances self-esteem and self-efficacy and reduces feelings of depression (Colton and Gore 1991). Various reports document higher academic performance, cognitive function and degree completion rates among girls and young women who engage in competitive sports (NCAA 2005). Furthermore, the use of tobacco and drugs, as well as sexual promiscuity, is lower among female athletes (Kulig et al. 2003).

Despite the many benefits, involvement in some sports — particularly those where a slender or lean body type (having less body fat) is important — has been associated with a higher risk of developing certain negative health outcomes. Girls and women participating in gymnastics and endurance sports such as cross-country running have demonstrated low bone mass, particularly at the lumbar spine; menstrual disturbances, including fewer cycles or no cycle (amenorrhea); and a high prevalence of disordered eating (Beals and Hill 2006; Nichols et al. 2007; Torstveit and Sundgot-Borgen 2005a).

Female athlete health issues

Beals and Hill (2006) reported the eating attitudes and behaviors, menstrual function and bone mass of 112 female U.S. collegiate athletes. The authors observed

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that compared to other athletes, those with slender/lean body types had higher frequencies of moderate to extreme body dissatisfaction and binge eating, and a trend ($P = 0.08$) toward a higher frequency of self-described eating disorders. Slender or lean female athletes also exhibited a higher prevalence of menstrual irregularity (cycles less than 28 days, nine or fewer cycles per year or no cycle at all) and lower bone mass.

Low bone mass. Low bone mass in young women can lead to the early onset of osteoporosis and increased risked for bone fractures. Mudd et al. (2007) compared data from 99 female collegiate athletes participating in 12 different sports and found that endurance runners had lower bone density throughout the body and particularly at the lumbar spine, compared to gymnasts and softball players, while swimmers exhibited significantly lower bone density in their legs compared to athletes in all other sports. Additionally, approximately 40% of collegiate or postcollegiate (Cobb et al. 2003) and adolescent endurance runners (Barrack et al. 2008) had low bone mass for their ages.

Menstrual irregularity. Adequate reproductive hormones, especially estrogen, are needed for bone mineralization during adolescence and for continued bone maintenance thereafter. Up to 66% of female competitive endurance runners exhibit menstrual irregularities, and 40% have low bone mass (Barrack et al. 2008; Cobb et al. 2003; Gibson et al. 2004; Warren and Chua 2008). These rates are about three to five times higher than reported estimates of these same conditions in healthy young women and girls who do not run competitively (van Hooff et al. 1998).

Gibson et al. (2004) observed a 66% prevalence of menstrual irregularity among 50 elite female endurance runners. In a sample of 423 California high school athletes, Nichols et al. (2007) found that girls participating in sports that benefit from a lean or slender body type had a higher prevalence of menstrual irregularity (26.7% and 16.6%, respectively; $P < 0.005$) compared to athletes in other sports but no increased evidence of disordered eating.

Eating disorders. Eating disorders have been shown to disrupt the menstrual cycle, resulting in low or no estrogen production. Adequate levels of estrogen are needed to increase bone mineralization and, in turn, bone density. In a sample of 300 female collegiate cross-country runners, Thompson (2007) reported that 19.4% either currently or previously had an eating disorder, while Hulley and Hill (2001) found that 16% of their sample of elite women distance runners had a current eating disorder. These estimates are considerably higher than the 0.5% to 2% occurrence of anorexia or bulimia nervosa in the population of young, healthy, adult female noncompetitive runners (Academy of Eating Disorders 2011).

Furthermore, among a sample of collegiate athletes, young women involved in aesthetic sports such as cheerleading, diving and gymnastics (lean or slender body-build sport types) had the lowest body weight and highest scores on the Eating Attitudes Test, which is indicative of abnormal eating attitudes and behaviors such as dietary restraint, in which the individual restricts food intake to control body weight (Beals and Manore 2002).

Female Athlete Triad syndrome. These interrelated conditions (eating disorders, amenorrhea and osteoporosis) were first officially recognized and defined as the "Female Athlete Triad" by the American College of Sports Medicine Task Force on Women's Issues in 1993 (Yeager et al. 1993).

Subsequent epidemiologic studies found that among female athletes, the prevalence of all three aspects of this triad was low (about 1% to 3%) (Beals and Hill 2006; Cobb et al. 2003; Nichols et al. 2006). Several studies identified a portion of girls with menstrual disturbances but no eating disorders, and a portion of girls with low bone mass but no clinical menstrual disturbances (Hilton and Loucks 2000; Torstveit and Sundgot-Borgen 2005b). This prompted more research to better understand the causes of exercise-related low bone mass and menstrual disturbances in female athletes.

Energy deficit vs. exercise stress

An important question is whether these exercise-related health problems occur from an energy deficit, in which calories consumed do not meet calories needed for the increased level of physical activity, or from excess stress that alters the hormones regulating menstrual function and bone metabolism. Several well-controlled animal and human experimental studies have confirmed that as long as energy is available for physiological needs other than exercise, it promotes normal hormone function. This suggests that intense exercise does not in itself exert an additional stress that disrupts menstruation and bone metabolism.

Williams et al. (2001) trained a group of female monkeys to do 2 hours of endurance exercise (running) per day. The resulting energy deficit caused the complete cessation of their menstrual cycles. But once the monkeys were fed adequate calories to adjust for the increased energy expended on their endurance exercise regime, normal menstrual function gradually resumed over a period of 12 to 57 days. These findings demonstrate the critical role of energy in maintaining normal hormonal levels and menstrual function during exercise.

Ihle and Loucks (2004) evaluated the roles of energy deficiency and exercise stress on hormone concentrations that regulate bone metabolism by examining markers of bone turnover in 29 healthy, moderately active young women. First, the women spent 2 months on a balanced diet and exercise regimen that required about 25% of their energy intake. Then

Grand Forks Human Nutrition Research Center/USDA ARS

An insufficient diet restricts the amount of energy available for metabolic processes. The deposition of bone may subsequently be reduced, and bone resorption increased.

Tim Hipps, FMWRC Public Affairs, U.S. Army

Dietary restraint, such as skipping meals or avoiding "fattening" foods to control the shape or weight of one's body, can be associated with failure to ovulate and low bone mass. Female athletes in lower-intensity sports such as baseball are less prone to these problems.

they were divided into three regimens where they continued to exercise at the same level but consumed a diet that was (1) adequate (met needs), (2) insufficient (met about 75% of needs) or (3) deficient (met about 55% of needs).

After just 5 days on an energy-restricted diet, women had significant changes in the hormones that regulate bone metabolism and turnover (such as thyroid hormone, insulin-like growth factor-1 and estradiol). Women on the insufficient diet had significantly lower thyroid hormone, insulin-like growth factor-1 and markers of bone deposition. Women on the deficient diet had even lower levels of these hormones and bone biomarkers, as well as significantly lower estradiol and increased bone resorption (the release of minerals such as calcium, magnesium and other nutrients).

Because the exercise stress remained constant, these findings indicate that energy deficits directly lowered metabolic rate, reduced bone deposition and increased bone resorption in a dose-dependent manner. Together, these studies and others (Loucks and Thuma 2003) provide strong evidence of the direct role of low energy availability, not exercise stress, on the hormone disruptions contributing to menstrual disturbances and bone loss in female competitive athletes.

Low energy availability

Energy availability is the amount of energy that is not expended on exercise and therefore is available for performing the basal metabolic processes involved in organ function and the nonexercise activities of daily living. This relationship is represented in the following equation:

Energy Availability = (Energy Intake – Exercise Energy Expenditure) / Fat Free Mass

This value is standardized for lean or fat free mass (the portion of body weight that is not fat) because lean muscle tissue accounts for more than 80% of an individual's resting metabolic rate (Stipanuk 2000).

When evaluating the relationship between energy availability and the hormones that regulate menstrual function, investigators found that hormone disturbances occurred below an energy availability level of 30 kilocalories per kilogram of lean tissue or fat free mass (kcal/kg FFM), regardless of whether this low energy availability occurred as a result of decreased energy intake or increased exercise energy expenditure (Loucks 2006; Loucks and Heath 1994). Low energy availability occurs when 66% or less of basal metabolic energy needs are met, which for young adults has been defined as below 30 kcal/kg FFM per day. The cutoff may be higher for adolescents, since they are in a period of growth and development; however, this has not been evaluated in young athletes. It is recommended that future studies employ similar research methods among healthy, regularly menstruating, noncompetitive adolescent athletes to identify the energy availability level below which hormone disturbances occur.

Disordered eating behaviors

Research has indicated that anorexia nervosa, a condition that entails extreme dietary restriction and compulsive exercise, as well as several other subclinical disordered eating behaviors, is highly related to low energy availability and the development of low bone mass. These subclinical disordered eating behaviors include dietary restriction, purging and overexercise without concurrent compensatory eating.

Anorexia nervosa. Patients with anorexia nervosa chronically exist in a state of severely low energy availability and exhibit marked menstrual disturbances. As a result, bone mass is classically low, with many exhibiting lumbar spine bone densities that are about two standard deviations below the average for their age groups. While the consequences epitomize those of Female Athletic Triad syndrome, the prevalence of this disorder among female athletes is so low that it likely represents a small percentage of those at risk.

Subclinical behaviors. More common are subclinical disordered eating behaviors such as dietary restraint, aimed at reducing overall energy (or fat) intake in an attempt to control one's body weight or shape. Behaviors practiced by restrained eaters include restricting "fattening" foods, avoiding eating for many hours, skipping meals and adopting rules or limitations regarding daily caloric intake.

Studies have identified an elevated prevalence of failure to ovulate (anovulation) and other subclinical menstrual disturbances (which disturb the menstrual cycle without necessarily causing amenorrhea) among several groups of adult women exhibiting excessive dietary restraint, including sedentary young adults, vegetarians and recreational runners (Barr et al. 1994). Additionally, both adult sedentary women and adolescent female endurance runners with restrained eating behaviors exhibited a greater degree of low bone mass (Barr et al. 1994; McLean et al. 2001). Reduced energy intake contributes to low energy availability, which likely accounts for the relationship between dietary restraint, menstrual dysfunction and low bone mass.

For optimal health and peak performance, it is critical to consume the three primary macronutrients for energy metabolism (carbohydrates, protein and fat) as well as a multitude of micronutrients (vitamins and minerals).

Amit Belani

Critical vitamins can be obtained in a variety of foods, or, if necessary, dietary supplements.

Menstrual disturbances, low bone mass

Menstrual disturbances have long been established as risk factors of low bone mass. While it was previously hypothesized that psychological or physiological stressors caused at least some cases of menstrual dysfunction, accumulating evidence indicates that most if not all menstrual disturbances in female athletes are preceded by an energy deficit due to dietary restriction or excessive exercise without adequate nutritional replenishment (Ihle and Loucks 2004).

Converging evidence from previous research studies has documented the direct effect of energy deficits on hormonal secretions from the pituitary gland, which controls many endocrine functions, including growth hormone production and the regulation of ovulation, as well as estradiol synthesis, all of which are needed for optimal bone mineralization and the attainment of peak bone density (Loucks 2006). Menstrual disturbances do not appear to cause low bone mass; rather, they are a sign of underlying hormone dysregulation, which may also negatively affect bone metabolism.

In addition, reduced bone mass can be associated with energy deficits that lower hormonal secretions, resulting in anovulation and other subclinical menstrual disturbances that often go unnoticed (McLean et al. 2001). Therefore, clinical menstrual function may not be an adequate signal of underlying hormone disruptions and associated bone-related risks. Because individuals with seemingly normal menstrual function may also be at risk of disrupted bone metabolism and consequently low bone mass, it is important to identify a proxy measure of low energy availability.

czalewski/iStockphoto

Antioxidants, found in vegetables such as kale, protect against oxidative cell damage and are important for high-intensity aerobic athletes.

Macronutrients for female athletes

Proper nutrition influences sport performance and overall health by, for example, reducing injury risk while increasing energy levels, recovery time and immune function. Given the high activity levels of athletes, their requirements for some nutrients are higher than those of the general population. It is important for female athletes to be aware of their unique requirements and maintain diets that satisfy their specific needs.

Just consuming enough energy (calories) is not enough. For optimal health and peak performance, it is critical to consume the three primary macronutrients for energy metabolism (carbohydrates, protein and fat) as well as a multitude of micronutrients (vitamins and minerals).

Energy. Consuming adequate energy is necessary to support high-intensity or endurance exercise. The 2005 Institute of Medicine Dietary Reference Intake report provides energy intake recommendations for all ages, but these should be carefully defined for each athlete based on age, development stage, activity level and proportion of lean tissue mass (IOM 2005)

Carbohydrates. Carbohydrates are important components of an athlete's diet, particularly an endurance athlete's. Besides being a primary macronutrient for energy metabolism, carbohydrates are needed to efficiently maintain the muscle glycogen that athletes use as fuel for vigorous exercise. It is recommended that athletes consume a high-carbohydrate snack or beverage 30 to 60 minutes after strenuous, glycogen-depleting exercise. This enhances both glycogen repletion and recovery from strenuous exercise. Depending on the athlete's training volume, carbohydrate requirements range from 6 to 10 grams per kilogram of body weight per day (IOM 2005), generally 50% to 60% of total energy intake.

Protein. According to the most current research, the protein needs of athletes not participating in power (such as wrestling and weight lifting) or endurance (such as cross-country running) sports are similar to those of the general population. Therefore, most athletes' protein needs can be met by consuming the recommended level of 0.8 grams per kilogram of body weight per day (IOM 2005). In addition, protein should make up 15% to 35% of total calories consumed. However, endurance and power athletes may require higher protein levels, ranging from 1.2 to 1.7 grams per kilogram of body weight per day, or almost twice the amount for sedentary individuals. This ensures that they are getting enough nitrogen, which is required for protein synthesis and muscle repair. To aid in postexercise repair, synthesis and maintenance of skeletal muscle, athletes should consume high-quality proteins (those with high bioavailability that contain the essential amino acids) such as soy and animal proteins, including egg and milk products (such as casein and whey).

Fat. Besides yielding a substantial amount of energy, dietary fat provides important fat-soluble vitamins (A, D, E and K) and essential fatty acids. Dietary fat

Suzanne Paisley

Endurance and power athletes may need twice as much protein as sedentary individuals. After exercise, high-quality proteins, such as those found in dairy products, are critical to muscle repair.

should make up 20% to 35% of the total diet and should provide no less than 20% of total energy needs to benefit athletic performance (Rodriguez et al. 2009).

Vitamins and minerals

Due to their relatively high metabolic and nutrient needs, athletes should consume diets that provide at least the recommended daily allowance (RDA) for all micronutrients for each individual's respective age group (IOM 2005).

B vitamins. B vitamins facilitate energy metabolism (thiamin, riboflavin, niacin, pyridoxine, pantothenic acid and biotin) and promote muscle repair (folate, vitamin B12). Some reports suggest that the B vitamin requirements of competitive athletes are slightly elevated, and if so these higher needs likely are easily met with increased energy intakes. However, several B vitamins are often low in the diets of female athletes, including riboflavin, pyridoxine, folate and vitamin B12 (IOM 2005; Rodriguez et al. 2009). This can impair performance because severe deficiencies in vitamin B12 or folate can lead to anemia, a condition causing severe fatigue. B vitamin anemia is different from iron-related anemia and can affect the size of red blood cells. Animal-derived foods such as red meats and milk are good sources of B vitamins.

Antioxidants. Antioxidant vitamins (such as C, E and the vitamin A precursor beta-carotene) and minerals (such as selenium) protect cell membranes from oxidative damage. These compounds become highly important during periods of increased exercise, particularly endurance-type exercise, since oxygen consumption increases 10 to 15 times with prolonged moderate- to high-intensity aerobic activity (IOM 2005; Manore 2002). Some reports suggest that vitamin E requirements for endurance athletes may be higher than for the general population. Vitamin E supplementation reduces inflammation and muscle soreness, speeding recovery times. It also deters exercise-induced DNA damage. Populations at risk of low vitamin C, vitamin E, beta-carotene and selenium intake are those with diets low in fat, energy, vegetables and whole grains (IOM 2005; Manore 2002); these foods should be incorporated into the diets of all athletes.

Calcium and vitamin D. Calcium serves many important functions and facilitates nerve conduction, muscle contraction, the maintenance of blood calcium levels and the mineralization of bone. Calcium promotes normal growth, development and the maintenance of strong bones. Vitamin D facilitates the absorption of calcium, is important for calcium utilization in the body and improves immune defenses. Female athletes with the highest risk of calcium deficiencies are those with low energy intakes or those who do not meet the dietary recommendation of 3 to 4 servings of dairy foods (such as milk, yogurt and cheese) per day (ACSM et al. 2000). Further, calcium absorption may be hindered due to low estrogen levels in girls and young women exhibiting disordered eating or amenorrhea (Cobb et al. 2003; Gibson et al. 2004; Rodriguez et al. 2009).

The calcium and vitamin D requirements for athletes exhibiting these conditions increase from the normal 1,300 milligrams calcium and 200 IU (international unit) vitamin D per day, to 1,500 milligrams calcium and 400 to 800 IU vitamin D per day (ACSM et al. 2000). While vitamin D can be synthesized when skin is exposed to the sun's ultraviolent rays, sunscreens inhibit this natural process, making adequate dietary intake essential.

Iron. Iron is an important mineral for optimal athletic performance due to its role in energy production as well as its oxygen-carrying capacity (ACSM et al. 2000). Iron is a component of the hemoglobin in red blood cells, which transport oxygen to working muscles. Inadequate iron is one of the most common nutritional deficiencies among female athletes and leads to impaired muscle function and reduced performance during workouts and competition (Rodriguez et al. 2009). The iron needs of endurance athletes may be up to 70% higher than those of the general population (ACSM et al. 2000). Endurance athletes who consume primarily a vegetarian diet take in non-heme iron, a form that is absorbed much less efficiently than heme iron, which is found in red meat (IOM 2005). Female athletes at risk of low iron levels are endurance runners, vegetarians, adolescents and athletes who frequently donate blood (IOM 2005; Rodriguez et al. 2009). The recommendation for iron is 18 milligrams per day and can be obtained most easily from red meats.

Zinc and magnesium. Zinc is important for normal growth, immune function, muscle repair and energy production. Zinc also reportedly influences basal metabolic processes, protein utilization and thyroid hormone function. Low zinc levels have been associated with reduced muscle strength, aerobic function and endurance (IOM 2005; Rodriguez et al. 2009). Female athletes with low animal protein and high fiber intakes are at increased risk of developing zinc deficiency.

Adequate magnesium levels in conjunction with zinc aid performance

because of their joint roles in facilitating cellular metabolism, and cardiovascular, immune and hormone function. Magnesium deficiencies increase the oxygen required for endurance exercise, hindering performance. Athletes with diets low in energy have been documented to consume low magnesium levels. The daily recommended amounts of zinc and magnesium for adolescents are 12 and 300 milligrams per day, respectively. Readily available sources of these nutrients are animal red meats and milk.

Unique nutritional needs

Adolescent athletes represent a distinct subpopulation, since their diets must satisfy nutrient requirements for growth and development as well as the demands of their sports. The optimal diet for a young, developing athlete should maximize sport performance, while reducing injury risk and facilitating overall health, growth and maturation. Due to the metabolic and physiologic demands of their sports, the nutrient needs of young athletes can be much higher than their nonathlete peers and higher than the needs of young adult athletes who have reached maturity. It is important to make competitive adolescent athletes aware of their specific needs as well as to identify those athletes at risk of developing deficiencies.

Counseling by a registered dietitian may be necessary to design diets that meets the unique needs of adolescent female athletes. While prior research has provided valuable information about athlete groups with a potential increased risk of developing menstrual disturbances and low bone mass, the majority of studies assessed collegiate and adult athletes and looked at groups at only one point in time. Therefore, it is recommended that future research evaluate adolescents over time to identify the nutritional and exercise behaviors that optimize bone mineral gains and reduce fracture risk. Further, it is necessary to educate athletes, parents, coaches and athletic trainers about behaviors that promote lifetime bone health.

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RESEARCH ARTICLE

Citrus can help prevent vitamin A deficiency in developing countries

by Betty J. Burri, Jasmine S. T. Chang, *and* Tami Turner

California is a major producer of tangerines and oranges, which contain carotenoids that form vitamin A. Deficiencies of this vitamin are common in southern Asia and Africa, causing blindness and more than one-half million deaths each year. We evaluated the potential of tangerines and oranges to prevent vitamin A deficiency worldwide by measuring their carotenoid concentrations, estimating the amounts needed to meet the recommended safe nutrient intake for vitamin A and determining their availability in countries with vitamin A deficiency. We conclude that tangerines — particularly Satsuma mandarins, which have high concentrations of the carotenoid beta-cryptoxanthin — but not oranges, could be useful in preventing vitamin A deficiency, though not as the sole source.

McKay Savage

Oranges and tangerines are major sources of beta-cryptoxanthin, which provides easily absorbed vitamin A. Increasing the consumption of tangerines, particularly Satsuma mandarins (*above*, in Vietnam), could help reduce vitamin A deficiency in developing countries.

Vitamin A is essential for normal eyesight, growth and development (IOM 2000). Deficiency in vitamin A rarely occurs in California, but it is a serious health problem for most of the developing world, especially southern Asia and sub-Saharan Africa (WHO 2009) (fig. 1). More than 600,000 people, mostly young children and pregnant women, die each year from vitamin A deficiency (Black et al. 2008). It is also the leading cause of preventable blindness in the world (Sommer et al. 1996).

The amount of dietary vitamin A that a person needs depends on age and sex, whether pregnant or lactating, and presumably on genetics and lifestyle. Recommended amounts of nutrients for healthy individuals are called dietary reference intakes (DRI) in the United States (IOM 2000) and recommended nutrient intakes (RNI) in most of the rest of the world. Although DRI and RNI are based on the same scientific evidence, they vary slightly (table 1).

Humans can get their RNI of vitamin A from animal-derived foods such as organ meats (the best source is liver). However, organ meats are scarce and too expensive for most of the world's population to eat regularly. Fortunately, vitamin A is also formed in the body from carotenoids, brightly colored phytonutrients found in fruits and vegetables. The most common vitamin A–forming carotenoids are beta-carotene, alpha-carotene and beta-cryptoxanthin. Of the three, beta-carotene is the most widely distributed in plants. It is found in many green or orange vegetables and fruits, such as carrots, orange sweet potatoes, mangos, spinach and pumpkin. Alpha-carotene is mostly in carrots. Beta-cryptoxanthin is found in orange fruits, such as tangerines, oranges, papaya and persimmons, and also in red peppers (USDA ARS 2009).

RNI reports in retinol equivalents (RE); DRI reports in retinol activity equivalents (RAEs). Both are measures of how much vitamin A is formed from carotenoids. In a test tube, one molecule of beta-carotene could be converted into two molecules of vitamin A, and one molecule of alpha-carotene or beta-cryptoxanthin into one molecule of vitamin A. In the body, these carotenoids produce far less RAE than in the test tube: current estimates are one-twelfth (FAO 2004) or one twenty-fourth (IOM 2000) of the test-tube RAE. The reason for this difference is because carotenoids are poorly absorbed from most foods (de Pee et al. 1998; Veda et al. 2006).

Absorption depends on many factors, including the type of carotenoid, the food it is contained within, and the digestive health, vitamin status and genetic makeup of the individual. Beta-carotene is better absorbed from orange fruits and vegetables than from leafy green vegetables (de Pee et al. 1998). Recent research has suggested that foods rich in beta-cryptoxanthin, such as tangerines and oranges, are surprisingly good sources

Recent research has suggested that foods rich in beta-cryptoxanthin, such as tangerines and oranges, are surprisingly good sources of vitamin A.

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of vitamin A, even though beta-carotene could produce more RAEs based on its chemistry. Burri et al. (2011) found that beta-cryptoxanthin appears to be absorbed significantly better than beta-carotene from mixed diets. Recent research in animals by Davis et al. (2008) suggests that foods rich in beta-cryptoxanthin are equivalent to those rich in beta-carotene as sources of vitamin A.

Encouraged by these findings, we decided to evaluate whether oranges and tangerines, the primary food sources for beta-cryptoxanthin in most of the world, could be used to prevent vitamin A deficiency. We determined the carotenoid concentrations of fresh oranges and fresh and canned tangerines, and calculated the amount of these fruits needed to meet the RNI for one at-risk individual (FAO 2004) as well as the amount of these fruits needed to meet the RNI for all at-risk populations (WHO 2009). We then reviewed the extent and distribution of orange and tangerine production, and identified countries that were major producers of each fruit. We evaluated the effect of increasing production in developing countries with food deficits (those which currently cannot produce enough food to feed their populations) and compared yields to those achieved in California. Production and nutrient data were collected from library and Internet searches.

Vitamin A deficiency programs

Providing vitamin A supplements or vitamin A–rich foods to people with vitamin A deficiency seems to decrease the severity of diseases such as influenza and diarrhea, and reduce infant and postpartum maternal mortality by about 30% (Black et al. 2008; FAO 2004). Vitamin A deficiency is usually prevented by food fortification or distributing high-dose vitamin A capsules twice a year.

Supplements. Vitamin A supplementation programs are cost-effective nutrient

TABLE 1. Recommended nutrient intake (RNI) and dietary reference intake (DRI) for vitamin A at different life stages

Life stage	RNI*	DRI
	μg/day	
Infants 0–6 months	375	400†
Infants 7–12 months	400	500†
Children 1–3 years	400	300
Children 4–6 years	450	400
Children 7–8 years	500	400
Children 9 years	500	600
Children 10–13 years	600	600
Adolescents 14–18 years	600	900 for males‡ 700 for females
Adult males 19–65 years	600	900
Adult males 65+ years	600	900
Adult females 19+ years (except when pregnant/lactating)	500	700
Adult females 65+ years	600	700
Pregnant females 14–18 years	800	750
Pregnant females 19+ years	800	770
Lactating females 14–18 years	850	1,200
Lactating females 19+ years	850	1,300

* RNI reports in retinol equivalents (RE); DRI reports in retinol activity equivalents (RAE).
† Recommended adequate intake, since no dietary reference intake has been established.
‡ Adolescent group is combined with adults.

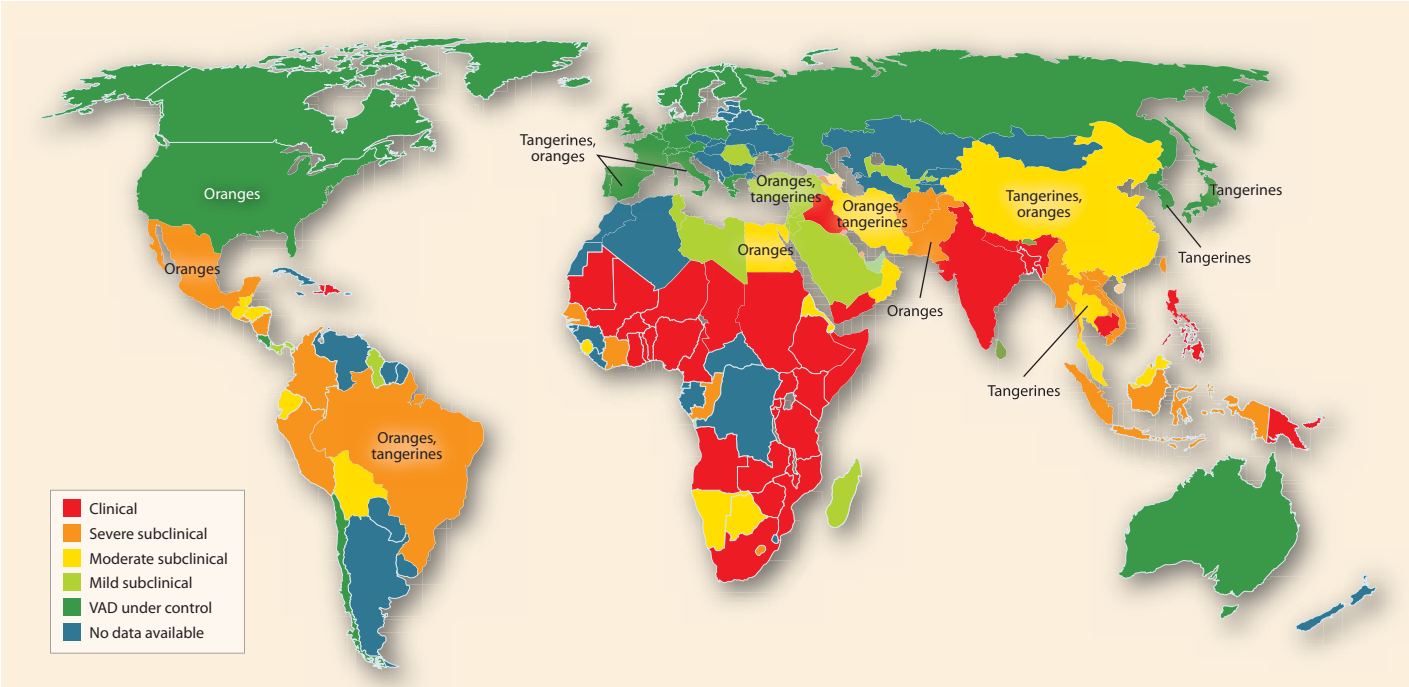

Fig. 1. Vitamin A deficiency (VAD) prevalence (clinical and subclinical) and regions of orange and tangerine production (WHO 2009).

M. Murtaza Farrahmand/Community Eye Health Journal

Some national governments run vitamin A supplementation programs; they are cost effective, but reaching most people at risk of vitamin A deficiency has proved challenging. *Right,* vitamin A distribution in Afghanistan.

interventions supported by several national governments and international charitable organizations (including the Bill and Melinda Gates Foundation and California-based Vitamin Angels), but these programs have been difficult to sustain. Coverage — the number of people who are given nutritional supplements compared to the number who should receive them — is often incomplete. For example, India has supported national vitamin A supplementation programs for 40 years but has attained less than 25% coverage (Stein et al. 2006). Furthermore, vitamin A capsule supplementation programs were suspected of causing death through inadvertent overdosing (Mudur 2001), although research suggests that these suspicions were unfounded (West and Sommer 2002).

Food fortification. Programs that fortify foods such as sugar and oil with vitamin A are another popular and cost-effective way to prevent vitamin A deficiency, and they have been quite effective in Central America. However, it has been difficult to expand these programs, mostly because of problems inherent in food fortification. The food must be consumed by almost everyone, including the poorest people. The amounts eaten must not differ greatly: the food must prevent vitamin A deficiency in most people but not cause toxicity in those who eat more than average amounts. Also, food processors and manufacturers generally want fortified foods to look and feel exactly the same as the unfortified foods, which limits the type of nutrient that can be added.

Fruits and vegetables. Growing produce rich in vitamin A–forming carotenoids is an attractive alternative to vitamin A supplements (Ruel 2001). Fruits and vegetables can provide a variety of nutrients in addition to vitamin A — citrus is also a well-known source of vitamin C and fiber — and generate income for small farmers and shopkeepers. Long-term sustainability might be achievable because fruit and vegetable seeds can be harvested and shared at the local level.

TABLE 2. Estimated carotenoids in 100 grams (edible portion) of tangerines and oranges, and two foods (mangos and sweet potatoes) already used to prevent vitamin A deficiency

Food (information source)	Estimated carotenoid content*		
	Beta-carotene	Alpha-carotene	Beta-cryptoxanthin
	RAE/100 grams		
Tangerine (~ ½ cup or 1 medium tangerine)			
Raw (USDA ARS 2009)	13	4.2	34
Raw (USDA ARS 1993)	13	4.2	88
Raw (Burri, this report)	1.1–23	0.6–4.6	41–247
Raw (Schlatterer and Breithaupt 2005)	Not measured	Not measured	63
Raw (Yano et al. 2005)	4.5–16	0–3.8	88–249
Canned, drained (USDA ARS 2009)	30	5.5	64
Canned, drained (Burri, this report)	9–22	1.7–4.8	83–233
Orange (~ ½ large orange)			
Raw (USDA ARS 2009)	5.5	0.5	9.6
(Schlatterer and Breithaupt 2005)	Not measured	Not measured	6.0–15
(Yano et al. 2005)	4.3–6	0–0.8	23–38
Mango (~ 3/5 cup)			
(USDA ARS 2009)	37	0.7	1.0
(Perkins-Veazie 2007)	41–217	Not measured	Not measured
Sweet potato, orange-fleshed (~ 2/5 cup)			0
Canned, drained (USDA ARS 2009)	458	0	0
Boiled (USDA ARS 2009)	787	0	0
Baked (USDA ARS 2009)	959	3.6	0

* 1 µg retinol activity equivalent (RAE) = 12 µg beta-carotene and 24 µg alpha-carotene from food (US IOM 2000) and 12 µg beta-cryptoxanthin from food (FAO 2004).

Vitamin A deficiency has decreased in several Asian countries, presumably because of dietary improvements (WHO 2009). It would be useful to know whether any country has succeeded in improving its vitamin A status by increasing fruit and vegetable production and consumption on a large scale. Unfortunately, such national programs do not exist. However, small-scale interventions using fruits and vegetables rich in vitamin A–forming carotenoids (usually beta-carotene) have increased vitamin A status in several populations in Africa and southern Asia (Haskell et al. 2004; Haskell et al. 2005; Ruel 2001). Some of these interventions have used a mixture of foods, such as goat liver, amaranth and carrots (Haskell et al. 2005). Others have used a single food, such as red palm oil, mangos or sweet potatoes (Burri and Turner 2009).

Tangerines and oranges

Tangerines and oranges are major crops in California (USDA 2008), and they have several desirable characteristics for a nutritional intervention program. They are relatively durable fruits, simple to prepare and popular with children. Tangerines are easier to peel than many other citrus fruits and are often seedless. Tangerines and oranges both grow on trees that produce for many years; the trees generally are more pest-free and require less labor than vitamin A–containing vegetables, and they are somewhat less dependent on rainfall and optimal growing conditions. Both grow well in subtropical temperatures (Ladaniya 2008). With optimal storage, they can be kept for up to 4 weeks. They can also be processed into juice and sold fresh, frozen, bottled or canned, extending their availability and shelf life. Tangerines are often canned, so they are available year-round, an advantage since vitamin A deficiency often occurs during

the winter when other fruits and vegetables are scarce.

Carotenoid concentrations

Oranges and tangerines have significant concentrations of beta-carotene and beta-cryptoxanthin (table 2). Concentrations vary with the species and variety of orange and tangerine, and with growing, harvesting and storage conditions (Rodriguez-Amaya 2003). Data compiled by the U.S. Department of Agriculture (USDA ARS 2009) does not specify the variety of tangerines tested. Other data shows that the variety of fruit tested is important (Perkins-Veazie 2007; Yano et al 2005). Satsuma mandarins (*Citrus reticulata unshui*), for example, have much higher amounts of beta-cryptoxanthin than clementines (*Citrus reticulata blanco*), which in turn have more than other citrus fruits such as oranges and grapefruits. This probably explains the high carotenoid concentrations seen in canned tangerines, which are often Satsuma mandarins.

We collected data from several laboratories and measured carotenoid concentrations in fresh and canned tangerines in our laboratory, then compared the results to data for mangos and sweet potatoes, two foods that have been used in successful small-scale interventions to prevent vitamin A deficiency. Carotenoid concentrations were measured by high-performance liquid chromatography, although the methodologies differed.

How much fruit must be eaten

We calculated the amount of tangerines and oranges needed to supply one person with the RNI of vitamin A (FAO 2004) (tables 1 and 2). For the best-case scenario, we used the highest estimated RAEs listed for oranges (6 from beta-carotene, 1 from alpha-carotene and 38 from beta-cryptoxanthin) for a total of 45 RAEs in 100 grams of orange. The best-case scenario of RAEs for tangerines was 30 from beta-carotene, 6 from alpha-carotene and 249 from beta-cryptoxanthin, for a total of 285 RAEs in 100 grams. We compared these results to mangos and sweet potatoes. The best case for mangos was 217 RAEs in 100 grams, while the best case for orange-fleshed sweet potatoes was 963 RAEs in 100 grams.

We then estimated the amount of carotenoid-rich food needed to meet the recommended safe nutrient intake of vitamin A (FAO 2004) in people at various life stages, based on the unrealistic assumption that all of the vitamin A in the diet would be derived from that food. The serving sizes used were 1 cup of tangerine slices, sweet potatoes or mango (which weighed 195, 255 and 165 grams, respectively); or 1 large orange (weighing 184 grams) (USDA ARS 2009).

Our calculations showed that the RNI for vitamin A could not be met by eating oranges. Even under the best-case scenario, the amount of orange needed to meet the RNI ranged from 396 grams (two oranges) per day for 1- to 3-year-old children, to 866 grams (4.25 oranges) per day for lactating women. It would also be difficult to achieve the RNI of vitamin A for lactating women solely by eating mangos or tangerines (table 3). They would have to eat as much as 8 cups of tangerines and 13 cups of mangos every day under the worst-case scenario. However, mangos have been used to increase vitamin A status (de Pee et al. 1998), and the best-case scenarios for lactating women show more realistic consumptions of about 2 cups per day. In contrast, people of all ages could meet the RNI by eating less than one cup of orange-fleshed sweet potato, which is quite realistic.

Tangerines can provide a substantial amount of vitamin A in the diet, and could theoretically supply enough vitamin A to be useful as interventions, but sustaining such high intakes of tangerines would be difficult.

Scale of production needed

We calculated the amounts of tangerines that would be needed to supply the

TABLE 3. Estimated grams and servings per day (cups) needed to meet recommended nutrient intakes (RNI) for vitamin A at different life stages

Life stage	Food needed to meet RNI	
	grams/day	*servings/day (cups)*
Infants 0–12 months	Assumed mostly breastfed	NA
Children 1–3 years		
Tangerine	140–784	0.7–4.0
Mango	184–1,026	1.1–6.2
Sweet potato	42–86	0.2–0.4
Children 4–6 years		
Tangerine	158–882	0.8–4.5
Mango	207–1,154	1.2–7.0
Sweet potato	47–96	0.2–0.4
Children 7–9 years		
Tangerine	175–980	0.9–5.0
Mango	230–1,282	1.4–7.8
Sweet potato	52–107	0.2–0.4
Adolescents 10–18 years; adult males 19–65+ years; adult females 65+ years		
Tangerine	210–1,176	1.1–6.0
Mango	276–1,538	1.7–9.3
Sweet potato	62–130	0.2–0.5
Adult females 19–65 years (not pregnant or lactating)		
Tangerine	175–980	0.9–5.0
Mango	230–1,282	1.4–7.8
Sweet potato	52–107	0.2–0.4
Pregnant females		
Tangerine	281–1,567	1.4–8.0
Mango	369–2,052	2.2–12.4
Sweet potato	83–173	0.3–0.7
Lactating females		
Tangerine	298–1,666	1.5–8.5
Mango	392–2,179	2.4–13.2
Sweet potato	88–186	0.3–0.7

RNI for vitamin A to 190 million preschool children and 19.1 million pregnant women most in danger of vitamin A deficiency (WHO 2009). We assumed that 75% of the preschool children were 1 to 3 years old, and the rest 4 or 5 years old. We also assumed that the tangerines contained high carotenoid concentrations (285 RAEs per 100 grams of edible fruit). In this best-case scenario, it would take 13,345,538 tons of tangerines to supply vitamin A to all the women and children most at risk for 1 year. We compared this number to the amounts of tangerines and oranges grown per year.

For both tangerines and oranges (table 4), we assessed the production, yield and land area harvested in the world, Asia, Africa, the 77 low-income countries with food deficits, the United States and California. Although the most current information is for 2007 (FAO 2009), today's production is believed to be similar.

It is evident that if all tangerines were bred for high carotenoid concentrations and a percentage of the production were used to provide vitamin A to those most at risk for vitamin A deficiency, then current worldwide production would be sufficient. Indeed, production would be sufficient in the low-income food deficit countries if the two groups of people most at risk ate 70% of the tangerines. Furthermore, current production levels could be increased. Several changes could make the production of sufficient tangerines more certain; for example, Satsuma mandarin varieties could be selected and bred for higher carotenoid concentrations. Also, if yields in low-income food deficit countries could be increased to those currently attained in California, then, without any changes in land use, their tangerine production would rise to an estimated 32,915,681 tons, or about 2.5 times more than needed to prevent vitamin A deficiency for those most at risk. In Africa, such efforts are being made; through ongoing outreach and education, citrus production is increasing and improving (US AID 2007). Finally, because the land devoted to orange production worldwide currently exceeds that of tangerines, some of the land, investment and knowledge used to grow oranges could be used for growing tangerines.

Current production of tangerines in tons is comparable to that of mangos (world production 36,867,502 tons, yield 3.23 tons per acre and 11,390,417 acres harvested). Tangerine production is dwarfed by sweet potato production (world production 118,682,401 tons, yield 5.93 tons per acre and 20,023,450 acres harvested), but most sweet potatoes eaten by humans in low-income food deficit countries are white ones with low concentrations of vitamin A. Orange sweet potatoes, which were introduced later into these countries, are more likely to be used as fuel or fed to livestock (USDA ERS 2009).

TABLE 4. Production quantity, yield and area harvested for tangerines and oranges

	Production	Yield	Area harvested
	tons	*tons/acre*	*acres*
Tangerines			
World	30,715,177	6.05	5,070,442
Asia	22,343,630	5.70	3,920,274
Africa	1,622,352	7.10	228,562
Low-income food deficit countries*	18,821,515	5.24	3,593,415
United States	361,554	8.12	44,500
California	82,466	9.16	9,000
Oranges			
World	71,388,969	7.32	9,754,824
Asia	19,626,668	5.53	3,549,937
Africa	6,356,288	6.82	932,283
Low-income food deficit countries	18,075,046	5.00	3,610,084
United States	8,109,621	12.15	667,200
California	2,415,983	13.42	180,000

Source: California data, USDA Agriculture Statistics Board 2008; all other regions, FAO 2009 (2007 data converted to tons and acres).

* These 77 countries meet criteria set by the World Bank and FAO as currently unable to grow enough food to feed their populations.

TABLE 5. Availability for consumption of oranges, tangerines and sweet potatoes, 2003*

	Oranges/tangerines		Sweet potatoes (all colors)	
	g/person/day	*cup/person/day*	*g/person/day*	*cup/person/day*
World	30.14	0.15	27.40	0.11
Asia	19.18	0.10	38.36	0.15
Africa	13.70	0.07	30.14	0.12
Low-income food deficit countries	13.70	0.07	41.10	0.16
United States	104.11	0.52	5.48	0.02

Source: FAO 2009.

* Assumes 100 grams citrus = 0.5 cup, and 100 grams sweet potatoes = 0.4 cup.

Problems of availability

Of course, the amount of a food that is produced can be much higher than the amount that is available for consumption, especially by the poor. For example, a crop might be exported to other countries, used for biofuel or animal feed, or spoiled. The amount available for consumption is also an important indicator of its acceptability and how well it might serve as a food-based intervention to prevent vitamin A deficiency. Food availability data is less recent than food production data and is subject to more uncertainty. No data has been gathered for mangos, and the white- and orange-fleshed types of sweet potatoes are not separated in the data, nor are tangerines and oranges. No data exists on the availability of oranges and tangerines for consumption in California, though it is likely to be at least as high as that of the United States overall since citrus is a major crop in California and good quality fruit is relatively inexpensive and abundant.

Even though the data is limited, it shows that oranges, tangerines and sweet potatoes are not available in sufficient amounts to prevent vitamin A deficiency in people most at risk (table 5). This is because most people at risk for vitamin A deficiency are poor and live in remote areas with bad roads and intermittent

electricity. Production at the village level is often inefficient and storage conditions inadequate, so spoilage and waste is high, increasing costs and decreasing availability. Major producers typically export tangerines to other countries, which is simpler and more profitable. Several major producers of tangerines, including China, Brazil, Iran, Thailand, Egypt and Pakistan, also have moderate levels of vitamin A deficiency (fig. 1).

Tangerines' potential to help

We used nutritional, agricultural and population data to evaluate the potential of oranges and tangerines to prevent vitamin A deficiency. Satsuma mandarin tangerines are good sources of vitamin A, providing up to 285 RAEs per 100 grams of fruit. In our best-case estimate, young children could obtain 100% of their recommended nutrient intake of vitamin A by eating 0.7 cup of tangerines per day, while pregnant or lactating women could meet their RNI by eating 1.5 cups of tangerines per day.

These amounts are reasonable, though perhaps not sustainable on a long-term basis. Tangerines could supply substantial amounts of vitamin A to individuals, which cannot be said for oranges, which contain far less vitamin A–forming carotenoids.

In our best-case estimate, current global tangerine production meets the RNI for all of the young children and postpartum women most at risk for vitamin A deficiency. Furthermore, production might increase substantially if farmers in developing countries were able to adapt for their own use the high-carotenoid varieties of tangerines bred by California food scientists and the high-yield farming procedures of California farmers. Currently, there are no major environmental issues associated with growing tangerines, and essentially all of the tangerines produced are eaten by people, not used for animal feed or fuel. Finally, tangerines are relatively durable, easy to prepare and store, and available during fall and winter when the mangos, peppers and green leafy vegetables that supply much of the vitamin A in countries with food deficits are scarce. In theory, tangerines have the potential to help prevent vitamin A deficiency worldwide.

Unfortunately, the current availability of tangerines and oranges for consumption is too low to prevent global vitamin A deficiency. Availability is also the major obstacle for other foods, such as orange-fleshed sweet potatoes, that have the potential to prevent vitamin A deficiency. Looking to the future, breeding strategies to increase concentrations of vitamin A–forming carotenoids in tangerines will be useful. However, it may be even more important to increase the availability and acceptability of tangerines and other foods rich in vitamin A–forming carotenoids for people at risk. The availability and acceptability of these crops may make the difference between the success and failure of food-based interventions to prevent vitamin A deficiency.

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Barbara Sutherland, Associate Staff Scientist, Children's Hospital Oakland Research Center (CHORI) (former Director, Expanded Food and Nutrition Education Program, UC Davis Department of Nutrition), served as Guest Associate Editor for this article. *This work was supported in part by the U.S. Department of Agriculture's Agricultural Research Service.*

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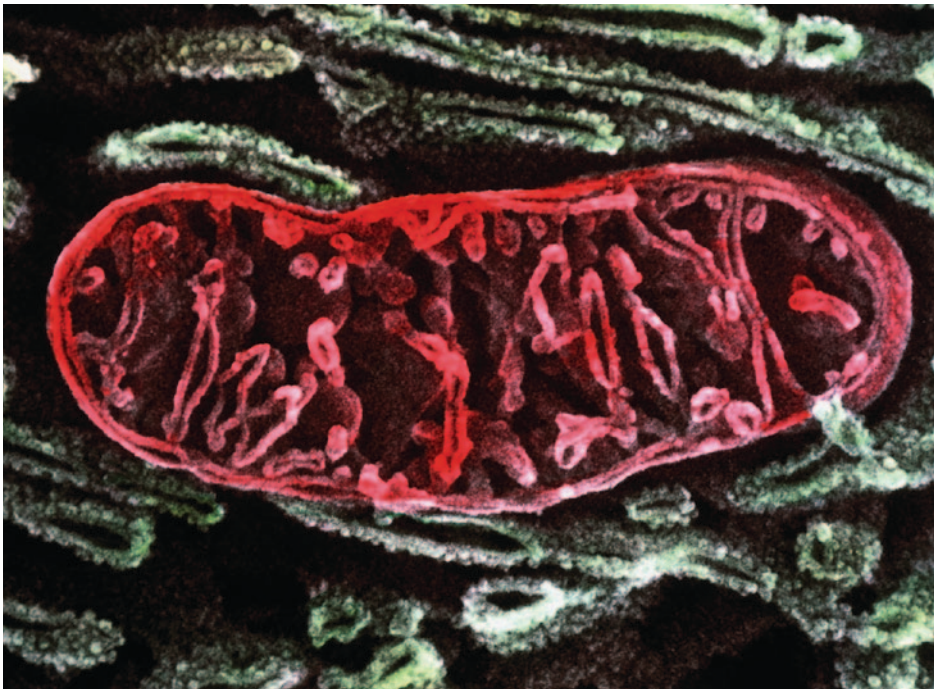
REVIEW ARTICLE

Well-functioning cell mitochondria promote good health

by Winyoo Chowanadisai, Sonia F. Shenoy, Edward Sharman, Carl L. Keen, Jiankang Liu *and* Robert B. Rucker

Mitochondrial function can be directly linked to protection from certain chronic diseases and conditions, such as heart disease, diabetes, metabolic syndrome and chronic inflammation, as well as the aging processes. Mitochondria are central to normal glucose, amino acid and fatty acid metabolism, in addition to antioxidant modulation and virtually all aspects of cell turnover and maintenance. Nutrition plays an essential role in optimizing such functions. We describe strategies for the regulation of mitochondria, as well as metabolic strategies for dealing with the thousands of compounds in plants and animal tissues that are metabolically important. Many of these compounds function to signal the up- or downregulation of mitochondria or act as antioxidants.



Professors Pietro M. Motta & Tomonori Naguro/Photo Researchers, Inc.

Mitochondria are the organelles in all cells that break down nutrients and produce energy for the body's myriad functions. Nutrition plays a critical role in the process of mitochondriogenesis, and poor nutrition can subsequently lead to health problems.

Aging and many chronic diseases and conditions such as heart disease, diabetes, metabolic syndrome and inflammation are affected by mitochondria, organelles that break down nutrients and produce most of the energy in our cells. These organelles act as the cell's chief metabolic control center, converting substances from the foods we eat into energy for essential functions. To generate the adenosine triphosphate (ATP) that powers most of the chemical reactions in cells, mitochondria use oxygen to help break down (metabolize) glucose, amino acids and fatty acids. Because this process requires oxygen, it is called cellular respiration. The control of oxygen use and respiration is central to normal growth and development, affecting virtually all aspects of the metabolism of glucose, amino acids and fatty acids and the modulation of reactive oxygen species (ROS) that can damage cells (Lane 2006).

Mitochondria regulate these processes with great precision. For example, consider that over the course of a year, whether slender or obese, each of us consumes hundreds of pounds of food, yet our body weight usually does not vary more than a pound. Mitochondria are also key to regulating body temperature: the human production and use of ATP is equivalent to about 10,000 times more heat per day than that produced by an equal mass of the sun. Mitochondrial control of ATP production helps keep us from boiling in our own juices or even raising our body temperature a few degrees, except on rare occasions (Lane 2006; Scheffler 2007).

Editor's note: This article describes important functions and characteristics of mitochondria. It is the basis for the next article, "Biofactors in food promote health by enhancing mitochondrial function" (page 141), which describes the actions of specific food components as cellular signals for mitochondriogenesis.

Regulation of mitochondria

The mechanisms for mitochondrial regulation include (1) changes in the number of mitochondria per cell, (2) control of the assembly and disassembly of mitochondria (turnover), (3) changes in the size and surface area of mitochondria and (4) regulation of the numerous catalytic steps important to the control of body fuel utilization, heat and ATP production. Besides the nucleus of the cell, mitochondria are the only cellular organelle in animals that contains a distinct set of genes as well as the machinery to manufacture some of its own messenger RNAs and proteins. Of the approximately 25,000 genes that make up the human genome, about 0.1% are found exclusively in the mitochondria (Scheffler 2007).

Indeed, mitochondrial DNA (mtDNA) has a separate evolutionary origin, being derived from the circular genomes of bacteria that were engulfed by early ancestors of the eukaryotic cells in today's

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plants and animals. These mitochondrial genes, plus another thousand or so in the nucleus (about 4% of the total nuclear genes), are associated with mitochondrial assembly and production. It is important to note that in most multicellular organisms, mtDNA is maternally inherited. This is partly because an egg contains 100,000 to one million mtDNA molecules, whereas a sperm cell contains only 100 to 1,000, and most of these are lost or degraded after fertilization.

Mitochondrial function and health

In addition to being key to the control of energy production and utilization, mitochondria are important to cellular regulatory signaling, cellular differentiation, apoptosis (programmed cell death or cellular turnover) and, when appropriate, cell replacement. The lifespan of a cell is directly linked to mitochondrial assembly and production (Spierings et al. 2005). Apoptosis occurs as part of the normal development, maintenance and renewal of tissue. In the average adult, between 50 billion and 70 billion cells turn over each day due to apoptosis (Lane 2006); this amounts to the proliferation and subsequent destruction of a mass of cells equal to one's body weight in a single year!

Consequently, when mitochondrial function is compromised at any level, a number of metabolic changes with health-related consequences can occur (table 1). In many cases, their direct causes are genetic in nature. However, the decline in mitochondrial efficiency with aging is thought to be a major contributor to metabolic syndrome, as well as neurological and psychiatric disorders such as Parkinson's and Alzheimer's disease (Guarente 2008). Understanding nongenetic factors such as diet and exercise is essential to correctly interpreting the health-related consequences of mitochondrial function.

Exercise intolerance (fatigue or breathlessness during exercise) is a common symptom of mitochondrial defects that are genetic as well as some of those that are due to normal aging (Tarnopolsky and Raha 2005). Such defects compromise the transfer of food energy into metabolic energy. This in turn reduces oxygen extraction by tissues and can result in the excessive generation of lactate and enhance the free radical production that may cause tissue damage.

Glossary

Adenosine triphosphate (ATP): A multifunctional nucleotide in cells often designated the "molecular unit of currency" of cellular energy transfer. When energy is derived from the breakdown of chemical bonds in food, a part of that energy goes into forming ATP, which is then used to drive reactions important to cellular and mechanical work (e.g., walking or running).

Antioxidant: A substance capable of inhibiting the oxidation of other molecules, particularly cellular components such as lipids, proteins and nucleic acids.

Apoptosis: The process of programmed cell death, in which the cell uses specialized cellular machinery to kill itself, so as to control cell numbers and eliminate cells that threaten the organism's survival.

Biofactor: Any material that has some level of biochemical function. The term is used as a general designation for functional chemicals, usually found in natural products, that contain, for example, vitamins, antioxidants or cell signaling agents.

Cell signaling: The relaying of molecular signals (chemical or physical, such as light) from a cell's exterior to its intracellular response elements and signaling molecules.

Messenger RNA (mRNA): Messenger ribonucleic acid, a molecule that encodes the chemical "blueprint" contained in genetic DNA, transcribing and carrying it to the site of protein synthesis to form a protein.

Metabolism (aerobic, anaerobic): The physical and chemical processes in an organism by which compounds are produced (anabolically formed), maintained (homeostatically maintained) or destroyed (catabolically altered). Energy for metabolic and mechanical work is derived from metabolism. Aerobic metabolism requires oxygen; anaerobic metabolism occurs in the absence of oxygen.

Mitochondria: Membrane-enclosed compartments (or organelles) found in most eukaryotic cells. They produce most of the cell's chemical energy, but as byproducts of this function, also generate significant amounts of damaging substances termed reactive oxygen species (ROS).

Mitochondriogenesis (mitochondrial biogenesis): The production of new mitochondria. This process can occur independently of normal cell division.

Phytoalexin: A substance (phytochemical) that is produced by a plant in response to bacterial or fungal pathogen attack. A number of substances that plants produce and utilize as phytoalexins are ingested by animals and function as antioxidants.

Reactive oxygen species (ROS): Chemically reactive, oxygen-containing molecules, most of which have unpaired electrons. ROS form as natural byproducts of normal oxygen metabolism and have important roles in cell signaling. ROS levels can also increase dramatically and produce significant damage to cell structures.

Respiration, cellular: The combined metabolic processes that are directed at capturing chemical energy from food and converting it to compounds, such as ATP, for eventual transfer to use in work-related functions (e.g., biosynthesis, locomotion or transport).

Transcriptional coactivator: A cofactor protein that stimulates mRNA transcription by binding at particular sites on DNA.

Xenobiotic metabolism: Sets of metabolic pathways that modify the chemical structure of xenobiotics — compounds foreign to an organism's normal biochemistry such as those produced by plants (phytochemicals), synthetic drugs and poisons. The same xenobiotic pathways are also important for the transformation and metabolism of certain vitamins, hormones and endogenous compounds important to integrated metabolism and cellular regulation.

Apoptosis. Mitochondrial-related apoptosis is often accelerated in response to inflammation or protection from abnormal cell proliferation (such as that associated with some types of cancer) (Mann et al. 2005). The presence of abnormally elevated mitochondrial DNA in serum can be diagnostic for certain cancers because of apoptosis (Ellinger et al. 2008). Although here we specifically focus on the cells that control ATP regulation and use (that is, those in muscles and the liver, and those that are important to nerve transmissions), the good news is that many biofactors that improve cellular energy relationships — such as the enhancement of lipid and glucose metabolism — also aid in improving inflammation and immune responses (Mann et al. 2005).

Cell signaling. Part of a complex system of communication, cell signaling helps govern the fundamental activities of cells (Gomperts et al. 2003). Errors in cellular information processing or signaling can be responsible for obesity and numerous diseases, such as cancer and diabetes. Higher numbers of mitochondria are often associated with improved function in neural and energy processing cells, such as liver and muscle cells.

Mitochondrial assembly. In cells primarily designed for energy utilization and nutrient processing, the first steps of mitochondriogenesis often involve cell surface receptors responding to extracellular signals. These signals can come to a given cell in the form of both internal (e.g., hormones and cell-derived factors from other or adjacent cells) or external messenger molecules (e.g., dietary factors) that interact with the receptors of the given or targeted cell. Examples of internal messenger molecules include cytokines, hormones, growth factors, neurotransmitters and adhesion molecules that aid in cell-to-cell communication. External messengers include many bioactive factors (see page 141) (Rice-Evans and Packer 2003).

In some cases, cell surface receptors communicate with other cellular proteins, ranging from membrane proteins that open and close ion channels in the cell membrane to cellular enzymes that act as on/off switches by chemically activating proteins important to signaling pathways. Examples of these enzymes include kinases and phosphatases that add or remove phosphate groups from specific signaling molecules, thereby changing their chemical characteristics and in turn the levels of signaling activity. Such complex signaling pathways provide opportunities for feedback, signal amplification and interactions with other signals and signaling pathways.

The master regulators of mitochondriogenesis are a family of proteins that control the transcription of messenger RNAs, which in turn control and regulate the production of proteins needed to manufacture mitochondria. These proteins are called the peroxisome proliferator-activated receptor (PPAR) gamma family of transcriptional coactivators, and include one called PGC-1α, which enhances the expression of intermediate transcription factors that are important to mitochondriogenesis (Handschin and Spiegelman 2006). These factors include nuclear respiratory factors (NRFs), PPARs and TFAM (transcription factor A, mitochondrial). PPARs and NRFs play essential roles in the integration and regulation of cellular differentiation and metabolism, and TFAM is a key activator of mitochondrial DNA gene transcription.

Other factors regulated by PGC-1α include CREB (cAMP response element binding) and STAT (signal transducer and activator of transcription). These transcription factors are important to mitochondriogenesis and can help coordinate

TABLE 1. Complications and disorders associated with mitochondrial mutations and defects in mitochondriogenesis

Condition	Signs and symptoms	Causes
Type 2 diabetes	Characterized by poor blood glucose control, elevated insulin and elevated triglycerides; often accompanied by increased thirst, hunger and/or fatigue; loss of visional acuity. Symptoms may develop slowly.	Obesity; decline in mitochondrial function.
Metabolic syndrome	High triglyceride levels with reduced HDL cholesterol levels; abnormal prothrombin levels; high blood pressure; abnormal fasting glucose and other symptoms common with type 2 diabetes.	Genetics and environment play important roles, as do a sedentary lifestyle, progressive weight gains and diminished mitochondrial function. Metabolic syndrome is present in about 5% of people with normal body weight, 22% of those who are overweight and 60% of those who are obese.
Obesity	Weight gain; faulty body temperature regulation; signs of metabolic syndrome; fatigue.	Genetics; hypothyroidism; overeating; diminished mitochondrial function.
Dyslipidemia	Presence of raised or abnormal levels of lipids and/or lipoproteins in the blood.	Genetics and the environment (high carbohydrate and/or lipid diets) play important roles, contributing to diminished mitochondrial function.
Alzheimer's disease	May start with slight memory loss and confusion but eventually leads to irreversible mental impairment that destroys a person's ability to remember, reason, learn and imagine.	Growing evidence implicates mitochondrial dysfunction with patterns of reduced expression of mtDNA- and nuclear DNA–encoded genes; consistent with a down-regulation of the respiratory chain in response to reduced neuronal activity.
Parkinson's disease	Early signs may be subtle and can go unnoticed; signs and symptoms may include tremor, slowed motion (bradykinesia), rigid muscles, loss of automatic movements and dementia in the later stages.	Increasing evidence that impairment of mitochondrial function, oxidative damage and inflammation are contributing factors. There is a deficiency of oxidative capacity and mitochondrial-related inflammation and oxidative damage.

mitochondrial function with the dozens of other processes important to cell growth and maintenance (Gough et al. 2009; Wegrzyn et al. 2009). In brief, these factors work together either by enhancing the binding of one or more factors or by decreasing their binding affinities, thereby modulating the production of messenger RNAs involved in mitochondrial assembly and disassembly.

Mitochondrial disassembly. Like any highly responsive and dynamic process, mitochondrial production must be coordinated with disassembly as necessary. Mitochondrial disassembly is mediated by cell-surface receptors in the Fas family of proteins that modulate apoptosis. These proteins are closely related to other apoptotic proteins, the tumor necrosis factor and the nerve growth factor receptor family of proteins (Nagata and Golstein 1995). When Fas proteins are activated, they in turn activate and mobilize other protein families involved in apoptosis, such as the Bcl-2 family. Bcl-2 proteins can either promote or inhibit apoptosis (Spierings et al. 2005; Wong and Puthalakath 2008). One of the best-understood Bcl-2 proteins is called BAX, which promotes apoptosis by competing with another Bcl-2 protein (called Bcl-2 proper) that inhibits apoptosis (Lane 2006; Wong and Puthalakath 2008). BAX inserts itself primarily into the outer mitochondrial membrane and induces the opening of voltage-dependent channels, which results in the release of proteins such as cytochrome c (an important mitochondrial respiratory protein) and other pro-apoptotic factors.

In addition, Bcl-2 proteins can activate any of a large family of caspase enzymes, which break down proteins and can degrade and modify various cellular components. The activation of caspases plays a role in both apoptosis and normal development. It is important to note that in some cells, caspases are required for normal immune and inflammatory responses. Consequently, the failure to control apoptosis is one of the main contributors to uncontrolled cancer proliferation and tumor development, as well as the expression of certain autoimmune diseases.

Moreover, to add to this complex arrangement, a family of enzymes in the nucleus also affects apoptosis (Alcain and Villalba 2009; Elliott and Jirousek 2008; Lane 2006; Sharman 2010). Called silent information regulators (or sirtuins), these enzymes modify histones, which are proteins in the nucleus that serve as the scaffolding that helps organize nuclear DNA. When histones are modified, different segments of DNA can be exposed and transcribed into mRNA. These sirtuins seem particularly important to the exposure of genes related to apoptosis control.

Other effects on mitochondriogenesis

Mitochondriogenesis and signaling are significantly affected by two other factors: (1) xenobiotic metabolism, which evolved mainly to aid in the disposal and transformation of hundreds of plant pigments in the diet and (2) ROS, which include free radicals and other chemically reactive oxygen-containing molecules.

Fig. 1. Examples of reactions involved in the modification of xenobiotic compounds and, in some cases, endogenous metabolites. These reactions alter the solubility and accessibility of targeted compounds so that they may be carried to a site to act as activators or deactivators (inhibitors) of a process. Alternatively, increasing solubility or adding a functional group may be a signal to dispose of the compound. Cytochrome P450 enzymes are most often involved in phase I. If more chemical modification is needed, compounds proceed to phase II, where additional chemical substituents (functional groups) are added. Phase I and II modifications chemically alter compounds to make them more or less potent as activators or deactivators of specific processes or to direct the modified compounds to specific transport systems for delivery into bile or blood for eventual disposal by the kidney.

Xenobiotic metabolism. Besides being critical to phytochemical availability and disposal, xenobiotic metabolism plays a role in activating many of the biofactors in food that act as cell signaling agents (Johnson 2008) (fig. 1). Although the strategic steps involved in xenobiotic metabolism are relatively few, an extraordinary aspect is its ability to tactically modify thousands of compounds with differing chemical properties for disposal and use.

Xenobiotic metabolism is divided into three phases. In phase I, enzymes (cytochrome P450 oxidases) catalyze new reactive or polar groups on compounds that range from substances created by the body's cells to dietary factors to xenobiotics. In phase II, transferase enzymes catalyze the conjugation of the phase I products to other compounds, which results in additional novel or unique properties. In phase III, these modified products are recognized by cellular efflux transporters and may be pumped out of cells for disposal via the kidney or bile. However, along the way some of these compounds can take on novel biological activities (see page 141).

Reactive oxygen species. ROS are products of normal metabolism, such as aerobic metabolism in mitochondria, and environmental stresses, such as excess exposure to ultraviolet light. ROS are important to many aspects of normal metabolism; for example, cells involved in the destruction of foreign substances may use ROS as a part of their arsenal (Bonekamp et al. 2009). However, excess ROS can be highly destructive to cell membranes, DNA and proteins, and can lead to the destruction of mitochondria and apoptosis if not controlled.

Phytochemicals can help protect against ROS, for example by inducing enzymes that reduce free radicals. In addition, when damage is severe, phytochemicals can induce signaling proteins such as Bcl-2 that lead to the turnover of

damaged mitochondria and apoptosis of their associated cells (Bonekamp et al. 2009; Shay and Banz 2005) (fig. 2).

Mitochondrial medicine

Mitochondria are responsible for most aerobic oxidative metabolic functions and the conversion of substances in the foods we eat into energy. The discovery of links between mitochondria and diseases has led to a new field of study called mitochondrial medicine. More than 40 genetic diseases are now recognized as involving mitochondria directly, affecting an estimated 1 out of 4,000 individuals. In addition, mitochondria also play a role in metabolic syndrome, obesity and diabetes, major chronic diseases affecting one-third to one-half of Western populations. A common feature of these diseases is that mitochondria, whether genetically defective or abnormally reduced in number, are unable to completely burn food and oxygen to generate energy. This in turn results in impaired oxygen utilization, which can lead to an increase in damaging ROS as side products as well as other toxic metabolic products (such as ammonia, which may not be efficiently incorporated into urea by metabolic pathways located in mitochondria) (fig. 2).

To understand exactly how phytochemicals may help protect against mitochondrial-related diseases, it is important to appreciate that highly regulated complex pathways have evolved to aid in their nutritional availability, disposal, and in some cases, biological activation. According to the free-radical theory, oxidative damage initiated by ROS is a major contributor to the functional declines associated with aging and many diseases. In this regard, many biofactors can act both in mitochondrial signaling and as ROS scavengers (see page 141). This exciting area of nutrition research provides evidence that California agriculture can play a beneficial role in oxidative metabolism, thus promoting good health.

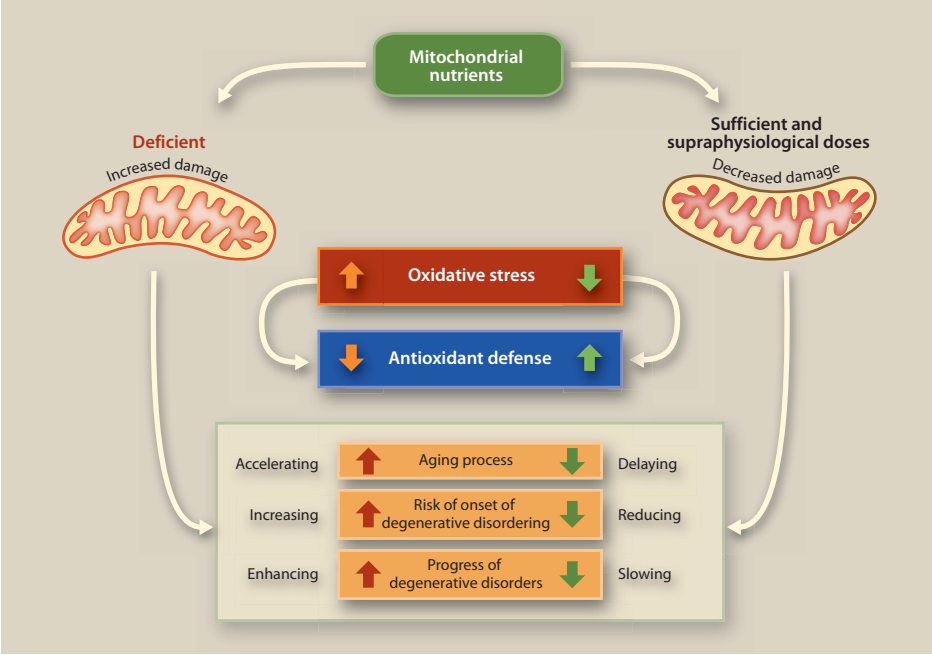

Fig. 2. Consequences of deficiency and sufficiency of mitochondrial nutrients. Deficiency can increase oxidative stress and decrease antioxidant defenses, thereby accelerating the aging process and increasing the occurrence of degenerative diseases. Conversely, sufficient mitochondrial nutrients decrease oxidative stress while fortifying antioxidant defenses, with the opposite effect.

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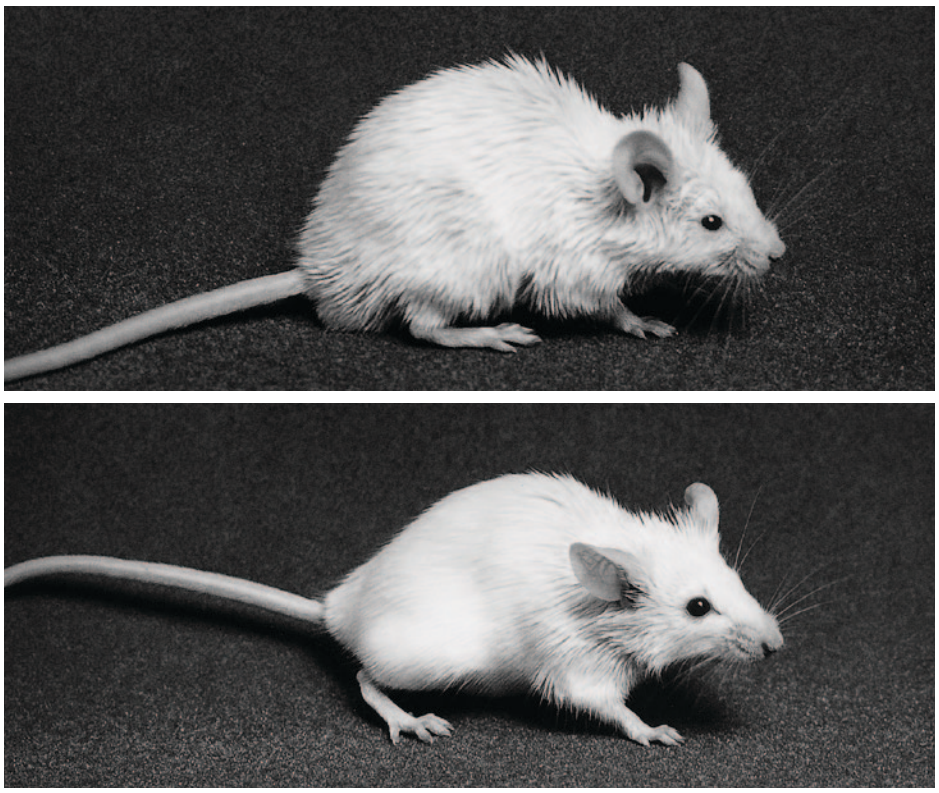
Biofactors in food promote health by enhancing mitochondrial function

by Sonia F. Shenoy, Winyoo Chowanadisai, Edward Sharman, Carl L. Keen, Jiankang Liu *and* Robert B. Rucker

Mitochondrial function has been linked to protection from and symptom reduction in chronic diseases such as heart disease, diabetes and metabolic syndrome. We review a number of phytochemicals and biofactors that influence mitochondrial function and oxidative metabolism. These include resveratrol found in grapes; several plant-derived flavonoids (quercetin, epicatechin, catechin and procyanidins); and two tyrosine-derived quinones, hydroxytyrosol in olive oil and pyrroloquinoline quinone, a minor but ubiquitous component of plant and animal tissues. In plants, these biofactors serve as pigments, phytoalexins or growth factors. In animals, positive nutritional and physiological attributes have been established for each, particularly with respect to their ability to affect energy metabolism, cell signaling and mitochondrial function.



Francine M. Steinberg, UC Davis

Biofactors in food play a role in enhancing mitochondrial function, thereby decreasing the risk of some chronic diseases. *Top,* a mouse that has been deprived of pyrroloquinoline quinone (PQQ), a ubiquitous bacterial compound found in fermented products, tea, cocoa and legumes. *Above,* a mouse fed a diet containing PQQ.

One of the most promising current areas of nutritional research focuses on plant compounds with positive health effects that extend beyond the functions of well-recognized essential vitamins, minerals and macronutrients (Rice-Evans and Packer 2003). Identifying such compounds and studying their mechanisms of action have been important activities of the UC Davis Center for Health and Nutrition Research (CHNR) (table 1).

Many of the human health–related biofactors in plants (e.g., various pigments, secondary metabolites and phytoalexins) have evolved to provide protective camouflage, repel predators or facilitate the transformation of specific wavelengths of light into chemical energy. Our food exposes us to thousands of such "xenobiotic" compounds (external chemicals that our body does not normally produce) that must be either eliminated or put to novel uses in the body. Many xenobiotics in foods can influence specific metabolic functions, acting as bioactive factors (biofactors). For example, epidemiological studies have shown a correlation between foods high in bioactive factors such as flavonoids and the decreased risk of chronic diseases such as vascular disease and gastrointestinal tract cancers (Rice-Evans and Packer 2003; Wallace 2011). While we still have only a rudimentary understanding of how these bioactive compounds work, they can have profound and often specific effects on mitochondria (see page 136).

Biofactors in food that enhance mitochondrial function include resveratrol, quercetin, procyanidins, catechins, hydroxytyrosol and pyrroloquinoline quinone (fig. 1). Although it is easy to overstate mitochondria-related health claims, a broad range of healthful attributes has been described and validated for each of these compounds.

Biological properties of resveratrol

Resveratrol is a stilbenoid (a type of natural polyphenolic compound) and a phytoalexin, a class of compounds produced by some plants when under attack by pathogens such as bacteria or fungi. It is found predominately in purple grapes and juice, red wine, peanuts and some berries (Xia et al. 2010). In animals, resveratrol also has potent biological properties that have been reported to range from cardio-protection to enhanced neuronal activity. As examples, resveratrol exposure has been associated with longer life spans in yeast and in short-lived invertebrates, such as *Caenorhabditis elegans*

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DOI: 10.3733/ca.v065n03p141

(a type of roundworm) and *Drosophila* (a genus of small fruit flies), which are often used as experimental models in aging studies (Bass et al. 2007). It has been proposed that the effect of resveratrol on lifespan in invertebrate models is due to the induction of phase 2 drug detoxification or the activation of AMP kinase (one of the signaling molecules important to mitochondriogenesis) (see page 136).

However, in more complex vertebrate animal models, the effects of resveratrol on longevity can be variable (Agarwal and Baur 2011). Resveratrol has little effect on the longevity of lean mice or mice whose caloric intake is restricted, but it does influence the expression of several genes associated with longevity (e.g., sirtuins) (Baur 2010). Dietary supplementation with resveratrol in mice appears to prevent age-related declines in cardiovascular function and can improve the ability of mice to respond to inflammation (Baur 2010; Baur et al. 2006). Moreover, treating mice with resveratrol significantly increases their aerobic capacity, as evidenced by increased treadmill running time and muscle fiber oxygen consumption, and improved mitochondrial function (Baur et al. 2006; Murase et al. 2009). Mice treated with resveratrol also accommodate better than nontreated mice to oxidative stresses induced by exposure to various chemical agents (Kovacic and Somanathan 2010).

Relatively little is known about resveratrol's effects in humans. Interest in exploring this compound's potential health benefits increased after speculation that

TABLE 1. Recent Center for Health and Nutrition Research publications examining plant phytochemicals

Phytonutrient	Publications
Pyrroloquinoline quinone	Chowanadisai et al. 2010; Rucker et al. 2009; Tchaparian et al. 2010
Epicatechins	Schroeter et al. 2006
Cocoa flavanols	Balzer et al. 2008; Keen et al. 2005; Lanoue et al. 2010; Ottaviani et al. 2011
Cocoa procyanidins	Keen et al. 2005
Lichee flavanols	Kalgaonkar et al. 2010
Hydroxytyrosol	Hao et al. 2010; Zhu et al. 2010
Monounsaturated fats	Rajaram et al. 2009

Glossary

Acrolein (propenal): A chemical that can play a role in the balance between cell proliferation and apoptosis. Acrolein has been used as a toxicant in experimental models to study age-related macular degeneration and neurological diseases.

Amino acids: Nitrogen-containing compounds that, when linked together, form proteins or function independently as precursors to chemical messengers and as intermediates in metabolism. The body cannot make some amino acids in sufficient quantities, making them essential in the diet.

AMP kinase (AMPK): An enzyme involved in regulating cellular energy homeostasis. Upregulation of AMPK results in a decrease in energy consumption pathways coupled with the creation of ATP or cellular energy.

Anti-inflammatory: A compound (drug or food substance) that acts to reduce inflammatory processes or responses to injury or infection. Potential markers of an anti-inflammatory response include changes in the levels of high-sensitivity C-reactive protein or various cytokines, intercellular mediator in the generation of an immune response.

Bioavailability: Measure of the ability for a particular tissue to absorb and utilize a nutrient. Factors potentially affecting bioavailability include the food matrix, chemical form or current nutritional status.

Cytochrome c oxidase: A mitochondrial protein involved in energy metabolism via the transfer of electrons derived from the oxidation of given substrates.

Epidemiological: Large-scale observational studies that provide relationships (e.g., correlations) between experimental variables and related factors (e.g., food and health).

Flavanol (a class of flavonoids): Contain multiple phenol groups in a specific configuration that is the chemical basis for antioxidant potential. Flavanols are different from flavonols; flavanols are building blocks for proanthocyanidins, which include many compounds and their polymers, such as epicatechin, catechin and tannins. Examples of flavanol-rich foods include tea, cocoa and grapes.

Flavonol (another class of flavonoids): Also contain multiple phenol groups in configurations that are the chemical basis for antioxidant potential. They differ from flavanols in part due to their 3-hydroxyflavone backbone. Examples of flavonol-rich foods include cranberries and onions.

Monomers, oligomers, polymers: A monomer is the fundamental chemical unit of a compound, which is capable of linking together to form polymeric chains. An oligomer is a molecule that consists of a specifiable number of monomers (usually less than five). Unlike a polymer, if one monomer is removed from an oligomer, its chemical properties are altered. A polymer is repeating units of a monomer, usually resulting in compounds of high molecular weight. For the epicatechin and catechins, extensive polymerization results in tannin formation.

mtDNA (mitochondrial DNA): Genetic code (DNA) located in the mitochondria.

Phytochemicals, phytonutrients: Plant compounds that may have health-protecting qualities such as antioxidant activity or immune system enhancement.

Proanthocyanidins (procyanidin). Polymer chains of flavanols, such as epicatechins and catechins. Proanthocyanidins have little color, whereas the monomer units of anthocyanidins are usually red.

resveratrol in wine could help explain the so-called French Paradox: Why the French seem to have a low incidence of coronary heart disease, despite diets that are relatively rich in saturated fats (Kopp 1998).

Daily dosage. Given the intense interest in resveratrol, surprisingly few human clinical trials have been conducted. However, it is known that a daily dose in the range of 300 to 3,000 milligrams is required to achieve a significant clinical response in people (such as bringing high levels of blood glucose back to normal) (Elliott and Jirousek 2008). About the same amounts of resveratrol are required for animals, relative to the unit of dietary energy utilized (when expressed as milligrams resveratrol per kilocalorie). For example, in mice genetically modified to express type 2 diabetes, normalizing blood glucose and plasma insulin requires 100 to 500 milligrams resveratrol per 1,000 kilocalories. Significant clinical responses in humans also require resveratrol in this range, about 200 to 800 milligrams per day when typical caloric intakes of 2,000 to 2,500 kilocalories are consumed.

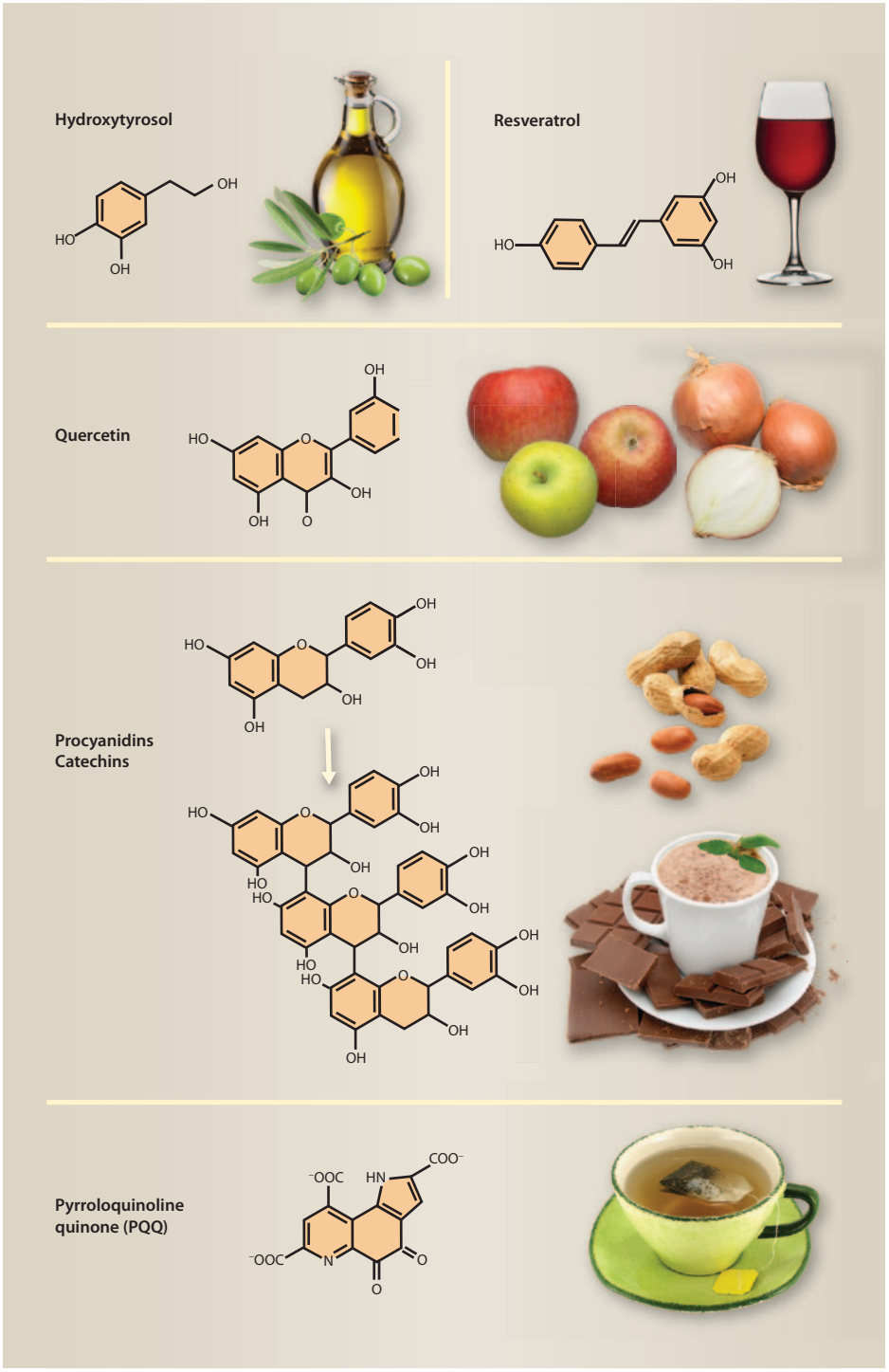
Food sources. The best food sources of resveratrol supply far less than the amounts required for significant clinical responses. For example, red wine, a major dietary source, seldom has more than 10 milligrams per liter, which raises questions about resveratrol-related health benefits based on wine consumption alone. As a consequence, researchers are currently pursuing questions related to resveratrol's bioavailability (ability of the body to absorb a substance).

Derivatives. Another area of active investigation is whether or not a derivative of resveratrol is more responsible than resveratrol itself for a given effect. Like other bioactive factors, once in the body resveratrol undergoes a number of metabolic transformations associated with fermentation, digestion and xenobiotic metabolism (see page 136). For resveratrol, the most abundant metabolites are resveratrol-3-O-glucuronide and resveratrol-3-sulfate (Xia et al. 2010).

Studies designed to elucidate resveratrol's actions have nevertheless been useful as research templates for the now hundreds of studies dealing with the role and function of biofactors in general. For example, it has been reasonable to ask whether given flavonoids, flavanols or other types of phenolic compounds provide cardiovascular or neurological protection by mechanisms similar or dissimilar to those for resveratrol. A principle example is studies on resveratrol's modes of action as they relate to the signaling important to mitochondriogenesis.

Quercetin and mitochondriogenesis

Quercetin is a flavonol (a subclass of flavonoid) widely present in fruits and vegetables, particularly onions and apples. Depending on one's food choices, the daily estimated intake of



Olive oil, Valentyn Volkov/iStockphoto; red wine, Serghei Platonov/iStockphoto; apples, Jack Kelly Clark; onions, Will Suckow; green tea, Borut Trdina/iStockphoto; peanuts, RedHelga/iStockphoto; chocolate, Kim Reinick/iStockphoto

Fig. 1. Parent chemical structures of biofactors in food, and common sources. Derivatives may also be present, created by food processing or as a result of xenobiotic metabolism (see fig. 2). All of the compounds highlighted are polyphenolic (with multiple aromatic -OH groups). Quercetin and procyanidins are examples of flavonols and flavanols, respectively.

quercetin ranges from 10 to 25 milligrams per day.

Mouse studies. Like resveratrol, quercetin has also been shown to induce mitochondrial biogenesis in mice (Davis et al. 2009, 2010). Mice fed quercetin have increased mitochondrial DNA, cytochrome c oxidase activity (a measure of mitochondriogenesis) and proteins key to mitochodriogenesis in both muscle and brain. Like resveratrol studies, these observations are supported by evidence of increased exercise tolerance following quercetin administration in mice.

Human studies. In humans, doses of quercetin similar to those for resveratrol are required to achieve an effect. In typical studies, usually 500 to 1,000 milligrams per day are given to elicit responses such as improvements in anti-inflammatory or antioxidant activity and mitochondrial-related functions (Davis et al. 2009). Quercetin also accumulates in mice and rat mitochondria, an observation consistent with reports that it can act as a protector of mitochondrial function. In humans, only short-term quercetin trials have been performed. In one study, 500 milligrams of quercetin twice per day for 7 days caused a modest but significant increase in endurance capacity and VO_{2max}, the maximum oxygen uptake during aerobic exercise (Davis et al. 2010). The 7 days of quercetin feeding was associated with an increase in VO_{2max} (about 4%) along with a 13% increase in bicycle ride time to fatigue (Davis et al. 2010).

For perspective, elite endurance athletes typically have a high VO_{2max}. Training has been shown to increase VO_{2max} up to 20% from an individual's baseline; thus a 4% increase from merely taking a supplement is an intriguing prospect. In competitive situations, even small differences may be of importance. In other studies, particularly those involving nontrained subjects, quercetin had little or no significant effect on endurance-related parameters (Curetin et al. 2009; Dumke et al. 2009).

Quercetin is readily absorbed from the gut, and absorption is dose-dependent in humans (Hollman et al. 1995). It has been estimated that around half the quercetin in onions is absorbed, along with appreciable amounts of important quercetin derivatives such as quercetin-3-rutinoside and quercetin aglycone. A single dose of quercetin leads to a rise in blood plasma levels of this biofactor, which peak about 2 to 6 hours after ingestion and gradually decline over a 24- to 48-hour period. This pattern of absorption is also typical of other members of the flavonoid family.


Pgiam/iStockphoto

Extra-virgin olive oil contains hydroxytyrosol, a biofactor associated with the healthful effects of a Mediterranean diet. Hydroxytyrosol has been shown to stimulate mitochondrial function.

Flavanols and health

Flavanols are another subclass of flavonoids and, like quercetin, can exist in a variety of chemical forms and derivatives (Rice-Evans and Packer 2003). Common flavanols include epicatechin and catechin, as well as their oligomers (polymeric chains of these compounds) that are often called proanthocyanidins or procyanidins. They are the predominant pigments in wine, tea and cocoa. The daily estimated intake of flavanols (both monomeric and polymeric forms) is higher than that for flavonols (i.e., compounds similar to quercetin) (fig. 1), amounting to 100 to 300 milligrams per day.

Despite relatively low amounts of catechin and epicatechin in foods, once absorbed and metabolized they have the potential to affect numerous important health parameters (Keen et al. 2005). The consumption of flavanol-rich cocoa and chocolate has been associated with the decreased risk of some vascular diseases, including cardiac events and strokes. Likewise, the daily consumption of foods containing flavanols has been associated with improvements in blood pressure (Desch et al. 2010; Janszky et al. 2009).

In the diet, the smaller or less polymerized forms of flavonoids (monomers and dimers) are more bioavailable than their larger oligomeric counterparts, such as tannins and procyanidins. Studies have


John Herschell

Flavanols are a kind of flavonoid, antioxidants in food that scavange free radicals that can damage cells. The consumption of flavanols — the predominant pigment in wine, tea and cocoa — has been associated with a lower risk of heart disease and stroke, and improved blood pressure.

shown that differences in the bioavailability of bioactive flavonoids are somewhat independent of the food matrix: monomer uptake is largely unaffected by the average diet, and consuming flavanols in water versus milk does not markedly affect their uptake and metabolism (Keen et al. 2005; Schramm et al. 2003).

Flavanols such as epicatechin are thought to influence mitochondrial function through both activation and control of programmed cell death (apoptosis). Epigallocatechin-3-gallate functions as an inhibitor of certain nuclear proteins important to the control (rate) of mitochondriogenesis and apoptosis.

While a good balance is apparently maintained between improved mitochondrial efficiency and orderly apoptosis in normal cells, epicatechins such as epigallocatechin-3-gallate may tip the balance toward apoptosis in certain cancer cell lines (Li et al. 2009). In addition, epigallocatechin-3-gallate has the potential to improve oxidative metabolism (glucose and fatty acid). Low concentrations of epigallocatechin-3-gallate (micromoles) both inhibit glucose production and reduce the expression of genes critical to producing glucose, increasing lipid oxidation and improving insulin sensitivity. At even lower concentrations (nanomoles), epigallocatechin-3-gallate is able to activate 5′-AMP-activated protein kinase (AMPK), a signaling factor that sets the stage for improved oxidative metabolism and fatty acid oxidation.

Generalizing from observations involving epigallocatechin-3-gallate, flavanols have the potential to improve oxidative metabolism as well as accelerate apoptosis in some lines of oncogenic (tumor-producing) cells.

Sources of hydroxytyrosol

Olive oil. The Mediterranean diet has been associated with a lower incidence of cardiovascular disease and certain cancers (Ordovas et al. 2007; Ortega 2006). Extra-virgin olive oil (produced by only physical and no chemical processing) is the principal source of fat in the Mediterranean diet and has been identified as an important contributor to the reduced mortality associated with this diet. Hydroxytyrosol, a phenolic constituent of olive oil, is considered one of the biofactors associated with the healthful effects of olive oil (Bendini et al. 2007; Bertelli 2007; Fito et al. 2007; Hao et al. 2010).

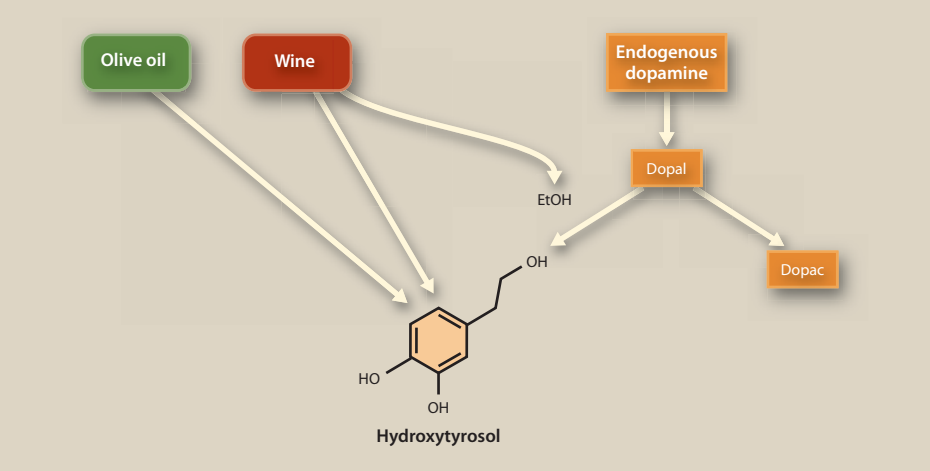


Fig. 2. Sources of the mitochondrial nutrient hydroxytyrosol associated with the Mediterranean diet. Primary nutritional sources are extra-virgin olive oil and wine. The alcohol in wine or distilled spirits may suppress the conversion of endogenous dopamine to dopac (3,4-dihydroxyphenylacetic acid) and redirect it, via a reductive pathway, to dopal (3,4-dihydroxyphenylacetaldehyde) and then to hydroxytyrosol.

Indeed, recent research indicates that hydroxytyrosol stimulates mitochondrial function — perhaps constituting a new mechanism by which olive oil lowers the risk of various diseases (Hao et al. 2010). Diabetes and obesity, for which cardiovascular disease is the most common and serious complication, both involve dysfunctional glucose metabolism. Because mitochondrial respiration plays a critical role in glucose metabolism, mitochondrial dysfunction likewise has been shown to be associated with these conditions.

Wine. Although extra-virgin olive oil is the principal source of hydroxytyrosol, this biofactor also occurs in wine — a second hallmark of the Mediterranean diet. Remarkably, it appears that alcohol in wine further boosts hydroxytyrosol levels in the body. A study comparing the effects of wine and olive oil consumption showed that even though the amount of hydroxytyrosol per serving of wine was less than in olive oil (about 0.3 to 0.4 milligrams per 200 to 300 milliliters wine, versus 1.7 milligrams in 50 milliliters oil), bodily amounts as assessed from urinary excretion measurements were 40% higher than could be attributed independently to either wine or olive oil intake (de la Torre et al. 2006).

This hydroxytyrosol increase, however, could be attributed to an additional effect of the wine. The ethanol in wine (and distilled spirits) apparently affects the pathways that produce the neurotransmitter dopamine, redirecting them to produce hydroxytyrosol instead (fig. 2). In a recent dietary study of 506 males who consumed a diet containing both olive oil and red wine, after adjusting for the contributions of olive oil and wine to hydroxytyrosol, urinary hydroxytyrosol levels increased linearly with alcohol intake (Schöder et al. 2009). In addition to the direct consumption of hydroxytyrosol in wine and olive oil, dietary alcohol can play an indirect role in raising levels of this beneficial biofactor.

The alcohol in red wine appears to boost hydroxytyrosol levels by redirecting the body to produce hydroxytyrosol instead of dopamine, a neurotransmitter.

In Mediterranean countries such as Italy and Greece, dietary intakes of olive oil polyphenols are estimated at around 10 to 20 milligrams per day with a daily intake of 25 to 50 milliliters of olive oil. If the olive oil is not chemically treated, about 0.5 to 1 milligram of the polyphenolic fraction is hydroxytyrosol or hydroxytyrosol derivative. A daily serving of one to two glasses of wine could add another 0.3 to 0.4 milligram of hydroxytyrosol (Granados-Principal et al. 2010).

PGC-1α and mitochondrial function. Heightened levels of hydroxytyrosol and other mitochondrial enhancers may improve mitochondrial function in two distinct ways. Each involves increasing the expression of a key mitochondriogenesis protein (PGC-1α), thereby reducing the risk of developing obesity, diabetes and cardiovascular disease. Increased expression of PGC-1α also indirectly improves mitochondrial function by interacting with and enhancing enzymes that protect cells against the oxidative damage caused by excessive levels of reactive oxygen species (ROS) (see page 136). For example, increased PGC-1α protects neuronal cells from oxidative stress. Mice lacking PGC-1α suffer greater drug-induced oxidative damage to brain and neural tissues (St-Pierre et al. 2006).

Healthful diets include an abundance of fruits and vegetables that contain these biofactors, providing measurable benefits to mitochondrial health and lessening some disease signs or symptoms.

Hydroxytyrosol is thought to be protective because it has the ability to increase PGC-1α and improve mitochondrial and cellular ROS-related functions. In cultured adipocytes (fat cells), hydroxytyrosol increases both PGC-1α expression and mitochondrial biogenesis (Hao et al. 2010). Furthermore, hydroxytyrosol has the same beneficial effects on the mitochondria of retinal pigment epithelial cells (derived from the retina) treated with the toxic chemical acrolein, a model for the slowly accumulating damage to the eyes that results in age-related macular degeneration (vision loss). This suggests that hydroxytyrosol may also be useful in treating or delaying age-related macular degeneration (Zhu et al. 2010). In summary, hydroxytyrosol could be potentially therapeutic in preventing and treating type 2 diabetes, obesity and age-related macular degeneration.

Ubiquity of bacterial PQQ

In addition to being a plant growth factor, the bacterial compound pyrroloquinoline quinone (PQQ) protects mitochondria from oxidative stress and promotes mitochondriogenesis (Rucker et al. 2009). Many soil bacteria provide plants with PQQ, which increases their height, flower number, fruit number and total fruit weight. Recent evidence suggests that compounds like PQQ are components of interstellar dust (Krueger et al. 2004), considered the precursor of organic materials in early life on the Earth. This ubiquitous presence of PQQ suggests that humans and animals are constantly exposed to this compound. However, although many bacteria make PQQ, most common intestinal bacteria (such as *Escherichia coli*) do not. Hence, an external source of PQQ may be important in sustaining its levels in human and animal tissues.

PQQ can play a role in pathways important to mitochondrial cell signaling, such as increasing production of the mitochondriogenesis protein PGC-1α (Chowanadisai et al. 2010; Tchaparian et al. 2010). In addition, PQQ deprivation results in a wide range of systemic responses, including growth impairment, compromised immune responsiveness and abnormal reproductive performance in experimental models. PQQ deprivation also alters lipid and amino acid metabolism, and reverses the effects of mitochondrial oxidation inhibitors.

Dietary sources of PQQ include many fermented products, wine, tea, cocoa and legumes. In animals, deficiency signs are observed at levels at or below 300 micrograms PQQ per kilogram of diet. Human dietary consumption may be estimated at 200 to 400 micrograms PQQ per day, similar to the dietary allowances for vitamins, biotin and folic acid. When mice are fed highly purified diets devoid of PQQ, repletion with 300 micrograms PQQ per kilogram to the diet results in improved growth, mitochondrial function and immune responsiveness. Expressed as a human equivalent, this amounts to 100 to 200 micrograms PQQ per day. In recent unpublished human studies, 10 to 20 milligrams PQQ per day resulted in changes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Legumes are an important source of PQQ, a growth factor provided to plants by soil bacteria. Such dietary biofactors stimulate mitochondriogenesis (the conversion of glucose into energy in cells) or inhibit apoptosis (cell death), ultimately improving metabolic health.

in triglyceride levels and measures of antioxidant and anti-inflammatory potential in plasma. However, such amounts are only obtained with supplements and were chosen somewhat arbitrarily. Although about 20 times more than what might be present in the diet, this is nevertheless 20 to 50 times less PQQ than the amounts of quercetin or resveratrol needed to elicit similar effects.

Mitochondria and diet

Mitochondria are responsible for oxidative metabolism and converting substances from the foods we eat into energy for essential functions. Dietary biofactors that optimize mitochondrial function work by either activating mitochondriogenesis or inhibiting apoptosis. This in turn improves oxidative defenses and has a major impact on efficient energy utilization by cells. Healthful diets include an abundance of fruits and vegetables that contain these biofactors, providing measurable benefits to mitochondrial health and lessening some disease signs or symptoms; California fruits and vegetables can have an enormous impact on health, ranging from the normalization and control of blood pressure to improving blood lipid and sugar regulation.

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RESEARCH ARTICLE

Blue oak stump sprouting evaluated after firewood harvest in northern Sacramento Valley

by Richard B. Standiford, Douglas McCreary, Sheila Barry *and* Larry Forero

California's hardwood rangelands, an oak-dominated woodland system, cover 10 million acres. More than 80% of these lands are privately owned, with two-thirds grazed by domestic livestock. Public concerns about long-term damage to habitat in areas harvested for firewood — particularly in the northern Sacramento Valley — led to this study of resprouting, to assess long-term trends in oak cover following harvesting and the potential of sprout (coppice) management to sustain woodlands. In field surveys on 103 sample plots at 19 ranches where oak firewood was harvested, we found that 54% of all oak stumps resprouted. Stump diameter, herbicide application, overstory crown cover percentage, and slope and aspect were significant variables in models developed to assess the probability of stump sprouting. Ten-year sprout height and crown growth models were developed, and livestock grazing, residual overstory canopy, herbicide treatment and stump diameter were found to be significant variables. These models can be used to predict stand development following firewood harvest and can be integrated with forage growth, wildlife habitat and residual tree growth models.

Oak woodlands support thousands of native plant and animal species, improve water quality, and provide aesthetic and recreational opportunities. More than 80% of California's oak woodlands are under private ownership, utilized for livestock grazing and, to a lesser extent, firewood harvesting. *Above*, a harvested study site in Tehama County.

California's hardwood rangelands cover 10 million acres, or 10% of the state (Bolsinger 1988). The overstory tree canopy is predominantly oaks (*Quercus* spp.) with an understory of exotic annual grasses and forbs, and occasional native perennial grasses (Bartolome 1987; Standiford 2001). The five major oak species occurring on hardwood rangelands include three deciduous white oaks — blue oak (*Quercus douglasii* Hook. & Arn.), valley oak (*Quercus lobata* Nee) and Engelmann oak (*Quercus engelmannii* Greene); and two evergreen oaks — coast live oak (*Quercus agrifolia* Nee) and interior live oak (*Quercus wislizeni* A. DC.).

Since European settlement of California, oak woodlands have been managed primarily for livestock production. These areas have high public value because of their rich species abundance, with over 300 vertebrate, 5,000 invertebrate and 2,000 plant species (Garrison 1996). Oak woodlands also contribute greatly to water quantity and quality, outdoor recreation and aesthetics. Over 80% of oak woodlands are in private ownership (Huntsinger et al. 1997).

Firewood has been harvested from oak woodlands since the late 1700s (Pavlik et al. 1991), and woodcutting continues to occur throughout the state. The primary reasons are to generate additional cash flow from the sale of firewood, primarily associated with periods of low livestock prices; to increase forage production; and to improve habitat for game species (Standiford and Howitt 1993). Aerial surveys in the early 1990s showed that firewood harvesting was concentrated in Shasta and Tehama counties, in the northern Sacramento Valley. These two counties contain about 5% of the state's total hardwood rangelands but produced over 40% of all firewood harvested between 1988 and 1992 (Standiford et al. 1996). The two primary species harvested were blue oak and interior live oak.

Basal or stump sprouting following wildfire or tree harvest is common for many oak species and is the primary mechanism for oak regeneration in this study area. (A separate study, not reported here, showed limited regeneration from seedlings derived from acorns.) Numerous studies of the U.S. East, Midwest and South have evaluated the sprouting phenomenon, including the relationship between sprouting and harvest season (Geisinger et al. 1989; Johnson 1977;

Online: http://californiaagriculture.ucanr.org/landingpage.cfm?article=ca.v065n03p148&fulltext=yes
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Roth and Hepting 1943); the relationship between tree size and sprouting response (Clark and Liming 1953; Dey et al. 2008; Johnson 1975; Lockhart and Chambers 2007; Roth and Hepting 1943); the sprouting capacity of different oak species (Johnson 1979; Liming and Johnston 1943; Weigel et al. 2006) and age classes (Rogers and Rogers 1959); and the effect of site quality (Weigel et al. 2006; Weigel and Peng 2002).

Little information has been available about native oak sprouting in California. Leiberg (1902) observed aggressive stump sprouting of oaks in the Sierra Foothills following extensive harvesting and fires. Several years later, Jepson (1910) also noted extensive sprouting of small blue oak trees harvested to increase pastureland. On coastal foothill woodlands, Longhurst (1956) found that evergreen oaks sprouted better than deciduous oaks, and that there was a tendency for sprouting to decline with age for deciduous species.

The sprouting of native California oaks plays a vital ecological role. Plumb and Gomez (1983) noted that sprouting of dormant buds was the most important adaptation of oaks to fire. McClaren and Bartolome (1989) determined that a large percentage of existing trees in their study area became established within a year of fire, suggesting that many trees in the stand originated from sprouts. Mensing (1992) came to a similar conclusion in an examination of the age class distribution of blue oaks in the Tehachapi Mountains, reporting that top killing of previously established seedlings and saplings by frequent, relatively intense fires resulted in pulses of regeneration.

Finally, McCreary et al. (2008) conducted a series of experiments at five field sites throughout the state to examine the relationship between blue oak sprouting and season of harvest, the height of cut stumps and protection from browsing. In spite of the common belief that blue oak is a relatively poor sprouter, sprouting at most study locations was quite vigorous. In addition, the time when trees were cut had little influence on sprouting, while stump height and protection from browsing were exceedingly important.

Our study was designed to address several unknown areas of hardwood rangeland sustainability. The primary objectives were to develop baseline information on oak sprouting following harvesting and to develop predictive models of sprout survival and growth for use by resource managers and landowners.

Study of harvested woodlands

We identified 40 private hardwood rangeland owners in Shasta and Tehama counties who had conducted firewood operations in the previous 10 years. For each operation, the location, elevation, soil type, cover type (Allen et al. 1991) and years since harvest were collected. From this list, a random selection of owners was made, stratifying by county and number of years since harvest. Nineteen study sites were selected, representing approximately half of all identified locations. Twelve study sites were selected in Tehama County and seven in Shasta County, approximately proportional to the relative oak woodland acreage in each county. Additional information was collected from each study site on types of grazing practices (season of use, type of animal), as well as other management factors (use of herbicides on harvested stumps, use of prescribed fire, supplemental feeding and range fertilization).

Five to seven 0.1-acre circular plots were randomly established at each study location. This plot size has been used in previous studies of blue oak stand structure and adequately captured site variability (Standiford 1997; Standiford and Howitt 1988; Standiford et al. 1997). An area was selected if there was at least one stump within the plot. The slope, aspect and elevation of each plot were recorded. Residual dry matter of the grass and forb species was estimated using a photo series for California annual grasslands (Clawson et al. 1982). For each stump within the sample plots, information was collected on species, stump height, stump diameter, presence and number of sprouts, dimensions of the sprout crown, height of the tallest sprout and diameter at breast height (DBH = 4.5 feet) of the sprout if it was tall enough.

Species, total height, basal diameter, DBH and crown dimensions were recorded for all uncut, living and dead trees on each plot. Trees inside the plots having special wildlife characteristics (snags, granary trees, trees with cavities, etc.) were also noted and used in the separately reported wildlife survey (Garrison and Standiford 1997). At each location, the oak site index was calculated using the height and diameter of each dominant tree (Standiford and Howitt 1988).

In a random direction from the plot centers, we also ran a 100-foot-long line transect and a 100-foot-by-4.36-foot (0.01 acre) belt transect. Within the belt transect, all seedlings were recorded by height class. The line transect was used to record the percentage cover of understory shrub species, rocks and brush piles, and the amount and size of dead and down woody debris.

The preharvest and postharvest tree stand structure was constructed for

Researchers studying the role of stump resprouting in the regeneration of harvested native oaks measured a 100-foot-long line transect.

Santa Clara County natural resource advisor Sheila Barry (standing) and staff research associate Jerry Tecklin examined sprouts from a harvested blue oak stump.

each sample plot. A relationship was developed to describe total tree height, DBH and canopy area as a function of the basal diameter of each living tree for each ranch. This relationship was used to develop the preharvest DBH, total height and canopy area for each stump in the sample plot. Individual tree volumes in cubic feet per acre were calculated from equations developed by Pillsbury and Kirkley (1987). The sum of the individual trees before and after harvest gave the tree basal area, canopy cover and tree volumes of each plot and provided a measure of the intensity of the tree harvest.

Statistical models developed

All study areas were predominantly blue oak woodlands, with a scattering of interior live oaks. We measured 1,194 individual stumps, of which 1,150 were blue oak. Of the 517 individual, living, residual overstory trees measured, 482 were blue oaks. Table 1 shows the intensity of the tree harvest in the study area. Statistical models were developed for the probability of stump sprouting, sprout height growth and sprout crown growth. Because of the limited sample size for interior live oak, the results were limited to blue oaks.

Sprouting percentage. We found a strong negative correlation between sprouting probability and stump diameter (fig. 1). Of 1,194 stumps in this study, 54% sprouted after harvest. The hypothesis tested was that stump-sprouting probability is a function of site and management factors, tree size (DBH) and residual overstory tree cover (equation 1). The predicted value of the dependent variable, SPROUT, can be interpreted as the probability of a particular stump sprouting.

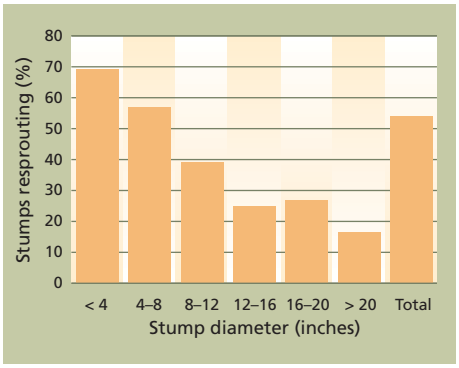

Fig. 1. Relationship between stump diameter and sprouting percentage for cut oak stumps in Shasta and Tehama county study areas.

(1) SPROUT = f (site factors, management factors, stump size, species, residual tree cover)

Since the dependent variable, SPROUT, is a discontinuous variable having a value of 0 (no sprouts) or 1 (successful sprouter), logistic regression was used to develop the statistical model (Wonnacott and Wonnacott 1979).

None of the other abiotic site factors (soils, oak site index), stand factors (brush cover, rock cover) or management factors (prescribed fire, grazing) were significant (equation 2; table 2). The negative sign on the coefficients for DBH, herbicides, crown cover percentage and slope percentage shows that these were negatively correlated with the probability of stump sprouting. Southerly slopes had a higher probability of sprouting than northerly slopes.

Height growth. With the data from the sample sites spanning a 10-year period following harvest, it was possible to develop a model of 10-year sprout height growth. Since the functional form of sprout height growth was not known, the Box-Cox transformation (λ) was utilized rather than imposing a linear or logarithmic form (Zarembka 1974).

The results of the analysis for blue oak sprout height growth are shown in equation 3. Other variables measured in the study, including site index, soil series, brush cover and prescribed fire were

TABLE 1. Average pre- and postharvest oak woodland stand structure on 19 study sites in Tehama and Shasta counties following firewood harvest

Characteristic	Preharvest	Postharvest
Oak canopy cover (%)	61 (28)*	15 (14)
Volume (cubic feet per acre)	737 (491)	259 (305)
Trees per acre (number)	219 (544)	49 (63)
Basal area per acre (square feet)	51 (29)	16 (17)

* Standard deviations in parentheses.

EQUATION 2. Logistic equation developed to evaluate sprouting probability

$$\text{SPROUT} = \frac{1}{1 + e^{-(1.4606 - 0.06799 \times \text{DBH} - 0.6581 \times \text{HERB} - 0.77631 \times \text{CC} - 0.00937 \times \text{SL} + 0.35120 \times \text{ASP})}}$$

TABLE 2. Logistic regression of blue oak sprouting probability

Variable	Coefficient (significance)
SPROUT = probability of stump sprouting (0 = no sprout, 1 = sprout)	(dependent variable)
Constant	1.60510 (***)
DBH = preharvest diameter at breast height in inches	–0.17186 (***)
HERB = cut stump treated with herbicide (1 = yes, 0 = no)	–0.73341 (***)
CC = postharvest overstory crown cover (expressed as decimal between 0 and 1)	–1.18680 (*)
SL = slope percent	0.00870 (*)
ASP = aspect of slope (1 = south, 0 = north)	0.22413 (**)
Equation significance	(***)
Proportion correct predictions	0.68

* = significant at 0.10 level; ** = significant at 0.05 level; *** = significant at 0.01 level.

not significant. This can be transformed directly to the functional form shown in equations 4 and 5 to calculate predicted blue oak sprout height in feet (table 3).

Oak sprout height growth was quite vigorous in the sample areas, with the tallest sprout per clump averaging 8.2 feet, 9 years after harvest. The positive sign of the grazing coefficient indicates that the sprout height growth in grazed woodlands was higher than in ungrazed woodlands. The reason for the higher height growth on the grazed sites in this study area will need additional study to determine if seasonal grazing practices in the northern Sacramento Valley positively affect microsites to favor sprout growth, or if there is some other site factor correlated with grazing that was not included in this study.

Other studies have shown that voles can girdle blue oak seedlings and saplings (Tecklin et. al 2002; Tecklin and McCreary 1993). In both these studies, reducing ground vegetation via herbicides or grazing significantly limited vole damage to planted oaks. However, the long-term impacts of grazing may offset initial reductions in vole damage. A 19-year study of sprouting that compared grazed and ungrazed plots found significant increases in damage on grazed plots; cattle and deer apparently browsed sprouts so much that sprout survival, height and diameter were significantly lower (McCreary et. al 2008).

In the current study, the positive effects of grazing may be partially explained by reductions in initial vole damage, but it is still hard to reconcile these finding with those of McCreary et. al (2008). Additional research is needed to further clarify the relationship between grazing and sprout performance. The negative coefficient for overstory crown cover shows the competitive effect of overstory trees on understory sprout growth. Areas that received operational stump applications of herbicides had lower height growth than those where no herbicides were applied.

Crown growth. The same Box-Cox transformation process was used to develop a model of the crown area of the sprout cluster following harvest. Equation 6 shows the significant variables for the Box-Cox equation. Other variables measured in the study, including site index, soil series, brush cover and

EQUATIONS 3–7: Blue oak sprout height and crown growth

(3) Variables in sprout height analysis:

$$HT_i^{(\lambda)} = a_0 + a_1AGE^{(\lambda)} + a_2HERB^{(\lambda)} + a_3GRAZE^{(\lambda)} + a_4CC^{(\lambda)} + a_5SPEC^{(\lambda)}$$

(4) Transformation to sprout height in feet:

$$\frac{HT^{(\lambda)} - 1}{\lambda} = 0.5958 + 0.3811\,\frac{AGE^{0.55} - 1}{0.55} - 0.4509\,\frac{HERB^{0.55} - 1}{0.55} + 0.3229\,\frac{GRAZE^{0.55} - 1}{0.55} - 0.9915\,\frac{CC^{0.55} - 1}{0.55}$$

(5) $$HT = (2.0657 + 0.3811AGE^{0.55} - 0.4509HERB^{0.55} + 0.3229GRAZE^{0.55} - 0.995CC^{0.55})^{(1/0.55)}$$

(6) Variables in crown growth model:

$$CROWN_i^{(\lambda)} = a_0 + a_1AGE^{(\lambda)} + a_2HERB^{(\lambda)} + a_3DBH^{(\lambda)} + a_4GRAZE^{(\lambda)} + a_5CC^{(\lambda)}$$

(7) Transformation to crown growth in feet:

$$CROWN = (3.6656 + 0.7609AGE^{(0.37)} - 0.5847HERB^{(0.37)} + 0.0761DBH^{(0.37)} + 0.5858GRAZE^{(0.37)} - 3.1357CC^{(0.37)})^{(1/0.37)}$$

TABLE 3. Box-Cox regression of blue oak sprout height growth and crown area

Variable	Sprout height	Crown area
	coefficient (significance)	
Dependent variable	HT = total sprout height, age *i*	CROWN = total crown area in square feet, age *i*
a_0 = constant	0.5958 (***)	0.9947 (**)
λ = Box-Cox transformation coefficient	0.55	0.37
AGE = age of sprouts in years since harvest	0.3811 (***)	0.7609 (***)
HERB = cut stump treated with herbicide (1 = yes, 0 = no)	–0.4509 (**)	–0.5847 (***)
GRAZE = livestock grazing present (1 = grazed, 0 = ungrazed)	0.3229 (***)	0.5858 (**)
CC = postharvest overstory crown cover (expressed as decimal between 0 and 1)	–0.9915 (**)	–3.1357 (***)
DBH = preharvest diameter at breast height in inches	NA	0.0761(**)
R^2	0.58 (***)	0.52 (***)

* = significant at 0.10 level; ** = significant at 0.05 level; *** = significant at 0.01 level.

Statistical models were developed to predict the probability of resprouting under various conditions, including stump diameter, site slope and aspect, and herbicide treatments.

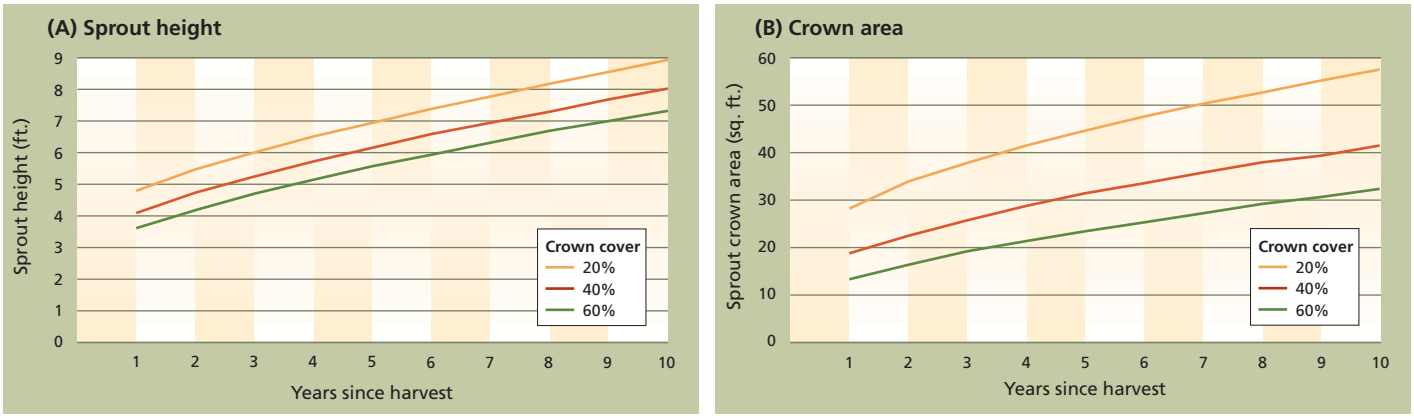

Fig. 2. Predicted blue oak (A) sprout height and (B) crown area in northern Sacramento Valley (10% south slope, grazed, no herbicides) for 20%, 40% and 60% crown cover (overstory canopy).

prescribed fire were not significant (table 3). Equation 7 uses the same transformation process as the sprout height model above, to provide a direct estimate of individual sprout cluster crown area in square feet.

The results for crown growth are similar to those for height, with positive effects from areas with grazing and tree size (represented by preharvest DBH), and negative effects from the operational herbicide stump treatment and overstory canopy competition.

Regenerating oak woodlands

The models that we developed provide tools that can be used to evaluate the development of stand structure on hardwood rangeland following harvest, based on site and tree characteristics. The results can predict the probability of stump sprouting, as well as the height and crown development of sprouts following tree harvest, and can be used to assess the impact of oak harvest on forage production (Frost et al. 1997) and wildlife habitat (Garrison and Standiford 1997). The models can also be used in conjunction with overstory tree growth models (Standiford 1997) to provide whole-stand dynamics of managed hardwood rangelands.

In general, smaller trees have relatively good sprouting potential, while larger trees have poorer capacity for sprouting.

Residual overstory canopy cover has a larger effect on crown area than height (fig. 2). Predicted sprout height is approximately 17% taller 10 years after harvest with an overstory of 20% (8.9 feet) than of 60% (7.3 feet). Crown area after 10 years is more than 44% greater with an overstory of 20% (57.7 square feet per stump cluster) than of 60% (32.3 square feet per stump cluster).

Predicting sprout development

The relationships developed in this study can be used to predict sprout development for a blue oak thinning prescription (see sidebar, page 153). The sprouting probability, height growth and crown development assessed can help to provide an assessment of the adequacy of stump sprouting as an effective regeneration tool.

This study showed a lack of significance for the oak site index — a standard measure of forest productivity — based on a height-diameter relationship (Standiford and Howitt 1988). This was surprising and merits further study. To refine future work on the importance of site quality, it may be necessary to rely on a more detailed evaluation of the soil and rainfall characteristics of a site, or the construction of more typical height-age site index curves.

Managers will need to consider grazing seasons and intensity when developing plans that allow for adequate oak regeneration.

continued on page 154

Predicting sprout development following thinning

The results of this study can be used to predict sprout development in a thinned blue oak stand. Our example stand is on a grazed property, where the objectives of thinning are to provide good forage for a cow-calf livestock enterprise, improve habitat for fee hunting, provide cash flow through the sale of firewood products and maintain long-term biodiversity (Standiford and Tietje 1990) (table 4). The harvest is designed to remove approximately 50% of all stems less than 12 inches diameter at breast height (DBH = 4.5 feet) and to retain all trees 12 inches and larger. All cut stumps will be allowed to resprout. This example stand is on a south aspect with an average slope of 10%.

An average of approximately 135 stems per acre will be harvested, representing the removal of just over 40% of the basal area of the initial stand, reducing the crown cover from 45% to 26% (table 4). The harvest removes 417 cubic feet per acre, representing just under five cords per acre of firewood.

Equations developed in this study can be used to predict the probability of stump sprouting, sprout height growth and canopy development (table 5). Equation 2 is used to estimate the probability of stump sprouting for the different-size trees harvested (see page 150). The 2-inch trees harvested have a 79.7% probability of sprouting, and the 10-inch trees have a 69.8% probability. The average sprout height after 10 years for this grazed stand, with no herbicides applied, is calculated using equation 5 (see page 151) and is estimated to average 8.6 feet. The predicted crown development of the stump sprouts is calculated from equation 7 (see page 151) for the various tree sizes harvested. The total crown area of stump resprouts is just over 5,144 square feet per acre, representing 11.8% canopy cover.

These projections of stump sprouting and sprout development can be evaluated to determine if adequate regeneration is anticipated to meet the landowner's objectives, or if the harvest prescription needs to be modified or additional tree planting needs to be considered.

In Shasta County, harvested firewood is stacked in the study area.

TABLE 5. Sprout development for cut trees (grazed, no herbicide, 26% residual overstory cover, 10% south slope)

DBH class, inches*	Stumps	Probability of sprouting†	Stumps with sprouts	Avg. crown area per class	Crown area in class‡
	number/acre		*number/acre*	*square feet/acre*	
2	4.44	0.799	3.55	49.25	174.90
4	28.89	0.776	22.43	50.16	1,124.95
6	53.33	0.752	40.10	50.82	2,037.67
8	35.56	0.726	25.80	51.35	1,324.84
10	13.33	0.698	9.30	51.81	481.98
Total	135.56		101.18		5,144.35§

* Diameter at breast height (4.5 feet).
† From equation 2; average sprout height at 10 years = 8.6 feet (from equation 5).
‡ From equation 7.
§ Sprout layer crown cover percentage at 10 years = 11.8%

TABLE 4. Inventory data for blue oak thinning project in California's northern Sacramento Valley*

DBH class, inches†	Trees			Basal area			Volume		
	Initial	Harvest	Residual	Initial	Harvest	Residual	Initial	Harvest	Residual
	number/acre			*square feet/acre*			*cubic feet/acre*		
2	6.67	4.44	2.22	0.27	0.16	0.11	1.32	0.76	0.56
4	57.78	28.89	28.89	6.23	3.06	3.17	47.35	23.25	24.09
6	104.44	53.33	51.11	20.51	10.49	10.02	223.52	111.65	111.87
8	71.11	35.56	35.56	24.36	12.31	12.05	330.61	168.45	162.16
10	26.67	13.33	13.33	14.06	12.18	1.89	221.27	112.90	108.37
12	8.89	0.00	8.89	7.00	0.00	7.00	120.17	0.00	120.17
14	2.22	0.00	2.22	2.11	0.00	2.11	43.72	0.00	43.72
16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	2.22	0.00	2.22	4.38	0.00	4.38	110.27	0.00	110.27
20	2.22	0.00	2.22	4.95	0.00	4.95	147.66	0.00	147.66
22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	2.22	0.00	2.22	6.58	0.00	6.58	153.87	0.00	153.87
Totals	284.44	135.56	148.89	90.44	38.20	52.24	1,399.77	417.02	982.75

* Crown cover: initial = 45%; harvest = 19%; residual = 26%.
† Diameter at breast height (4.5 feet).

continued from page 152

Our sample size of interior live oak was insufficient in the study area to make conclusive statements about its sprouting performance. This will require future studies focused directly on interior live oak. Furthermore, this work was only able to evaluate the presence or absence of grazing as a management strategy. As mentioned, McCreary et al. (2008) showed that herbivory of unprotected blue oak sprouts from both livestock and wildlife had a major effect on sprout survival and growth when compared to ungrazed controls. The positive effect of the grazing coefficient in this study contrasts with McCreary et al.'s (2008) statewide, controlled experiment. Further refinement of the grazing strategy, in terms of season and intensity of use, is necessary to determine an appropriate grazing prescription that provides for adequate stand regeneration.

In general, smaller trees (less than 12 inches DBH) have relatively good sprouting potential, while larger trees have poorer capacity for sprouting. Since sprouting vigor decreases for larger tree sizes, the retention of larger trees (over 12 inches DBH) for habitat value (mast production/acorns used as a wildlife food source, cavity sites, snag and woody debris recruitment) is appropriate. Where large trees are removed, it may be necessary to plant oak seedlings to ensure the replacement of harvested trees.

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Low hybrid onion seed yields relate to honey bee visits and insecticide use

by Rachael F. Long *and* Lora Morandin

Onion thrips, previously considered of minor importance to hybrid onion seed production in California, vector the newly introduced iris yellow spot virus, a serious pathogen of onions that can cause significant yield losses. Insecticide use to control onion thrips has increased in onion seed fields, coincident with a steep decrease in yields, especially in Colusa County. We examined a number of possible contributing factors and found a strong positive correlation between honey bee activity and onion seed set, indicating that a lack of pollination may be contributing to the reduced yields. In addition, honey bee visits to onion flowers were negatively correlated with the number of insecticides applied per field and field size. Reduced onion seed yields in recent years could be associated with the increase in insecticide use, which may be repelling or killing honey bees, important pollinators of this crop.

Onion seed yields have declined significantly in recent years in Colusa County, where the crop plays a small but important role in the rural economy. *Above,* a honey bee visits an onion umbel.

The production of hybrid onion seed in California has traditionally exhibited variable and unpredictable yields, but in recent years they have declined markedly (fig. 1). This additional uncertainty around the crop makes it difficult for the industry to contract with growers and maintain inventory for onion seed orders.

Hybrid onion production for a seed crop involves encouraging onion bulbs to flower, via a combination of factors including variety, planting date and temperature. Onion seed is primarily grown in Colusa County and the Imperial Valley on about 2,000 acres, although some production in Colusa County has recently shifted to other regions because of the yield declines. The market for seeds of red, yellow and white onion varieties extends worldwide. The value of the seeds is $12 million to growers, according to agricultural commissioner county crop reports, and they generate an additional $40 million in subsequent retail sales. While clearly a specialty, small-acreage crop, onion seed production is important to the rural economies in California where onion seed is primarily grown.

The causes of yearly variability in the production of seed onions are unknown. In the absence of any significant changes in landscapes, agronomic practices, acreage or onion seed varieties during the past 10 years that would explain yield variations and the recent dramatic drop-off in yields, we turned our attention to a lack of pollination and seed set. To produce hybrid seed, distinct male (male fertile) and female (male sterile) onion lines are planted in the same field. Honey bee (*Apis mellifera*) hives are brought in to the fields for cross-pollination at a recommended rate of 10 to 12 hives per acre (Voss et al.

The strong positive correlation between honey bee activity and onion seed set indicates that a lack of pollination may be contributing to reduced yields.

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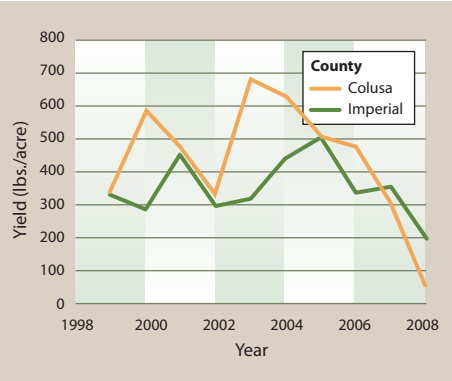

Fig. 1. Hybrid onion seed yields in Colusa and Imperial counties, 1999–2008. Source: Agricultural commissioner county crop reports.

1999). Wild bees also can be important; if they are present and visiting flowers, higher seed set results (Parker 1982).

One cause for poor recent seed harvests may be the increased use of insecticides to control onion thrips (*Thrips tabaci*), the number used possibly affecting the activity of wild bees and honey bees. Onion thrips were previously of minor importance in onion seed production. However, iris yellow spot virus is a new pathogen for California onions that vectors onion thrips, and it can cause significant onion seed yield losses if left unmanaged (Gent et al. 2004). We examined the activity of honey bees and other insect pollinators in commercial onion seed fields to understand the causus of the yield declines in recent years.

Onion seed fields

Our research was conducted in May and June 2009 in 13 commercial hybrid onion seed production fields in Yolo and Sacramento counties, which varied in size from 6 to 50 acres. Nine of the study fields were planted with different female cultivars, and nine fields had different male cultivars. Within each field, six monitoring sites were established along two transects at about 10, 200 and 500 feet from the field edges, where honey bee hives were placed, so we could conduct analyses with distance from hives as a factor.

At each of the six sampling sites per field, we observed the numbers and types of insects visiting onion flowers that were potential pollinators of onions. These included honey bees, wild bees (mostly *Halictus* and *Bombus* spp.), flies (Syrphidae and other Diptera) and wasps (Hymenoptera). Five-minute observations were made in each of two 1-square-meter quadrats, mostly between 10 a.m. and 5 p.m., with one observation in the female row and the other in the male row. These observations were made every 3 or 4 days for a total of 10 minutes per site, or 60 minutes per field. We began our observations of insect activity in each field when the female umbels (flower heads) were at about 5% flower bloom and ended when the flowers finished blooming and seeds were set, about 21 days later. Weather conditions were recorded during monitoring, and pollinators were observed when temperatures averaged about 75ºF to 85ºF (25ºC to 30ºC).

To assess onion seed yields relative to insect pollinator activity, we collected eight onion umbels at each of the six sites per field, for a total of 48 umbels per field. These were placed individually in labeled paper bags and dried. Seeds were then threshed and counted to obtain average yield data (number of seeds per umbel).

One to two days before nectar collection, plastic bags were placed over four male and four female umbels per field that were approximately the same size and at the same stage of bloom (Silva and Dean 2000). Bagging was done to minimize nectar evaporation and prevent honey bee foraging. Paper bags were placed over the plastic bags to minimize temperature increases. Nectar was collected from each umbel using 10-microliter capillary tubes and dispensed onto a refractometer to measure the percentage of sugar solids.

Ground mapping was done once around each field during midbloom to determine whether other preferred floral resources were available to honey bees, perhaps luring them away from onion flowers. Observers drove a 1-mile radius around each field, mapping out all other crops, particularly noting any crops in which bloom was concurrent with onion bloom. Observers also recorded any significant areas (more than 50 square feet) of natural or weedy vegetation that had plants in bloom.

The insecticides used on our field production sites for thrips control included spinosad, spinetoram, methomyl, cypermethrin, lambda-cyhalothrin and sodium tetraborohydrate decahydrate. The number of insecticides applied per field ranged from one to seven, including

Edwin Reidel

Honey bee hives are brought in to onion fields to pollinate the crop. Above, Yolo County farm advisor Rachael Long conducts observations of onion pollinators.

Davis Enterprise

The researchers analyzed the relationships between field size, number of insecticides applied and honey bee visits. They also monitored seed yields and mapped nearby floral resources. Above, Yolo County field assistant Mark Kochi examines onion umbels.

tank mixes, with all pesticides applied prebloom (per agricultural commissioner county pesticide use reports). The number of hives per acre ranged from four to 14, with the exception of one field that had resident hives at 42 per acre (local beekeepers, personal communication).

In order to assess which factors were most predictive of honey bee activity, we performed a best subset regression analysis in R (HH package). Honey bee activity was the response variable, and the predictor variables were sugar solids in male and female flowers, hives per acre, number of bloom-applied pesticides (only fungicides were used during bloom), total number of insecticides applied, total number of systemic or translaminar (moving into the plant) insecticides applied and total number of pesticides applied (insecticides and fungicides).

Honey bee observations

In our flower visit observations, we measured a large amount of variation in honey bee activity among fields. The variation was highly correlated with onion seed set, with higher honey bee visitation showing higher numbers of seeds per umbel. This explains 77% of the variation among sites (fig. 2). These data strongly indicate that lack of pollen transfer is causing poor seed set in some onion fields.

There was no interaction between field and distance from the honey bee hives on the abundance of honey bees observed on flowers (Kruskal-Wallis chi-squared = 0.9214, df = 2, $P = 0.6308$). There was also no difference in honey bee abundance on male and female flowers (Kruskal-Wallis chi-squared = 0.2596, df = 1, $P = 0.6104$).

The best subset models of honey bee activity included size of field (higher bee activity with smaller fields, $P = 0.0011$); total number of insecticides applied (higher bee activity with fewer insecticides used, $P = 0.0219$); hives per acre (higher bee activity with more hives, $P = 0.0232$); and sugar solids of male flowers, 15% to 27% (less bee activity with higher sugar solids, $P = 0.0360$; adjusted $R^2 = 0.704$, $F_{5,6} = 6.232$, $P = 0.0228$).

Univariate analyses of each of these factors showed that on their own, field size ($R^2 = 0.36$, $F_{1,11} = 6.15$, $P = 0.0306$) and number of insecticides applied ($R^2 = 0.24$, $F_{1,11} = 3.14$, $P = 0.0923$) were the strongest predictors of honey bee activity (fig. 3). This is consistent with a significant correlation found between increased insecticide use and decreased onion seed yields between 2003 and 2008 in Colusa County ($R^2 = 0.87$, $F_{1,4} = 25.77$, $P = 0.007$) (fig. 4).

Although wild bees, flies and wasps are important pollinators of onions for seed, few were observed on the onion flowers in our study (less than 15% of the total number of pollinators), so no relationship was found between wild pollinator visits and seed set. Percentages of sugar solids were generally lower in our study than those reported in the literature (Gary et al. 1977; Hagler et al. 1990), possibly due to dilution of the nectar from condensation inside the bags. We did not analyze landscape data, because we did not find any significant vegetation in bloom within a mile of the flowering onion fields, except for the occasional alfalfa field that was late in a cutting cycle.

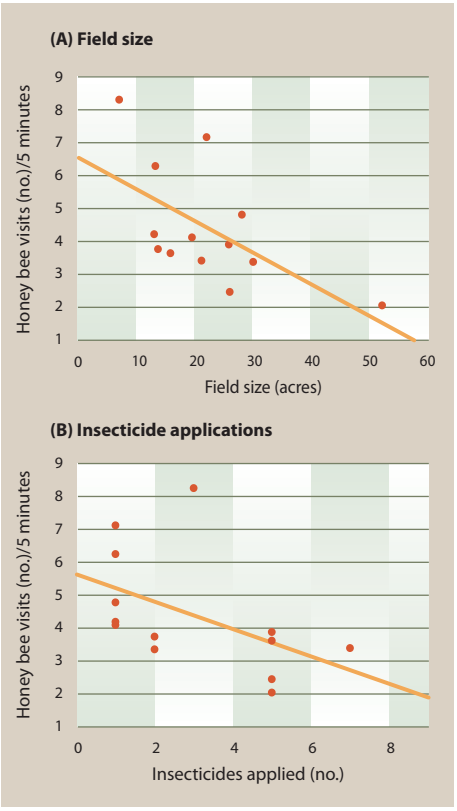

Fig. 3. Number of honey bee umbel (flower) visits per 5-minute observation versus (A) field size and (B) number of insecticides applied per field, Yolo and Sacramento counties, 2009.

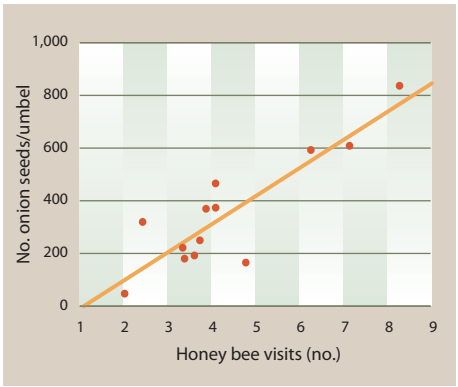

Fig. 2. Number of onion seeds per umbel per field versus number of honey bee umbel (flower) visits per 5-minute observation, Yolo and Sacramento counties, 2009.

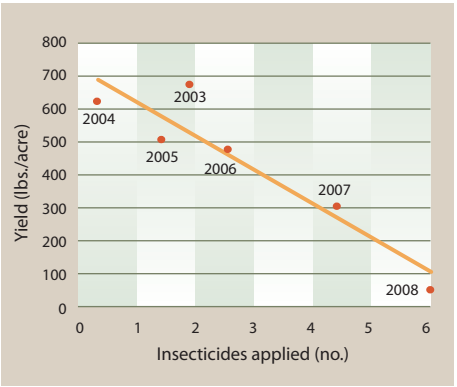

Fig. 4. Onion seed yields (pounds/acre) versus number of insecticides applied per field, Colusa County, 2003–2008. Source: Colusa County agricultural commissioner crop reports.

Davis Enterprise

This study found that the number of insecticides applied and field size were the strongest predictors of honey bee activity and onion seed yields.

Honey bee activity in onions

Onion flowers are well known to be unfavorable to honey bees. Their unattractiveness may lie in the high level of potassium in onion nectar and limited nectar rewards (Silva and Dean 2000; Waller et al. 1972). Onion floral resources can vary by genotype, with some onion varieties being more attractive to honey bees than others (in part due to a higher sucrose content), as well as by environmental factors, including field and weather conditions (Carlson 1974; Hagler et al. 1990; Nye et al. 1971). For example, under higher temperatures, as onion nectar increases in viscosity it becomes less preferred by honey bees (Voss et al. 1999).

In our studies, fields with the same female or male cultivar did not cluster together in their level of honey bee activity, but with limited varietal replication it was not possible to draw conclusions about the impact of cultivar on honey bee activity.

We did not see any significant differences in honey bee activity on male versus female flowers to explain differences in umbel seed set between fields. Since the introduction of hybrid onion cultivars, the female lines may be less attractive to foraging honey bees (no pollen available) than the adjacent male fertile lines, possibly reducing pollination (Parker 1982). However, individual honey bees have been shown to move freely between hybrid onion male and female lines with good pollination (Gary et al. 1977).

Competing, more-favorable floral resources in the landscape surrounding onion fields may draw honey bees away from onion seed crops, affecting pollination. As a result, the seed industry is careful to isolate fields so that competing flower resources do not occur at the same time within a mile of blooming onion fields. This field isolation was well observed in our study; the only competing resource that we occasionally observed was alfalfa in bloom, and the fields were subsequently harvested as soon as growers were notified of the problem.

Caution over insecticides

The negative correlation that we found between honey bee activity and number of insecticides applied per field site (including tank mixes with more than one insecticide) (fig. 3B) suggests that insecticide use to control onion thrips may be affecting crop pollination and seed set, perhaps by killing or repelling honey bees. This would help explain the significant yield declines in Colusa County during the past 5 years, which occurred concurrently with an increase in insecticide use. However, to confirm a causal relationship, more information is needed on the specific effects of different classes and rates of insecticides on honey bee activity. In addition, cultivar choice can play a role in honey bee activity and needs to be further investigated with respect to pesticide use and bee activity.

Our study suggests that growers should exercise caution when using insecticides, applying them only when needed as opposed to preemptively, to better protect both wild and honey bee pollinators. Also, the negative correlation between field size and honey bee activity suggests that spreading honey bee colonies around onion fields rather than grouping them may increase honey bee activity and pollination in larger fields.

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We thank local bee keepers, the onion seed production industry and growers for their help with this project.

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RESEARCH ARTICLE ABSTRACT

Transgenic rice evaluated for risks to marketability

by Dustin R. Mulvaney, Timothy J. Krupnik *and* Kaden B. Koffler

The California Rice Certification Act mandates specific planting and handling protocols for rice varieties, including transgenic rice, that may pose economic risks to California rice growers. Based on a literature review and extensive interviews, we describe this policy's evolution as a system for identity preservation and explain how it shapes the potential commercialization of transgenic rice. Several studies suggest that transgenic rice would be profitable for California growers, but the challenges in assuring 100% identity preservation — especially when access to export markets is at risk — means that the commercial approval of transgenic rice in California is unlikely until there is widespread market acceptance and growers are assured of no sales interruptions.

Ten years after the first regulatory approval of genetically engineered, or transgenic, rice in the United States, none is grown commercially. This contrasts with high adoption rates for transgenic soy (93%), corn (70%) and cotton (78%) (ERS 2010). The trend is similar globally: the transgenic rice closest to market is 'Xianyou 63', an insect-resistant (Bt, *Bacillus thuringiensis*) variety that was approved by China in 2009 and is expected to be available by 2012 (James 2009). Some experts contend that commercialization in China will usher in a wave of transgenic rice approvals and adoption (Serapio 2010), which could have implications for the California rice industry.

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RESEARCH ARTICLE ABSTRACT

Switchgrass is a promising, high-yielding crop for California biofuel

by Gabriel M. Pedroso, Christopher De Ben, Robert B. Hutmacher, Steve Orloff, Dan Putnam, Johan Six, Chris van Kessel, Steven Wright *and* Bruce A. Linquist

Ethanol use in California is expected to rise to 1.62 billion gallons per year in 2012, more than 90% of which will be trucked or shipped into the state. Switchgrass, a nonnative grass common in other states, has been identified as a possible high-yielding biomass crop for the production of cellulosic ethanol. The productivity of the two main ecotypes of switchgrass, lowland and upland, was evaluated under irrigated conditions across four diverse California ecozones — from Tulelake in the cool north to warm Imperial Valley in the south. In the first full year of production, the lowland varieties yielded up to 17 tons per acre of biomass, roughly double the biomass yields of California rice or maize. The yield response to nitrogen fertilization was statistically insignificant in the first year of production, except for in the Central Valley plots that were harvested twice a year. The biomass yields in our study indicate that switchgrass is a promising biofuel crop for California.

Switchgrass is a perennial, warm-season (C4) grass native to North America. One of the dominant species of the North American tallgrass prairie, switchgrass (*Panicum virgatum*) grows naturally in remnant prairies and native grass pastures, and along roadsides throughout the continental United States, except in California and the Pacific Northwest (USDA 2006). Agriculturally, it has been used primarily as forage and for grazing and groundcover. Recently, however, it has emerged as one of the most promising cellulosic biofuel crops for ethanol production.

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COMING UP *in California Agriculture*


A hedgerow lines fields at Fong Farms in Yolo County.
Lora Morandin

Hedgerows enhance beneficial insects on California farms

Hedgerows are trees, shrubs, forbs and perennial grasses bordering farm fields. Their many documented benefits in agricultural landscapes include enhanced weed control, water quality, soil fauna, erosion control, sediment retention, game hunting and biodiversity. In the next issue of *California Agriculture* journal, UC researchers present the results of a 2-year study comparing hedgerows and weedy areas near farms in the Sacramento Valley. The hedgerows had greater numbers of beneficial insects than pests while adjacent weedy areas showed the opposite trend, demonstrating that hedgerows may serve as replacement vegetation for weedy areas to encourage beneficial insects rather than pests in field crop edges.

Transgenic rice evaluated for risks to marketability

by Dustin R. Mulvaney, Timothy J. Krupnik *and* Kaden B. Koffler

The California Rice Certification Act mandates specific planting and handling protocols for rice varieties, including transgenic rice, that may pose economic risks to California rice growers. Based on a literature review and extensive interviews, we describe this policy's evolution as a system for identity preservation and explain how it shapes the potential commercialization of transgenic rice. Several studies suggest that transgenic rice would be profitable for California growers, but the challenges in assuring 100% identity preservation — especially when access to export markets is at risk — means that the commercial approval of transgenic rice in California is unlikely until there is widespread market acceptance and growers are assured of no sales interruptions.

Herbicide-tolerant rice has been developed to help farmers with costly weed problems. *Above,* Colusa County rice with at least three severe weed species.

Ten years after the first regulatory approval of genetically engineered, or transgenic, rice in the United States, none is grown commercially. This contrasts with high adoption rates for transgenic soy (93%), corn (70%) and cotton (78%) (ERS 2010). The trend is similar globally: the transgenic rice closest to market is 'Xianyou 63', an insect-resistant (Bt, *Bacillus thuringiensis*) variety that was approved by China in 2009 and is expected to be available by 2012 (James 2009). Some experts contend that commercialization in China will usher in a wave of transgenic rice approvals and adoption (Serapio 2010), which could have implications for the California rice industry.

We review the economic benefits and risks from transgenic rice and explain how market concerns shape California growers' perceptions of transgenic rice. Several studies suggest that transgenic rice would benefit California rice growers — particularly herbicide-tolerant varieties to help manage weeds (Annou et al. 2000; Bond et al. 2005). However, transgenic rice also presents economic risks to California rice growers, who rely on exports for half of their sales. Buyers could refuse to purchase stocks contaminated by transgenic rice, impose costly testing requirements or shut down markets permanently.

In 2001, UC Cooperative Extension surveyed 213 California rice growers, and 37% stated that if herbicide-tolerant rice were available they would not plant it. A subgroup of 78% suggested that this was due to "market concerns" (UCCE 2001). California growers produce over 1,980 tons (1,800 metric tons) annually valued at over $200 million, and close to $500 million when government payments are factored in. Many rice growers rely on export markets; as much as 40% of California rice is sold to Japan annually (Fukuda et al. 2003). These export markets, however, are not entirely secure, and the U.S. Department of Agriculture's Economic Research Service (2001) has described U.S. involvement as "thin, volatile and risky" (see sidebar, page 163).

To manage risks to marketability, the California Rice Certification Act (CRCA) regulates rice with "characteristics of commercial impact," including transgenic rice. The CRCA relies on a risk management scheme called identity preservation (IP), which refers to "production, handling and marketing practices that maintain the integrity and purity of agricultural commodities" (Sundstrom et al. 2002). Many crops — such as cotton, where keeping varieties of different fiber consistently separate is critical to marketability — require identity preservation for quality control. Identity preservation is also used to manage "genetic pollution" risks from transgenic crops (Ellstrand 2006), particularly those not approved for human consumption or used to make pharmaceuticals (Marvier 2007). In these latter cases, identity preservation must be 100% effective.

To explore the CRCA's evolution and effectiveness, all published accounts were surveyed, including journals, reports, media coverage, industry newsletters and regulatory agency publications. Forty-eight semistructured interviews

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were conducted with key policymakers, scientists, activists and growers working on issues related to transgenic crops and the rice industry. Using snowball sampling methodology, interviewees provided new contacts until additional interviews yielded no new informants, and informants yielded no new pertinent information (Salganik and Heckathorn 2004). Interviews and follow-up conversations were conducted from 2004 to 2010 and included 14 UC and industry scientists, eight rice growers, six rice marketers, six activists and 18 policymakers. This approach captured the range of views expressed by key individuals, firms and public institutions.

Glossary

Adventitious presence: A low-level, inconsequential presence of unintended genetic materials (e.g., transgenic seed).

Breeder, foundation and certified seed: Crops grown to produce seeds for planting.

Coexistence: The dual production, distribution and marketing of transgenic and nontransgenic varieties, with an emphasis on keeping them separate.

Commingling: The inadvertent mixture of seed or grain products.

Containment: Using spatial, temporal or biological isolation to prevent gene flow by keeping biological materials inside a set boundary.

Gene flow: The movement and exchange of genetic traits or biological organisms from one population to another.

Genetically engineered, genetically modified or transgenic organism: An organism produced by combining DNA from different sources, either from within the organism's genome or from another organism.

Identity preservation (IP): Handling practices to ensure that a product can be traced to its genetic/biological source (seed) and production location (e.g., a specific agricultural field).

Precautionary principle: When the consequences of a proposed action are severe or irreversible, policy should err on the side of caution.

Potential transgenic rice benefits

Weeds strongly affect yields and profitability in California rice production. Weed management requires multiple herbicide applications, which can be costly. Growers can spend up to $200 per acre on herbicides (Bond et al. 2003). One proposed weed control strategy is herbicide-tolerant rice modified to contain genes resistant to broad-spectrum herbicides. Herbicide-tolerant rice allows herbicides to be sprayed shortly after seedlings emerge, when rice-weed competition is highest and the potential for weed-inflicted yield losses is greatest. Reducing weed density and biomass early gives herbicide-tolerant rice seedlings a competitive advantage for solar radiation, nutrients and water.

Herbicide-tolerant rice could simplify weed management because it requires just one herbicide, rather than multiple selective herbicides for specific weed biotypes. One study suggests that herbicide-tolerant rice could decrease herbicide requirements by up to 84% (Bond et al. 2005). This would reduce costs and provide environmental benefits by promoting alternatives to more toxic herbicides (Ueji and Inao 2001). After accounting for additional costs — including seed technology fees, identity preservation costs, short-term yield reductions and CRCA fees — such research suggests that herbicide-tolerant rice would be profitable for California growers (Bond et al. 2003).

However, important impacts are obscured when costs and benefits are analyzed without considering how the adoption of transgenic rice would affect the marketability of exported rice. These studies assume no change in marketability or prices, and that transgenic herbicide-tolerant rice would be broadly accepted. However, market rejection of exports could have severe economic repercussions, so profitability analyses will ultimately hinge on successfully containing risks.

LibertyLink contamination

In August 2006, the U.S. Food and Drug Administration announced that Bayer's long-grain LibertyLink transgenic rice (LL601) — not yet approved for human consumption — extensively commingled with long-grain 'Cheniere' rice and foundation seed grown in five southern U.S. states (FDA 2006). Over the ensuing months, major importers of U.S. rice — Japan, South Korea, Taiwan, Mexico, Russia and the European Union (EU) — banned or halted all imports of long-grain rice from the United States (Vermif 2006). The FDA retroactively approved LL601 for human consumption to reassure consumers that it was safe. But by that time, rice futures prices had fallen on the Chicago Board of Trade, and growers had entered into futures contracts at lower prices than anticipated (GAO 2008). University of Arkansas economists later confirmed a large and adverse (but short-lived) price drop (Li et al. 2010).

Another of Bayer's LibertyLink varieties (LL604) was later found in 'Clearfield 131' rice seed marketed by a competing seed company, BASF. It was recalled after USDA asked that it not be sold or distributed, costing BASF millions of dollars in seed sales (GAO 2008). These events prompted additional testing requirements in export markets, and significant resources were mobilized to remove LibertyLink rice from the seed supply. An industry executive estimated domestic impacts to growers between $80 million to $100 million (Cole 2006), while an attorney representing affected growers in a class-action lawsuit against Bayer estimated damages at $1 billion (GAO 2008).

The LibertyLink incidents did little to instill confidence that experimental field trials of transgenic crops could always be adequately contained.

Drawing on USDA data, the U.S. Rice Federation suggested that rice exports to the European Union fell 68% from 2005 to 2007 (Cummings 2009). EU importers who deliver U.S. rice to market were also affected, losing an estimated €52 million to €111 million in 2006 and 2007 (Brookes 2008). While USDA did not definitively identify the contamination source, a jury awarded a dozen growers a $48 million judgment against Bayer, which owned the LibertyLink varieties grown in experimental field trials from 1999 to 2001 at a research station in Louisiana. Bayer

has lost six similar cases so far, and hundreds more are pending (Cronin Fisk and Whittington 2010).

Rice futures prices eventually recovered, as energy costs and commodity speculation drove bidding to record highs in 2008. But LibertyLink was detected in subsequent shipments, preventing marketers from taking advantage of high prices and effectively restricting U.S. ride trade with the European Union (GAO 2008). California rice growers were largely unaffected by the LibertyLink incident, because they primarily grow short- and medium-grain rice (table 1). Only one

TABLE 1. Regulatory status and field-test locations for Bayer's LibertyLink (LL) rice

Variety	Grain type	Federal regulatory status*	Calif. certification status†	Field-trial locations
LL62	Medium	Approved 1999	Approved for greenhouse trial in 2008	La. (2); Puerto Rico (2); Calif.
LL06	Medium	Approved 1999	Not approved	Calif. (10); Puerto Rico (2)
LL601	Long	Approved 2006‡	Not approved	Ark., Miss., Mo., Texas, La., Puerto Rico
LL604	Long	Not approved	Not approved	Ark., Miss., Mo., Texas, La., Puerto Rico

* Includes Food and Drug Adminstration, Environmental Protection Agency and U.S. Department of Agriculture Animal and Plant Health Inspection Service. Federal approval does not automatically entail approval at the state level.

† State regulatory agencies include California Department of Food and Agriculture and California Rice Commission task force.

‡ Approval came after discovered mixed with nontransgenic rice varieties in southern United States.

Biosafety or trade barrier? Japan's tenuous trade with California

Japan is the California rice industry's largest international customer, purchasing more than $421 million in 2009 — over 40% of the industry's exports (FAS 2010). If tests on imports find transgenic traits, Japan has suggested that it would deny rice shipments. Some observers question whether such policies are about biosafety or if they constitute a barrier to trade.

Rice is culturally, religiously and politically significant in Japan. Japan has invoked national food-security policies that promote self-sufficiency and smallholder paddy production (Ohnuki-Tierney 1993); nonetheless, it is a leading food importer, deriving about 40% of its total calories from imports (Sato 2007). Like California, Japan produces temperate, medium-grain japonica varieties. In Japan, however, per-acre production costs are 10 or more times higher, and consumer prices are two to three times higher than in California (Fukuda et al. 2003). Post–World War II land reforms divided rice-growing areas into holdings of less than 7 acres. In contrast with other food commodities, Japan is self-sufficient in rice production and tends to guard its domestic rice markets against imports.

During the 1994 Uruguay Round of international trade negotiations, Japan yielded to U.S. pressure and agreed to phase out rice import restrictions, reduce government subsidies and annually increase the amount of rice it imported. Japan is required to import more than 680,000 tons of rice per year (Fukuda et al. 2003). About 100,000 tons of this rice is used in food and beverage manufacturing; the rest is directed to government warehouses for eventual re-exportation as foreign food aid (Fukuda et al. 2003). In 2006, Japan announced that the rice surpluses stored in government warehouses would also be used for biofuels (Annon 2006), and production commenced in 2009 (Takada 2009).


Churl Han

Japan's strict policies on transgenic rice are rooted in both concerns about biosafety and economic protectionism. The Cartagena Protocol on Biosafety — part of the United Nations Convention on Biodiversity — allows countries to base decisions about transgenic organisms on the precautionary principle. When risks from biotechnologies are severe and potentially irreversible, nations can implement labeling and other regulatory requirements. Codex Alimentarius, the international standards-setting organization for food safety, has developed guidelines for food derived from biotechnology (i.e., transgenic foods) and suggests labeling as an appropriate approach to risk management (Codex 2003). Both institutions seek to ensure that restrictions on trade are not rooted in protectionism and are informed by the best available information about food safety and environmental consequences.

Unfortunately for California rice growers, other foreign customers that also import japonica rice, such as Taiwan, Turkey and South Korea, maintain rice import protocols similar to those of Japan. Both Taiwan and South Korea also have Uruguay Round commitments that are contradictory to the interests of domestic rice farmers in those countries, and both have asserted strict import restrictions on transgenic rice. Turkey banned transgenic rice altogether. While it is difficult to determine whether protectionism, culture or biosafety are the main forces driving such policies, all play a role in influencing the deployment of transgenic rice.

— D.R. Mulvaney, T.J. Krupnik, K.B. Koffler

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importer who resells rice from the affected region lost sales (B. Lundberg, Lundberg Family Farms, personal communication, 2006).

The LibertyLink incidents did little to instill confidence that experimental field trials of transgenic crops could always be adequately contained. Contamination from transgenic rice field trials was involved in 20 of 39 international commingling incidents in 2009 and 2010 (GMO Contamination Register 2010). Since 1996, more than 100 field trials of transgenic rice have been conducted in California (table 2), although to date seasonal testing of California rice seed for transgenic traits has found no contamination (CRC 2010).

Regulating risks

In 1999, the seed company AgrEvo, which is now owned by Bayer, petitioned to deregulate another LibertyLink variety (LL62). At that time, controversies over transgenic crops were making headlines. Exports of U.S. transgenic soy and corn were refused at European ports (Goldberg 2000), and from 1997 to 2005, U.S. exports of corn to the European Union fell $211 million annually to $200,000, while U.S. soy fell from $2.3 billion to $511 million (Zerbe 2007). A number of other high-profile, unauthorized releases of regulated transgenic crops into the food supply followed (table 3).

California rice growers and industry representatives were aware that the improperly managed introduction of transgenic rice could have economic repercussions, including poor consumer confidence, and lost sales and market access. With the support of a number of nonprofit organizations, the California Rice Commission (CRC), which represents about 1,000 rice growers, pursued a regulatory mechanism to control such risks. By 2000, the California Rice Certification Act (CRCA) was law (see box).

Under the CRCA, the CRC can propose regulations to the California Secretary of Agriculture for rice with characteristics that affect its marketability, including those difficult to identify without specialized testing and those considered expensive or impossible to remove. Though not named explicitly, transgenic rice is clearly regulated by the CRCA. When rice with problematic characteristics is identified, the CRC convenes a task force — representing growers, marketers, UC, the California Warehouse Association and the Cooperative Rice Research Foundation — to develop identity preservation protocols and keep it out of commodity streams for conventional rice.

Key provisions of the California Rice Certification Act (AB2622)

Rice industry partners will work cooperatively to maintain consumer confidence and the acceptance of rice produced and milled in the state.

The task force shall recommend regulations relating to rice identified as having characteristics of commercial impact.

The CRC has the authority to establish terms and conditions for the production and handling of rice to minimize the potential for commingling of various types of rice, and to prevent commingling where reconditioning is infeasible or impossible.

Identity preservation requires special planting, handling and auditing procedures along the path from the rice field to customer. Gene flow — the movement and exchange of genetic traits or biological organisms from one population to another — must be contained in rice fields. This requires physical or biological barriers to prevent rice pollen and seed from moving via wind, wildlife or flooding (Lu and Snow 2005). For rice, pollen-mediated gene flow is restricted by short dispersal (Song et al. 2003) and brief viability (Lu and Snow 2005). Seed-mediated gene flow can occur over longer distances, because rice seed remains viable for much longer, and dispersal can be assisted by high winds during aerial seeding, floods, wildlife or human error. California's rice fields are habitat to hundreds of millions of waterfowl migrating along the Pacific Flyway, making them potentially important gene-flow vectors. Seed dispersal can be minimized through spatial isolation, prohibitions on aerial seeding, closed-loop water recirculation requirements and wildlife exclusion nets (see box, page 165), although such precautions are likely to significantly

TABLE 2. Applications to USDA for transgenic rice trials in California, 1996–2009*

Trait	Institution	Applications
Herbicide tolerance	UC Davis, Louisiana State University, Aventis, Bayer Crop Science, Syngentia, AgrEvo and Monsanto	61
Pharmaceutical	Ventria Biosciences	7
Salinity tolerance	Arcadia Biosciences	7
Nitrogen-use efficiency	Arcadia Biosciences	6
Sterility	Bayer	4
Yield enhancements	Research for Hire, Monsanto	8
Bacterial/disease resistance	UC Davis	10
Altered metabolism/proteins	Aventis	6
Visual markers	UC Davis	6

Source: ISB 2009.

* Organisms with multiple transformations were counted multiple times. Not all applications were approved.

TABLE 3. Unauthorized releases of regulated transgenic crops into the food supply

Year	Product	Crop	Trait	Cause	Detection
2000	StarLink	Corn	Insect resistance, herbicide tolerance	Cross-pollination, commingling after harvest	3rd-party testing
2002	Prodigene	Corn	Pharmaceutical protein	Cross-pollination, uncontrolled volunteers	USDA inspection
2004	Syngenta Bt10	Corn	Insect resistance	Misidentified seed	3rd-party testing
2006	LibertyLink 601	Rice	Herbicide tolerance	Not determined	3rd-party testing
2006	LibertyLink 604	Rice	Herbicide tolerance	Not determined	3rd-party testing
2008	Event 32	Corn	Insect resistance	Under investigation	Developer testing

Source: GAO 2008.

raise production costs. Monitoring for rice crop volunteers in subsequent seasons and leaving land fallow between transgenic and conventional cultivation can also reduce gene flow. Pollen and seed management are most critical where commercial seed is produced, since the distribution of contaminated seed would have far-reaching impacts. Breeders provide foundation seed to contract growers of certified seed, who in turn produce seed for growers. While breeders already employ practices that limit gene flow, the prospect of incidental transgenic contamination raises the stakes for maintaining purity. This point was underscored in the LibertyLink incidents, where 'Cheniere' and 'Clearfield 131' foundation seed (representing 39% of the certified seed acreage in Arkansas) were contaminated (Schultz 2006). Growers seeking nontransgenic seed can not use these varieties until the transgenic traits are no longer detected, which can be several years.

Ultimately, the extent to which identity preservation can mitigate risk depends on the enforcement of standards set by buyers. Most identity preservation systems allow for a low-level presence of unintended characteristics, referred to as adventitious presence. Postharvest buyers typically only permit the adventitious presence of traits posing no human health risks. For example, Japan allows up to 5% of soy imports to contain transgenic soy that has been approved by its food safety regulators. But there is zero tolerance for unapproved crops, which often include experimental crops; with no margin for error, identity preservation is considerably more challenging.

Japan, the largest foreign importer of California rice, maintains some of the world's strictest food safety standards. Incoming shipments of rice are routinely tested for transgenic traits, which if found can result in refusal of the shipment. The LibertyLink incidents suggest that Japan will continue to maintain zero-tolerance policies for transgenic rice and would reject contaminated rice imports.

Many forecasts that determine potential impacts from the adoption of herbicide-tolerant rice base their claims on farm budget analyses that assume market acceptance (Bond et al. 2005). These studies assume modest cost increases for identity preservation but do not incorporate the economic risks associated with contamination incidents. These assumptions are important, because rice contaminated by transgenic traits can cause severe, long-lasting and potentially irreversible impacts on marketability.

The CRCA has drawn attention as a model policy for managing economic risks from transgenic crops (Taylor et al. 2004). While such management is unique, there are precedents for employing identity preservation systems: the cotton industry ensures consistent fiber quality, organic certification tracks crops for labeling, and seed purity is maintained in seed certification and quality control programs (Sundstrom et al. 2002).

Lundberg Family Farms clearly labels its products as "non-GMO," meaning that they contain no genetically modified organisms. ***(Lundberg Family Farms rice are Non-GMO Project verified. Lundberg Family Farms does not support the deregulation of "transgenetic" rice.)***

Pharmaceutical rice protocols

The CRCA first applied identity preservation to transgenic rice in late 2003, when Ventria Biosciences sought approval to commercially plant out two rice varieties engineered to produce pharmaceutical compounds that have antimicrobial qualities. The proposed varieties were engineered with recombinant (r-) human proteins, r-lacto-ferrin and r-lysozyme, which were intended for use in the production of iron supplements and antidiarrheal medicines. "Pharm" rice was grown in experimental plots in California from 1999 to 2003 (ISB 2009). When Ventria notified the CRC of its intent to commercialize production, a task force

Containment and identity preservation practices

Containment

- Spatial/temporal isolation or buffer zones between transgenic and nontransgenic crop fields.
- Clearly labeled and dedicated equipment for seeding, harvesting, transporting and handling.
- Netting to keep birds and other wildlife from entering fields.
- Screens to keep seed and seedlings from moving into drainage ditches and other waterways.
- Prohibitions against aerial seeding.
- Monitoring for crop volunteers on fields and margins.
- Postharvest tillage to reduce the regrowth of rice from stubble.
- Seed sterility.

Identity preservation

- Transgenic/nontransgenic labeling on rice bags, silos and trailers.
- Dedicated equipment for drying, hulling, processing and shipping.
- Inspections and documentation demonstrating that shared equipment has been properly cleaned out between processing transgenic and nontransgenic products.
- Clear product custody reports along the distribution path.
- Preventing seed spillage when transferring seed or grain in and out of equipment.

Raymond Panaligan, IRRI

***Above,* leaf samples of transgenic rice lines that were subjected to DNA extraction in the laboratory.**

was convened to develop planting and handling protocols. These protocols were not yet complete when Ventria asked the CRC to permit planting on an emergency basis so they could cultivate during the 2004 growing season (Moschini 2006).

In March 2004, the CRC task force voted six to five to approve Ventria's request, but with several restrictions. Pharm rice was permitted only in counties that were geographically isolated from California's primary rice-growing regions. Aerial seeding was prohibited, and practices to discourage wildlife movement and dedicated equipment were required.

Since these protocols modify the California agriculture code, the Secretary of the California Department of Food and Agriculture (CDFA) can veto decisions by the CRC task force within 10 days. CDFA received a letter from the Japanese Rice Retailers Association that stated, "It is certain that the commercialization of [pharm] rice in the United States will evoke a distrust of U.S. rice as a whole among Japanese consumers, since we think it is practically impossible to guarantee no rice contamination . . . If the crop is actually commercialized in the United States, we shall strongly request the Japanese government to take measures not to import any California rice" (Taylor et al. 2004). Such threats were not without precedent: in January 2002, Japan briefly blocked imports of rice bags with lead-based pigments because of food safety concerns (Fukuda 2002).

According to our interviews, concerns about market losses strongly influenced the CDFA decision to veto the planting protocol. The official decision was justified on the grounds that insufficient time was provided for public comment, and that Ventria had not obtained the relevant federal-level permits (Marvier 2007). Ventria subsequently moved operations to Missouri, and Anheuser-Busch said they would refuse to purchase Missouri rice if pharm crops were planted. Ventria next moved to North Carolina, a state without commercial rice production; however, the pharm rice field trials are reportedly taking place near the Tidewater Research Station, where many rice varieties are bred and tested (UCS 2006).

Gaps in CRCA oversight

Even before the LibertyLink incidents, many of our interviewees expressed concern about experimental field trials for transgenic crops (see box). These trials are the responsibility of the USDA Animal and Plant Health Inspection Service (APHIS), which requires strict containment protocols. Early in CRCA implementation, additional regulation was considered redundant. But in the wake of the LibertyLink incidents, which originated from experimental trials, there were calls for the CRCA to regulate field trials (RPC 2006). The CRC responded, and now experimental field trials require CRCA approval.

Another area of concern was the proximity of experimental field trials to the foundation rice seed supply. The California Rice Experimental Station in Biggs (Butte County) hosts many breeding programs and is where much of the industry's seed originates. Although no transgenic rice field tests have occurred there since 2003, there is no formal policy on future research. Tests of Biggs foundation seed found no transgenic-rice traits from 2007 to 2010 (CRC 2010). Nonetheless, several interviewees proposed a prohibition against transgenic tests near California rice seed production sites.

An additional gap in oversight is due to an exemption for UC researchers. The CRCA states that it "shall not apply to research conducted by the University of California except for rice produced directly from the research that enters the channels of trade" (AB2622 2000). While the industry works closely with researchers to follow proper protocols (T. Johnson, California Rice Commission, personal communication, 2006) not all growers are convinced that this adequately mitigates risk (RPC 2006).

Managing risks to rice marketing

Food safety concerns such as BSE (bovine spongiform encephalopathy, or mad cow disease), *E. coli* and *Salmonella* have led buyers to implement testing requirements and even reject food shipments (O'Neill 2005). Unapproved transgenic crops receive similar scrutiny. To manage the economic risks from transgenic rice, the CRCA requires strict identity preservation for any crop that might affect the marketability of rice. While deemed a model policy, the extent to which other commodities might adopt similar policies is limited because of three unique circumstances surrounding California rice.

On the LibertyLink incident

"Any rational person would probably say, yeah, those protocols probably didn't work. I think there is an even-keeled, good case to be made that we have a good example that didn't work, and we need to look at that."

— Rice grower

On containment

"No one is going to control the birds . . . there is nothing we can do unless we eliminate the plot."

— Rice grower

On export market risks

"We have a policy in place that speaks very clearly to the fact that GE [genetically engineered] rice should not be planted until such time as there is commercial acceptance."

— Rice industry representative

First, the California rice industry recognized the economic risks of transgenic rice contamination, prompting preemptive legislation to ensure that the problem could be managed. Second, the rice supply chain is amenable to identity preservation because the industry already separates rice based on grain type and color. Third, the reproductive biology of rice makes identity preservation more feasible. For crops that pose greater gene-flow risks, or that have more homogenous supply chains and distribution channels, similar policies are less feasible. For example, corn plants shed significant amounts of pollen to produce kernels, and much of the corn supply is delivered to elevators with homogenous supplies of corn from various sources. Identity preservation requirements are also unlikely in commodities facing little opposition to transgenic crops, such as cotton.

Herbicide-tolerant rice may be beneficial for California rice growers. But predictions of the economic benefits from transgenic rice must be considered alongside market risks. Rice shipments testing positive for transgenic traits could be rejected, likely with long-lasting repercussions. California growers could consequently face severe oversupply, lower prices and possibly decreased production (Childs and Burden 2000), underscoring the importance of a precautionary approach to market risks.

In the meantime, alternative weed-management options include breeding for weed-suppressive crop traits (Gibson et al. 2003); alternative tillage and stand establishment methods to pregerminate and then control weeds (Linquist et al. 2007); and long-term strategies aimed at shifting weed communities to make broad-spectrum herbicides more effective (Fischer 2004). Until transgenic rice gains market acceptance, these management strategies are likely to be less risky options for California growers.

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RESEARCH ARTICLE

Switchgrass is a promising, high-yielding crop for California biofuel

by Gabriel M. Pedroso, Christopher De Ben, Robert B. Hutmacher, Steve Orloff, Dan Putnam, Johan Six, Chris van Kessel, Steven Wright *and* Bruce A. Linquist

Ethanol use in California is expected to rise to 1.62 billion gallons per year in 2012, more than 90% of which will be trucked or shipped into the state. Switchgrass, a nonnative grass common in other states, has been identified as a possible high-yielding biomass crop for the production of cellulosic ethanol. The productivity of the two main ecotypes of switchgrass, lowland and upland, was evaluated under irrigated conditions across four diverse California ecozones — from Tulelake in the cool north to warm Imperial Valley in the south. In the first full year of production, the lowland varieties yielded up to 17 tons per acre of biomass, roughly double the biomass yields of California rice or maize. The yield response to nitrogen fertilization was statistically insignificant in the first year of production, except for in the Central Valley plots that were harvested twice a year. The biomass yields in our study indicate that switchgrass is a promising biofuel crop for California.

Switchgrass is a grass native to North America that has been utilized primarily as a forage crop and groundcover. Because of its high biomass yields, switchgrass (*above*, in El Centro, Imperial County) is considered a good candidate for dedicated energy crops (biofuels).

Switchgrass is a perennial, warm-season (C4) grass native to North America. One of the dominant species of the North American tallgrass prairie, switchgrass (*Panicum virgatum*) grows naturally in remnant prairies and native grass pastures, and along roadsides throughout the continental United States, except in California and the Pacific Northwest (USDA 2006). Agriculturally, it has been used primarily as forage and for grazing and groundcover. Recently, however, it has emerged as one of the most promising cellulosic biofuel crops for ethanol production.

California consumes more ethanol than any other U.S. state. California gasoline contains about 6% ethanol by volume (since the substitution of MTBE [methyl tert-butyl ether] in 2004), resulting in annual ethanol consumption of nearly 1 billion gallons (3.78 million cubic meters). Of that, about 80% is maize-based ethanol transported by rail from the Midwest; 12% is sugarcane-based ethanol shipped from Brazil; and 8% is ethanol from maize grains produced in-state (CEC 2010). The proportion of ethanol blended in California gasoline is expected to increase to 10% by 2012, resulting in ethanol demand of 1.62 billion gallons (6.12 million cubic meters) per year if no gasoline consumption changes occur (CEC 2007). If in-state production does not increase, California will need to import more than 95% of its ethanol by 2012.

Currently, most of the ethanol used in California is produced by fermenting the sugar in Brazilian sugarcane and the starch in Midwestern grains (Macedo et al. 2008). Technology is being developed to produce ethanol from cellulose, the most abundant structural carbohydrate in plants; the cellulose content of switchgrass, for example, is about 40% (Isci et al. 2008). Cellulose cannot be directly fermented to produce ethanol; it needs to be broken down into more simple sugars. There are different processes of lingo-cellulose conversion into ethanol, such as strong acid hydrolysis followed by fermentation (SHF) and simultaneous saccharification and fermentation (SSF). Significant challenges remain and need to be overcome before the technology can be commercially used (CEC 2010).

Compared to maize grain–based ethanol, which is the only ethanol currently produced in California, cellulosic ethanol has higher productivity and net energy value, and lower net greenhouse-gas emissions (Adler et al. 2007). Cellulosic ethanol can also be produced from non-food crops, waste and forest products, diminishing the possible inflationary effects on food prices if land used to produce food were diverted to ethanol crops.

Potential of switchgrass

There are many possible crop sources for cellulosic ethanol production in California, including agricultural and urban wastes, rice and wheat straw, wood

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chips from tree pruning and dedicated energy crops. Crops with high biomass yields, such as switchgrass, elephantgrass (*Pennisetum purpureum*) and miscanthus (*Miscanthus* × *giganteus*) are good candidates for dedicated energy crops. The advantages of switchgrass are its high yield potential, excellent soil conservation attributes and good compatibility with conventional farming practices (McLaughlin et al. 1999). Like most field crops, for example, switchgrass is established from seeds, whereas elephantgrass and miscanthus are established by transplanting billets and rhizomes. Also, switchgrass is no longer on the California Department of Food and Agriculture list of noxious weeds (CDFA 2010).

Under rain-fed conditions of the Midwest and southern United States, switchgrass biomass yields have ranged from 2.5 to 11 tons per acre (5.5 to 25 metric tons per hectare) (Fike et al. 2006; Heaton et al. 2004; McLaughlin et al. 1999; Schmer et al. 2008). The responses of switchgrass crops to nitrogen fertilizer have been variable and conflicting. Some studies report limited or no yield response (Christian et al. 2001; Thomason et al. 2004), while others have found significant yield increases due to nitrogen fertilization (Lemus et al. 2008; Muir et al. 2001; Stroup et al. 2003). Nitrogen response is an important area of study because nitrogen fertilizer is the main energy input and the main source of greenhouse-gas emissions during switchgrass cultivation (Adler et al. 2007; Schmer et al. 2008). Understanding how switchgrass responds to nitrogen will help researchers develop energy-efficient and environmentally benign production systems for biomass energy crops.

Because it can be used both as forage and as a biofuel crop, switchgrass may be well suited to California, a state with a large livestock industry and higher ethanol consumption than any other. However, there is little information about switchgrass production in California, nor in other irrigated regions. Irrigated Western regions are significantly different in climate and cropping patterns than the Midwest or southern United States, where most switchgrass research has been carried out. California's Mediterranean climate suggests greater yield potential but also higher water and nitrogen requirements.

Switchgrass can be separated into two ecotype groups: lowland and upland. Lowland ecotypes are found in floodplains and are taller (around 6 feet), coarser with a more bunch-type growth habit and may be more rapid growing than upland ecotypes. In contrast, upland ecotypes are found in drier upland sites and are finer stemmed, broad based and often semi-decumbent (Porter 1996). Usually, lowland ecotypes flower and mature later than upland ecotypes.

Switchgrass is not native to California, and no information has been available about the adaptability of lowland and upland ecotypes in California. In addition, California has different ecozones — areas defined by distinct climate patterns, landscapes and plant species. Therefore, it is possible that one ecotype is better adapted to one ecozone than another. The objectives of our research were to identify (1) how well the lowland and upland switchgrass ecotypes would adapt to the major ecozones in California, (2) the biomass yield potential for each ecozone and (3) the response of upland switchgrass to various nitrogen fertilizer rates.

Switchgrass ecotype trials

To identify the suitability of lowland and upland switchgrass ecotypes for California, and to test the adaptation of the crop itself, we established trials in July 2007 at four California locations with different climate characteristics and soil attributes (tables 1 and 2). Tulelake (Siskiyou County), the northernmost site on the border with Oregon, is typical of the intermountain regions of California and other parts of the Pacific Northwest. Davis (Yolo County) is situated in the Sacramento Valley, and Five Points (Fresno County) is in the San Joaquin Valley. El Centro (Imperial County) was the southernmost site and represents a low desert agricultural region typical of the Sonoran deserts of California, Mexico and Arizona.

In each location, we evaluated five lowland and five upland varieties. However, the lowland varieties slightly varied across sites, so we tested six lowland varieties total, but only five per site. Lowland ecotypes included two released varieties (Alamo and Kanlow) and four experimental varieties. The upland ecotypes included four released varieties (Trailblazer, Cave in Rock, Blackwell and Sunburst) and one experimental variety. The trials were a completely randomized block design with six replications. At all locations, the seedbed was prepared to provide a firm and fine soil surface. Switchgrass was drill seeded in July 2007 at a rate of 5 pounds per acre (5.6 kilograms per hectare) of pure live seeds, at a depth of

TABLE 1. Climatic characteristics of California ecozones used to evaluate switchgrass production, 2007-2008

Characteristics	Tulelake	Davis	Five Points	El Centro
Altitude (feet)	4,033	52	230	43
Latitude (°N)	41.7	38.5	36.4	32.7
Annual average max./ min. temp. (°F)	62/31	74/46	77/48	89/56
Annual precipitation (inches)	10.9	17.6	6.9	2.7
Frost-free days	164	307	320	365

Source: CalClim 2009.

TABLE 2. Soil properties of California ecozones used to evaluate switchgrass production, 2007-2008

Soil attributes*	Tulelake	Davis	Five Points	El Centro
Clay (%)	32	28	31	42
Silt (%)	45	48	34	42
Sand (%)	23	24	35	16
pH	5.9	7.2	7.6	8
CEC (meq/100 grams)†	45.5	35.4	30.7	31.6
Olsen-P (phosphorus) (ppm)	62.5	13.6	7.4	10.7
Potassium (K) (ppm)	367	375	439	409
Organic carbon (%)	4.85	1.91	0.95	0.98
Nitrate-nitrogen (NO_3-N) (ppm)	26.5	9.9	10.6	8.8
Total nitrogen (%)	0.34	0.13	0.07	0.07

* Soil samples were taken before planting at average depth of 4 inches (10 centimeters).

† CEC = cation exchange capacity.

In the first full production year, plots of lowland switchgrass in El Centro yielded 17.6 tons per acre. The crop's productivity tends to increase through the third and fourth years.

0.25 inch (0.6 centimeter) and with 10 inches (25.4 centimeters) between rows. Plot size was 10 by 15 feet (3 by 5 meters). The plots were sprinkler irrigated after seeding to ensure good germination. No fertilizer was applied at planting, but at the three-leaf stage nitrogen (N) was applied in the form of ammonium sulfate at a rate of 50 pounds per acre (56 kilograms per hectare).

All fields were harvested in November 2007, after which the crop entered winter dormancy (table 3). The last harvests at Tulelake, Davis and Five Points were driven in part by the onset of the winter rains or snow; later harvests would have been impractical due to wet soils. Winter dormancy was observed at all sites, including El Centro. El Centro was the first site to break dormancy in early February, followed by Five Points in late February, Davis in early March and Tulelake in early May.

In Tulelake, only one lowland ecotype variety, Kanlow, survived the winter. Lowland ecotypes developed in the southern United States where winters are mild, although frost is not uncommon. Tulelake has average minimum temperatures of 22°F (–5.5°C) during winter and receives 21 inches (53.3 centimeters) of snow annually (CalClim 2009). Therefore, lowland ecotype varieties were generally expected to be unsuitable at this location, which was confirmed by the winter mortality of all except one.

In spring 2008, nitrogen, phosphorus (P) and potassium (K) fertilizers were applied. Nitrogen was applied in the form of ammonium sulfate at a rate of 100 pounds per acre (112 kilograms per hectare). Phosphorus (P_2O_5) and potassium (K_2O) were each applied at a rate of 50 pounds per acre (56 kilograms per hectare).

During the 2008 growing season, the number of switchgrass harvests varied among sites due to climatic differences. The plots were harvested once at Tulelake (October), twice at Davis and Five Points (July and October/November) and three times at El Centro (July, September and November). After the first and second harvests, 100 pounds nitrogen per acre (112 kilograms per hectare) was applied in the form of ammonium sulfate.

Flowering differences

In 2007, we recorded flowering differences among ecotypes and locations at harvest, because early flowering has been associated with lower yields (Hopkins et al. 1995). The upland varieties exhibited higher flowering percentages (percentage of flowering tillers) than the lowland varieties at all locations, and flowering percentages were higher in the southern locations due to the longer season and warmer temperatures. In Tulelake, 5% to 10% of tillers on average were flowering in the upland ecotypes across all varieties, and no flowering was observed in the lowland ecotypes. At Davis and Five Points, there was 15% to 25% flowering for upland and 0% to 10% for lowland ecotypes. The highest flowering percentages were found at El Centro, ranging from 50% to 75% for upland and 10% to 40% for lowland ecotypes.

In 2008, the upland ecotypes once again exhibited higher flowering percentages at the first harvest than the lowland ecotypes at all locations: average flowering percentages observed in lowland and upland ecotypes respectively were 2% and 48% at Davis, 4% and 66% at Five Points, and 6% and 70% at El Centro. At Tulelake, the single-harvest location, the only surviving lowland variety exhibited 30% flowering, while all the upland

TABLE 3. Management operations in 2007 and 2008, and switchgrass dormancy break in 2008

Year	Management	Tulelake	Davis	Five Points	El Centro
2007	Planting date	7/24	7/5	7/17	7/19
	N fertilization	3-leaf stage	3-leaf stage	3-leaf stage	3-leaf stage
	Harvest	11/8	11/19	11/13	11/26
2008	Dormancy break	Early May	Early March	Late February	Early February
	N, P and K fertilization*	5/5	4/9 7/21	3/25 7/25	3/27 7/14 9/8
	Harvest	10/1	7/18 10/30	7/23 11/19	7/11 9/5 11/6

* Nitrogen, phosphorus and potassium.

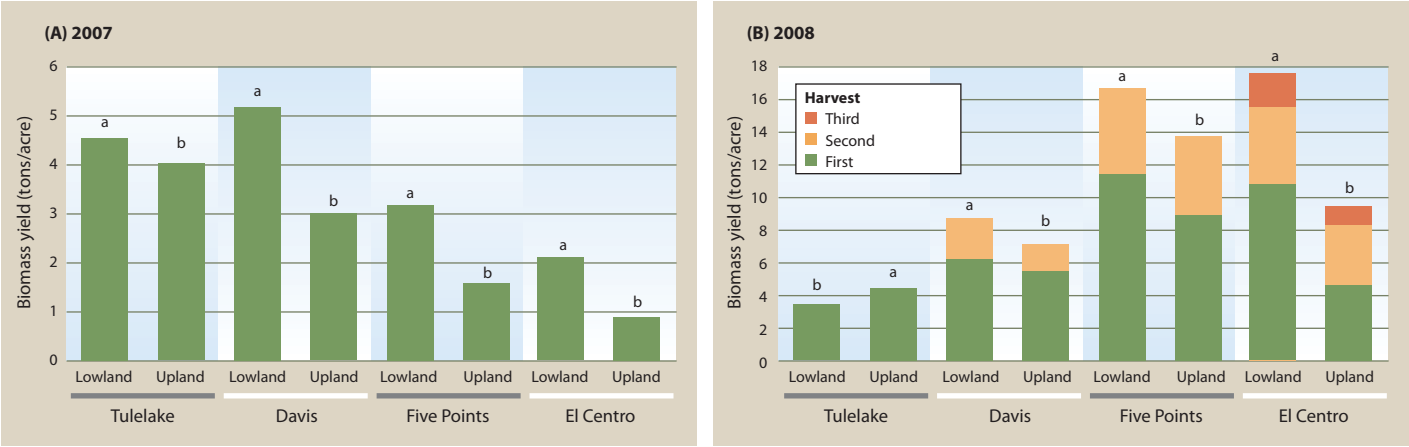

Fig. 1. Switchgrass yields during (A) establishment year (2007) and (B) first full production year (2008) by ecotype and ecozone. Each bar is mean of five varieties and six replications, except for the lowland ecotype at Tulelake, which represents one surviving variety. All locations were planted in July 2007. The same letter above an ecotype indicates that yields are not statistically different (Tukey at $P \leq 0.05$) within each location.

ecotypes showed 100% flowering. Only small differences in flowering among ecotypes were seen at the second harvest. Davis showed 47% and 35% flowering, Five Points 77% and 71%, and El Centro 29% and 8% for lowland and upland ecotypes, respectively. In our trials, early flowering was also correlated with lower yields, in accordance with other studies (Hopkins et al. 1995).

Biomass yields

In the 2007 establishment year, the lowland ecotypes yielded significantly more biomass than upland ecotypes at all locations, but these differences were less evident at Tulelake (fig. 1A). The highest biomass production was achieved by lowland ecotypes in Davis at 5.2 tons per acre (11.6 metric tons per hectare). El Centro had the lowest yield, with upland ecotypes producing only 0.8 ton per acre (1.7 metric tons per hectare). Biomass yields of switchgrass in the establishment year are expected to be lower than in subsequent years. Our establishment yields were similar to those reported in other U.S. studies (Muir et al. 2001) and in Mediterranean climates of Europe (Alexopoulou et al. 2008). We planted the switchgrass in July, and it is likely that an earlier planting date would have resulted in higher yields. Vassey et al. (1985) compared early, mid- and late-spring planting dates and found that the highest yields were achieved with the earliest planting dates.

In 2008, the first full production year, yields were generally higher than in 2007 (fig. 1B). The lowland ecotype yields were higher than upland ecotypes at all locations except for Tulelake, where only one lowland ecotype survived the first winter. Yields of lowland ecotypes averaged 8.7, 16.7 and 17.6 tons per acre (19.4, 37.4 and 39.4 metric tons per hectare) in Davis, Five Points and El Centro, respectively. The upland varieties were not well suited to the warmer locations, but in Tulelake all the upland varieties produced similar yields, which were higher than yields of the single surviving lowland variety.

At locations with multiple harvests, the first harvest produced significantly more biomass than subsequent harvests for all varieties. Of the total biomass, 73%, 67% and 57% was obtained in the first harvest at Davis, Five Points and El Centro, respectively (fig. 1B). At El Centro, the third harvest produced only 12% of the total annual biomass yield.

The 2008 yields achieved in our trials were substantially higher than those reported by Heaton et al. (2004) from 21 studies of mature switchgrass stands around the United States (3 years or older). In their studies, yields averaged 4.6 tons per acre (10.3 metric tons per hectare) and ranged from approximately 0.5 to 9.8 tons per acre (1.1 to 22 metric tons per hectare). Furthermore, we expect yields in our California plantings to increase in the following 1 to 2 years. Productivity tends to increase until the switchgrass stand reaches maturity and full yield potential at 3 or 4 years old (Sharma et al. 2003).

Nitrogen trials

Trials were conducted in the same four locations in 2008 to evaluate the yield response of the upland ecotype variety Trailblazer to different levels of nitrogen fertilizer. Nitrogen fertilizer was applied at each location at annual rates of 0, 70, 140, 210 and 280 pounds per acre (0, 75, 150, 225 and 300 kilograms per hectare) in Davis, Five Points and El Centro. In Tulelake, the nitrogen rates were half that of the other locations (0, 35, 70, 105 and 140 pounds per acre), due to the single harvest and shorter growing season. Five Points and El Centro received all nitrogen fertilizer in March and April, in two applications spaced 4 weeks apart. In Davis, the first nitrogen fertilizer application was in April and the second in July, after the first harvest. Tulelake received nitrogen fertilizer in one single application in May. The plots were adjacent to plots of the ecotype trials and were established at the same time in 2007 and harvested at the same time.

Overall yields were lowest at Tulelake and El Centro, averaging only 6.5 and 7 tons per acre (14.5 and 15.6 metric tons per hectare), respectively (fig. 2, page 172). Trailblazer is an upland variety, and in the ecotype variety trials it usually had lower yields than the lowland varieties (fig. 1B). Total annual biomass yields showed no response to nitrogen treatment at any location. This may be due to switchgrass's deep root system, reportedly up to 10 feet (3 meters), which is able to explore large amounts of soil for nitrogen (Parrish and Fike 2005).

While there was no response to nitrogen fertilizer in the first harvest yields at any location, the second harvest at both Davis and Five Points showed a

Nitrogen fertilizer will likely be needed for switchgrass crops to achieve their full yield potential. *Above,* switchgrass is tested in Davis in June, about 1 month before the first harvest.

With the high yield potential of switchgrass in California and consequent high nitrogen removal, California growers would most likely need to apply nitrogen fertilizer (and other nutrients) to sustain yields.

significant yield response when the two highest nitrogen treatments were compared to the plots that received no nitrogen. This suggests that over time switchgrass depleted the native soil's nitrogen reserves and required fertilizer nitrogen to achieve its yield potential. Muir et al. (2001) reported just such a situation: switchgrass depleted native soil nitrogen reserves when receiving no nitrogen fertilizer, and over time yield differences between unfertilized and nitrogen-fertilized switchgrass increased. Christian et al. (2001) found no yield response to nitrogen fertilizer during a 5-year study, but that was mainly because soil supply and deposition were adequate to support the low average yields of 4 tons per acre (8.9 metric tons per hectare), and the researchers affirmed that long-term management strategies would be necessary to avoid deficits in soil nitrogen. With the high yield potential of switchgrass in California and consequent high nitrogen removal, California growers would most likely need to apply nitrogen fertilizer (and other nutrients) to sustain yields.

Further research is still needed to improve nitrogen management, but it is likely that a switchgrass variety with higher yielding potential than Trailblazer would show a greater response to nitrogen fertilizer. We found that the plants were green at the first harvest, with high nitrogen content, resulting in high rates of nitrogen (and possibly other nutrients) removal from the soil, which in production fields would need to be replenished. Likewise, multiple-harvest systems are likely to require more fertilizers than single-harvest systems. In multiple-harvest situations, the crop's nutrient content is high at the first harvest, generally in midsummer. The last harvest (or the one harvest of single-harvest systems) takes place in the fall after the plants have senesced. During senescence, most nutrients, including nitrogen, likely retranslocate to the roots, becoming available for next year's growth. Retranslocation to the roots can increase nitrogen conservation within the plant-soil system and may reduce nitrogen fertilizer requirements in subsequent years. Further research is necessary to quantify nitrogen removal and develop nitrogen management strategies for single- and multiple-harvest crops.

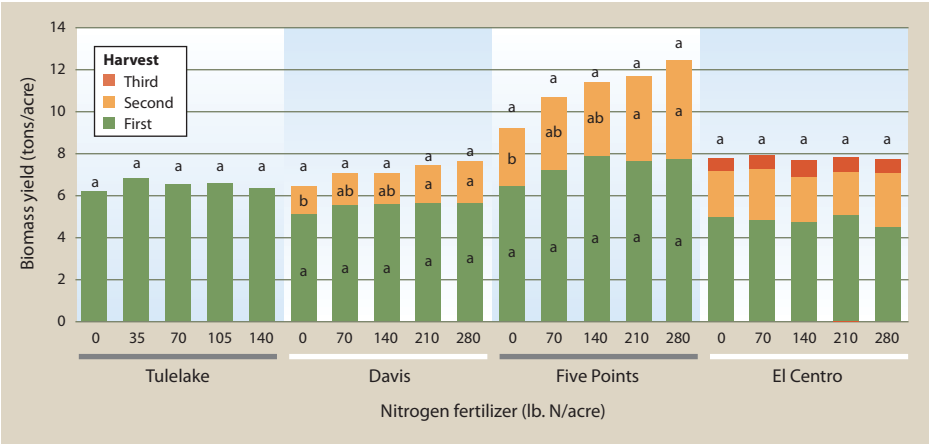

Fig. 2. Response of switchgrass variety Trailblazer (upland ecotype) to nitrogen fertilization in 2008. Letters above yield bars refer to total biomass for the full year, and letters inside bars refer to that harvest. The same letter indicates that yields were not statistically significant (Tukey at $P \leq 0.05$) within each location and harvest. No differences were seen in Tulelake or El Centro for each harvest or total biomass.

Promising biofuel crop

Our results suggest that switchgrass has high yield potential in California. Although its productivity in the state's cooler mountain regions is limited, productivity is considered good in the San Joaquin and Imperial valleys. Switchgrass had moderate yields in the establishment year and up to 17 tons per acre (38 metric tons per hectare) in the second year. By comparison, California maize and rice produce approximately 9 tons per acre (20.1 metric tons per hectare) of total biomass (grain plus stover), and alfalfa produces 7 to 8 tons per acre (15.6 to 18 metric tons per hectare). Therefore, the switchgrass yields reported here are promising both for forage and use as a biofuel crop.

Our results show that switchgrass requires little or no nitrogen in the establishment year, suggesting that it can efficiently use the native soil nitrogen pool; but switchgrass may require nitrogen fertilizers in multiple-harvest systems in the second and subsequent years in order to sustain high yields. In addition to the

While productivity in the cooler regions of California was limited, switchgrass did well in the warm San Joaquin and Imperial valleys. Average biomass yields were nearly double that of maize, rice and alfalfa. *Above,* Francisco Maciel shows the height of switchgrass at the El Centro field trial.

effect of multiple harvests on nitrogen removal, the harvesting and transportation costs (machinery, fuel and labor) of multiple harvests should be considered when evaluating the production and economic feasibility of switchgrass as a biofuel crop. Water use also elicits concern in California. Switchgrass is a C4 plant, and it is expected to show high rates of transpiration efficiency due to its more efficient photosynthesis pathway. Although not determined in these trials, we are currently researching water use in switchgrass production.

Productivity is dependent on ecotype varietal selection and proper fertility management. Upland varieties were best suited for Tulelake and the cooler mountain regions; most lowland varieties did not survive the first winter. For all other locations, where winter survival was not a concern, the lowland varieties were better adapted and achieved higher yields than the upland varieties.

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