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May 2015

The Microbiome and Overall Health Part 5: The Oropharyngeal Microbiota's Far-Reaching Role in Immunity, Gut Health, and Cardiovascular Disease

By Cass Nelson-Dooley, MS and Stephen F. Olmstead, MD

[This is the fifth in a series of articles about how the gut and the microbiome influence all areas of health. The first in the series appeared in the January 2015 ProThera®, Inc. Practitioner Newsletter. Previous installments have covered the microbiota and obesity, brain health, and the gut's role in cardiovascular and autoimmune disease. In the coming months, Dr. Olmstead will discuss such topics as the microbiota and gastric health and the gut's connection to the skin.]

INTRODUCTION

People often forget that the digestive or alimentary tract begins in the mouth. Even gastroenterologists frequently seem to view the oropharynx as little more than a portal for fiber-optic scopes headed down the esophagus for the proximal gastrointestinal tract. But the mouth and pharynx form an integral part of the alimentary tract and functionally are the beginning of the gastrointestinal tract. The oropharynx is home to teeming, variegated microbiota second only to the colon in density.[1] Unlike the normal colon microbiota which is not associated with intestinal disease, the oral microbiome is regularly associated with two common diseases, dental caries and gingivitis.[2] The oral microbiome has also been implicated in a range of systemic diseases including tumors, diabetes mellitus, cardiovascular disease, bacteremia, and low infant birth weight as well as preterm delivery. The tremendous microbial complexity of the oropharyngeal cavity is only now being fully investigated using high-throughput DNA sequencing technologies.[2,3] The microbiota in the oropharyngeal cavity is composed of a variety of microenvironments that select for different microbial populations. It holds at least 700 species of aerobic and anaerobic organisms organized in complex biofilm communities.[1-5] Immunologic tissues in this cavity such as the tonsils and adenoids are a first meeting place where antigens and microorganisms encounter the immune system and the host's microbial symbionts.[6] Finally, from its position at the beginning of the gastrointestinal tract, the oropharyngeal microbiota may influence the composition of the gastrointestinal microbiome downstream.[7,8] Modulating the oropharyngeal microbiota is therefore an integral part of a comprehensive health strategy. Diet, orodental hygienic practices, probiotics, and supplements may be used to improve oropharyngeal barrier function and reduce inflammation, thereby favorably supporting oral and overall health.

The oral microbiome has been implicated in a range of systemic diseases including tumors, diabetes mellitus, cardiovascular disease, bacteremia, and low infant birth weight.

THE OROPHARYNGEAL CAVITY: SETTING THE STAGE

ENVIRONMENT

Two primary surfaces are colonized by bacteria in the oral cavity: shedding mucosal surfaces and nonshedding solid surfaces (teeth).[4] The mucosal surfaces of the mouth consist of nonkeratinized, stratified, squamous epithelium just as found in the esophagus. The gingiva, hard palate, and parts of the tongue have a keratinized, stratified, squamous epithelium. Microorganisms growing around the tooth surfaces are divided into supragingival and subgingival plaques. These different oral tissues have

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PROTHERA®, INC.
PRACTITIONER NEWSLETTER
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Medical Editor
Chris D. Meletis, ND

Chief Science Officer
Stephen F. Olmstead, MD

Contributors
Stephen F. Olmstead, MD
Chris D. Meletis, ND
Cass Nelson-Dooley, MS
Marina MacDonald, PhD
Shana Rheault, MS

Editor
Kimberly Wilkes

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differing characteristics and therefore present diverse niches for microbial colonization.

From birth, the environment of the mouth changes, directly impacting the evolving microbial communities.[9] The oral cavity microenvironment changes with age, because of distinct phases of oral development: emergence of permanent teeth, cavities, extractions, toothlessness, and dentures. The temperature, oxygen levels, access to nutrients, antibiotics, and the redox potential profoundly affect the microecology of the oral cavity.

A healthy flow of saliva is a critical ingredient for a balanced oral microbiome.[4,10,11] It provides nutrients to microorganisms. Saliva determines oropharyngeal pH, enabling mineralization and remineralization (repair) of teeth. The saliva flow can disrupt bacterial biofilm communities on teeth. Bacteria continually shed into the saliva, making salivary microbes a representative sample of the oral microbiome fingerprint.[2] Saliva is an antimicrobial and antiviral, containing lysozyme, lactoferrin, lactoperoxidase, and immunoglobulins A, G, and M. It also contains proline-rich glycoproteins that allow bacterial attachment to the tooth surface.[12]

Diet has a profound effect on the microbial inhabitants of the digestive tract. Long heralded as the determining factor for optimizing health in the gut environment, the oral cavity is the first part of the body to experience a person's dietary choices. At infancy, the introduction of milk affects the environment, followed by solid foods, and dietary changes during growth and development. Breastfed infants have different microbial compositions in the nasopharynx than formula-fed infants consisting of increased numbers of the lactic acid bacterium *Dolosigranulum* as well as *Corynebacterium* and reduced numbers of *Staphylococcus* and anaerobic bacteria, such as *Prevotella* and *Veillonella*.[12] Unfavorable food choices can lead to oral cavity dysbiosis and pathology, most notably the impact of sugar on dental caries. Oral nitrate-reducing bacteria act on leafy greens and beets, making nutrients more bioavailable to the host.

IMMUNITY IN THE ORAL CAVITY

The gingival tissues are relatively porous, more so than the gut epithelia, and are more widely exposed to an array of microbes and their products.[6] Most lymphoid tissue in the oropharynx is found in lingual and pharyngeal tonsils and adenoids.[3] Dendritic cells are scattered throughout the oral mucosa and are critical in front-line recognition of microbes and in stimulating T-cell responses. Dendritic cells in the mucosa help determine tolerance or active immune response toward a given antigen.[3] The oral mucosa is rich in secretory immunoglobulin A (sIgA), a first-line defender against antigens.

In a healthy mouth, proinflammatory and anti-inflammatory mechanisms are kept in check and balanced.[6] Certain oral commensal microbes may affect this balance by decreasing nuclear factor-kappa B (NF-kB), promoting anti-inflammatory regulatory T cells (T_{REG}) or activating immune cells such as neutrophils, macrophages, and monocytes. *Lactobacillus* species can decrease NF-kB and promote IL-10 secretion.[6] Streptococci from the tongue or plaque may have immunosuppressive activity, as they were able to inhibit neutrophil attraction to gingival tissues by reducing NF-kB.[6] These mechanisms may facilitate host immune tolerance and successful colonization of the tissues.

BIOFILMS

Dental plaque was the first recognized medical bacterial biofilm. These are complex, multispecies structures located throughout the oropharynx consisting of microbes surrounded by self-produced extracellular polymeric substances.[5] Layers of microorganisms adhere to each other in the biofilm, affording the community protection from antimicrobials and host immune defenses. When members of the biofilm break free, they can form biofilms on other sites, on teeth, in gingival sulci or they can transform to free-floating planktonic forms to be swallowed. Oral biofilms provide a continuous source of bacteria into the oral cavity, the gastrointestinal tract, the airways, and lungs.

TAKEAWAY POINTS

- The oral cavity has rich species diversity, biofilms, and environmental conditions that can hinder or enhance disease.
- The oral microbiome seeds the rest of the gastrointestinal tract.
- The oropharyngeal microflora is intimately linked with systemic health.
- Probiotics can suppress certain oral pathogens and improve outcomes.
- Oropharyngeal microbial health is part of a comprehensive strategy for gastrointestinal health and overall health.

Infants delivered by C-section had less oropharyngeal microbial diversity and a high prevalence of colonization by Slackia exigua, a periodontal pathogen absent in vaginally delivered babies. Inoculation with maternal vaginal microbiota during birth may suppress Slackia populations.

THE ORAL MICROBIOME

BIRTH, INFANCY, AND ADULTHOOD

Vaginal birth increases oral microbiome diversity in infancy.[13] Infants delivered by C-section had less oropharyngeal microbial diversity and a high prevalence of colonization by *Slackia exigua*, a periodontal pathogen absent in vaginally delivered babies. Babies delivered by C-section were also colonized by more *Lactobacillus* Cluster I. Inoculation with maternal vaginal microbiota during birth may suppress Slackia populations. This study was consistent with other studies of gastrointestinal microbiota, showing that C-section delivery predicts reduced species diversity compared to vaginal delivery. It is not known if the impact of C-section on the oropharyngeal microbiota predisposes to disease later in life. However, it is widely agreed that greater microbiome diversity is associated with better health.[14]

The oral microbiome in infancy differs from the adult microbiome. Infants are predominantly colonized with *Streptococcus* as well as *Veillonella*, *Neisseria*, *Rothia*, *Haemophilus*, *Gemella*, *Granulicatella*, *Leptotrichia*, and *Fusobacterium*. In contrast, their adult caregivers were colonized predominantly with *Haemophilus*, *Neisseria*, *Veillonella*, *Fusobacterium*, *Oribacterium*, *Rothia*, *Treponema*, and *Actinomyces*.[9]

REGIONAL OROPHARYNGEAL MICRODIVERSITY

The buccal mucosa, keratinized attached gingiva (gums at the neck of the teeth), hard palate, saliva, tongue, supragingival and subgingival tooth plaque, throat, and palatine tonsils represent distinct ecological niches despite their proximity.[4] Microbial profiles overlap for certain oropharyngeal sites. Buccal mucosa, keratinized attached gingiva, and hard palate have similar microbiomes. These niches are dominated by

Firmicutes phyla, specifically *Streptococcus* and *Gemella*. The saliva, tongue, throat, and palatine tonsils constitute another group with similar microbial profiles. These sites have high numbers of *Prevotella*, *Veillonella*, *Porphyromonas*, and *Neisseria*. Saliva can serve as a microbial "fingerprint" for the oropharynx and contains bacteria at $1.4x10^8$ CFU/mL.[2] Saliva normally contains microbes from the phyla Actinobacteria, Bacteroides, Firmicutes, Fusobacteria, Proteobacteria, Spirochaetes, and TM7.

Tonsils have microbial populations similar to those found in the saliva and on the tongue.[4] In addition, the throat and tonsils are inhabited by small numbers of *Butyrivibrio* and *Mogibacterium* (Firmicutes phyla). The tongue is regularly colonized by *Streptococcus salivarius*, *Rothia mucilaginosa*, and an uncharacterized Eubacterium.[2]

The microorganisms in subgingival and supragingival dental plaques of healthy individuals are distinct from microbes colonizing the cheeks, gums, and tonsils. The differences may be due to the nature of hard, non-shedding tooth surfaces and oxygen availability.[4] In healthy people, dental plaque is dominated by Firmicutes and Actinobacteria phyla.[2] Supragingival plaque is primarily composed of facultative anaerobic genera such as *Streptococcus*, *Capnocytophaga*, *Neisseria*, *Haemophilus*, *Leptotrichia*, *Actinomyces*, *Rothia*, *Corynebacterium*, and *Kingella*.[4] Subgingival plaques often contain gram-negative, anaerobic bacteria.[5] Subgingival microbes include obligate anaerobes: *Fusobacterium*, *Prevotella*, *Treponema*, *Dialister*, *Eubacterium*, *Selenomonas*, and *Parvimonas*.[4] Other beneficial, commensal microbes found in periodontal tissues are *Streptococcus*, *Veillonella*, *Abiotrophia*, *Campylobacter*, *Capnocytophaga*, *Gemella*, and *Neisseria*.[2]

COMMENSAL OR PATHOGEN?

Commensal organisms in the oropharyngeal microbiome may be pathogens or pathogenic under certain circumstances.[4] Healthy individuals commonly harbor low numbers of oral pathogens such as *Porphyromonas gingivalis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Neisseria meningitidis*, and *Haemophilus influenzae*. *Treponema* species are commensals that may be associated with periodontal and endodontic disease. *Lactobacillus* and *Bifidobacterium* species have been implicated in development of caries. The genus *Moraxella*, which includes *M. catarrhalis*, a common sinus pathogen, can be detected in the throats of some healthy people. The current consensus is that normal commensals may become pathogenic when oropharyngeal dysbiosis is present. Oropharyngeal dysbiosis may arise when various interrelated factors such as diet, salivary flow, pH, immune defenses, and microbial interactions are not kept in balance.

DENTAL CARIES AND ENDODONTIC DISEASE

Nearly one-third of Americans have untreated tooth decay, and the vast majority of the population have some form of gum disease.[15] Caries and endodontic infections are widely recognized as the result of oral dysbiosis, particularly within microbial communities in plaques.[6,16] Frequent intake of refined carbohydrates, especially sucrose, selects for acid-loving and acid-producing bacteria in dental plaques. This results in demineralization of the tooth enamel by bacterial acids.[5] Gram-positive supragingival bacteria are responsible for dental caries.[5] These include *Streptococcus mutans*, *S. salivarius*, and *S. mitis*. *Lactobacillus* species may contribute by virtue of lowering ambient pH. Traditionally, *S. mutans* has been viewed as the primary cause of dental caries. However, it is now thought that changes in the oral microecology lead to cavities. For example, nonmutans streptococci such as *S. sanguinis*, *S.oralis*, and *S. mitis* contribute to a healthy, balanced oral microbiota and in a healthy mouth make up 95% of dental plaque while *S. mutans* normally comprises 2% of the plaque.[5]

However, poor oral hygiene, high sugar intake, and/or low salivary flow can lower pH in the mouth and increase numbers of acid-producing bacteria and acid-loving streptococci and lactobacilli, eventually making a hospitable environment for *S. mutans*. This acidic environment (pH <4.0) primes the development of dental caries allowing other microbes including *S. sobrinus*, *Actinomyces*, bifidobacteria, and yeast to move in.[5] This population shift is accompanied by a loss of microbial diversity.[2]

Endodontic disease, decay, or infection in the tooth pulp is considered to be primarily caused by pathogenic bacterial biofilms in the root canal system.[16] Proteobacteria, Firmicutes, Bacteroidetes, Fusobacteria, and Actinobacteria are abundant phyla in infected root canals.[2] *Olsenella uli*, *Prevotella baroniae*, *Porphyromonas endodontalis*, *Fusobacterium nucleatum*, and *Tannerella forsythia* species are often found in infected root canals.[2]

GINGIVITIS AND PERIODONTAL DISEASE

Subgingival plaques are made up of gram-negative, anaerobic bacteria including *Actinobacillus*, *Campylobacter*, *Fusobacterium nucleatum*, *Porphyromonas* gingivalis.[5] *F. nucleatum* is particularly important because of its ability to coaggregate with other pathogens. Gingival inflammation is caused by progressive plaque accumulation. As gingivitis develops, *Streptococcus* predominance subsides and *Actinomycetes*, *Capnocytophaga*, *Campylobacter*, *Eikenella*, *Fusobacterium*, and *Prevotella* increase.[2]

Periodontitis is a chronic inflammatory disease of the tissues around the teeth.[2] Periodontal disease is caused by the immune response to excessive subgingival plaque leading to destruction of the gum, periodontal membrane, and alveolar bone. Periodontal disease may be associated with systemic autoimmune or inflammatory diseases, as exemplified by its high prevalence in people with inflammatory bowel disease.[17]

Patients with H. pylori treated for dental plaque had a lower H. pylori gastric reinfection rate (19.4%) compared to those who received no treatment for plaque (84.3%).

THE OROPHARYNGEAL MICROBIOTA AND THE GUT

Oropharyngeal microbes "seed" the rest of the gastrointestinal tract. It is estimated that 10^{11} bacterial cells per day flow from the mouth to the stomach.[4] There was a 45% overlap between the oropharyngeal and colonic microbiota suggesting that the oral microbiome strongly influences the composition of the gut microbiome.[4] Pathogenic biofilms in the mouth, if not treated, potentially provide a continual source of pathogenic microbes to the gut. This may contribute to chronic or recurrent stomach, small intestine, or colon dysbiosis.

Studies of *Helicobacter pylori* suggest that its presence in dental plaque may be a source for reinfection after medical treatment.[7] In subjects with gastric *H. pylori* infection, high levels of *H. pylori* were found in dental plaques. These levels were not attributed to periodontal disease or poor oral hygiene. Another study showed that patients with *H. pylori* treated for dental plaque had a lower *H. pylori* gastric reinfection rate (19.4%) compared to those who received no treatment for plaque (84.3%).[18]

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INFLAMMATORY BOWEL DISEASE AND PERIODONTAL DISEASE

Inflammatory bowel disease (IBD) and periodontal disease often present as comorbidities and similar immune pathogenesis have been hypothesized.[19] People with IBD are more likely to have periodontitis than healthy subjects.[20] Patients with IBD and untreated periodontitis have higher opportunistic bacteria populations in inflamed periodontal tissue than those with periodontal disease alone.[8] Some authorities suggest treating both oral and systemic inflammation as a way to approach inflammatory bowel disease and periodontitis.[17]

THE OROPHARYNGEAL MICROBIOME AND SYSTEMIC DISEASE

CARDIOVASCULAR DISEASE

The role of the oropharyngeal microbiota in systemic health is most notable in its apparent link to cardiovascular disease.[21] People with poor dental hygiene practices have increased markers of inflammation and cardiovascular disease such as adiponectin, fibrinogen, CRP, and cellular adhesion molecule-1. In people with coronary heart disease, regular flossing and brushing are associated with a reduction in new cardiovascular events.[22] Good oral hygiene significantly lowers cardiovascular disease risk.[23] Maintaining a healthy oropharyngeal microbiome is therefore a way to prevent and treat cardiovascular disease.

NITRIC OXIDE PRODUCTION

Another compelling link between systemic health and oropharyngeal health is the significant contribution by oral bacteria to systemic nitric oxide levels.[24] Nitric oxide has a well-known role in regulating endothelial function. Low levels increase the risk of atherosclerotic disease and cardiovascular events. Humans lack the enzyme to reduce nitrate to nitrite but if supplied with nitrite, people readily convert it to nitric oxide. Nitrate-reducing bacteria in the mouth have a significant impact on physiological levels of nitrite and on clinical parameters associated with nitric oxide vascular activity. Up to 25% of nitrite production occurs in the mouth under the influence of oral bacteria. And this nitrite is taken up by salivary glands and concentrated 20-fold. Facultative and obligate anaerobes in the deep crypts of the tongue produce nitrate-reducing enzymes during anaerobic respiration. *Veillonella* and *Actinomyces* appear to have the strongest nitrate-reducing activity. Antiseptic mouthwash disrupts the oral microbiota and abolishes the effects of a diet high in nitrates, decreases oral and plasma nitrite levels, and even increases blood pressure.[24]

ORAL PROBIOTICS

Lactobacillus species have been estimated to make up approximately 1% of the commensal oral microbiota.[3] The *Lactobacillus* strains found in salivary specimens are *L. fermentum*, *L. rhamnosus*, *L. salivarius*, *L. casei*, *L. acidophilus*, and *L. plantarum*. It is unclear whether certain species are indigenous or transient due to dietary intake. Probiotic strains packed on periodontal dressings decrease the numbers of commonly isolated pathogens such as *Bacteroides*, Actinomyces, *S. intermedius*, and *C. albicans*. Probiotics reduced oral *Candida* in elderly patients.

Probiotics, particularly *Lactobacillus* species, antagonize pathogenic organisms implicated in dental caries and periodontitis.[3] *L. rhamnosus* GG and *L. reuteri* have been shown to reduce *S. mutans* numbers. Studies show *L. rhamnosus* also inhibits the growth of at least six oral pathogens and confers significant protection against the development of dental caries. Other *Lactobacillus* probiotics shown to inhibit or reduce numbers of *S. mutans* include *L. paracasei, L. salivarius*, and *L. plantarum*.

CONCLUSION

Dental caries, periodontal disease, gingivitis, and endodontic disease are commonplace pathologies caused by oral dysbiosis. No longer relying on the one-organism-one-disease model, microbiologists are recognizing that multiple environmental and microbial changes precede oral pathology. There is good evidence that the oral microbiome influences the organisms downstream and might be a hidden source of infection in people with chronic gastrointestinal dysbiosis. Further, mounting evidence shows the link between the health of the oral cavity and systemic disease such as cardiovascular disease. Considering these findings, the oral microbiome must be considered as part of a comprehensive approach to gut health and overall health. *Lactobacillus* and *Bifidobacterium* probiotics, a low-sugar diet, oral hygiene, and other interventions to reinforce mucosal integrity and reduce inflammation will help promote a healthy oropharyngeal microbiota and general health.

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The full list of references is available upon request.

CLINICAL APPLICATIONS

Here are some key clinical points to keep in mind about the management of oral diseases:

- Oral hygiene is important to balance the oral microbiome: brushing, flossing, and dental cleanings.
- Diet, especially low in refined sugar and plant-based, is critical for a healthy oropharyngeal microbiome.
- Healthy salivary flow is needed for a balanced oral microbiome.
- Periodontal disease should be considered when gastrointestinal dysbiosis is suspected.
- Treatments of the oral cavity including mucosal health, barrier integrity, and immune function can affect the gastrointestinal and systemic health of the patient.
- *H. pylori* in oral biofilms might contribute to continuous gastric exposure and reinfection.
- Exercise caution when using antiseptic mouthwash and antibiotic treatment.
- *Lactobacillus* probiotics can treat oral dysbiosis and provide support in the setting of periodontal disease, dental caries, and halitosis.

How Aging Affects the Body Part 5: Addressing the Causes and Consequences of Aging Skin

By Chris D. Meletis, ND

[This is the fifth monthly installment of an 11-part series discussing how aging affects various parts of the body. The first in the series appeared in January 2015. To date, cognitive decline, the aging heart, liver, and kidney health have been covered. Future installments will discuss aging and the lungs, eyes, stomach, small intestine and colon, mouth, and adrenal glands.]

The 21 square feet of skin on the average human is subject to a host of age-related changes including thinning, sagging, wrinkling, loss of elasticity and water content, and a reversal of the collagen type I/III ratio[1] (reduced synthesis of the stronger collagen type I but enhanced production of the weaker collagen type III). Wound healing also is delayed in the elderly[2] because dermal fibroblasts involved in skin healing are not able to proliferate as easily and are thus less able to migrate to the site of a wound.[3]

With age, skin tissue becomes progressively stiffer and less able to recoil, changes that parallel those occurring in the cardiovascular and pulmonary tissues. Researchers have hypothesized that age-related dysfunction of elastic fibers may be at least partly responsible for the fact humans are only able to achieve a 100 to 120-year lifespan.[4]

Patients often are concerned about their skin's appearance as they age. However, the obvious alterations that occur in the aging skin go hand in hand with less obvious and highly detrimental changes progressively happening beneath the surface such as a reduced ability to manufacture vitamin D and the initiation of skin carcinogenesis. In this article, I will discuss the causes and consequences of age-related skin damage and propose approaches for strengthening the health and the appearance of aging skin.

A REDUCED ABILITY TO MANUFACTURE VITAMIN D

When the skin is exposed to sunlight, the ultraviolet B (UVB) rays from the sun are absorbed by 7-dehydrocholesterol and subsequently converted to previtamin D_3, which is then rapidly transformed to vitamin D_3. The skin of elderly people is markedly less efficient at producing previtamin D_3,[5] due to a drop in 7-dehydrocholesterol levels with age.[6] This predisposes older adults to vitamin D deficiency, the consequences of which include an exacerbation of osteopenia and osteoporosis, and increased risk of fractures as well as certain cancers, autoimmune and cardiovascular diseases, and impaired immunity.[7] There is also good evidence in the medical literature for an association between vitamin D deficiency and neurocognitive decline.[8]

TAKEAWAY POINTS

- With age, skin tissue becomes progressively stiffer and less able to recoil, changes that parallel those occurring in the cardiovascular and pulmonary tissues.
- The skin of elderly people is markedly less efficient at producing previtamin D_3, due to a drop in 7-dehydrocholesterol levels with age.
- The fibrillar collagens and elastic fibers in the extracellular matrix (ECM) are significantly altered with age, resulting in reduced skin elasticity and an associated remodeling of the dermal ECM.
- As a result of glycation, extracellular matrix components stiffen, causing a decline in skin elasticity.
- A number of natural agents including silymarin, pyridoxamine, and benfotiamine, have been shown to inhibit glycation and the formation of advanced glycation end-products (AGEs).
- AGEs have also lowered levels of hyaluronic acid (HA) by up to 49% during in vitro studies.
- UV radiation suppresses immunity in part by depleting the skin of dendritic cells and enhancing the production of the cytokine interleukin-10.
- Omega-3 fatty acids, green tea, lycopene, melatonin, and silymarin all have properties that can protect against photodamage.
- With age the thickness of the dermis and epidermis is reduced, allowing more toxic substances to elude the skin's barrier defenses, predisposing older adults to enhanced absorption of pesticides, herbicides, and environmental toxins and their release into systemic circulation.
- Topical HA can significantly improve skin hydration, overall elasticity, and reduce wrinkle depth.

WEAKENING OF THE EXTRACELLULAR MATRIX

The extracellular matrix (ECM) plays a critical part in the resilience of many soft tissues, including the skin. The ECM is comprised of collagen fibers and hydrated proteoglycans, which resist tensile and compressive forces. Fibers in the ECM are able to store energy and to use this energy to recoil, allowing for elastic deformation of the skin after pressure is applied.[9,10] Because elastic fiber ECM proteins have a significantly elongated lifespan compared with intracellular proteins, they are predisposed to progressively accumulate damage over time.[11]

The fibrillar collagens and elastic fibers in the ECM are significantly altered with age, resulting in reduced skin elasticity and an associated remodeling of the dermal ECM.[11] This causes older adults to be more vulnerable to skin tears and pressure ulcers.[12] Age-related changes of collagen fibrils also prolongs wound healing and encourages initiation and growth of skin cancer.[13]

ADVANCED GLYCATION END PRODUCTS AND THE SKIN

Aging causes cross-linking between the amino groups of skin proteins and the hydroxyl groups of sugars.[14] This process, known as a glycation, produces toxic compounds called advanced glycation end products (AGEs), which are thought to play a role in age-related ECM dysfunction.[14] Furthermore, AGEs accelerate photoaging of the skin through increasing production of reactive oxygen species during UVA irradiation.[15]

As a result of glycation, extracellular matrix components stiffen, causing a decline in skin elasticity.[14] Diabetes promotes glycation within the skin and body.[14] Exposure to AGEs also occurs after eating AGE-containing foods.[14,16] Some of the more common AGEs include carboxymethyl-lysine (CML), pentosidine, and N-carboxyethyl-lysine (CEL). Levels of each of these AGEs rise by approximately three fold between 20 and 80 years old.[14]

Fibrillin-1, a glycoprotein critical for the formation of elastic fibers found in connective tissue, is particularly susceptible to AGEs.[17]

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Fibroblasts secrete fibrillin-1 into the ECM, where it becomes a part of microfibrils, building a framework for depositing elastin. Increased alteration of fibrillin-1 is linked to the appearance of the AGE CML.[18] Fibrillin-1 dysfunction related to AGE deposition is involved in wrinkle formation and loss of elasticity.[17]

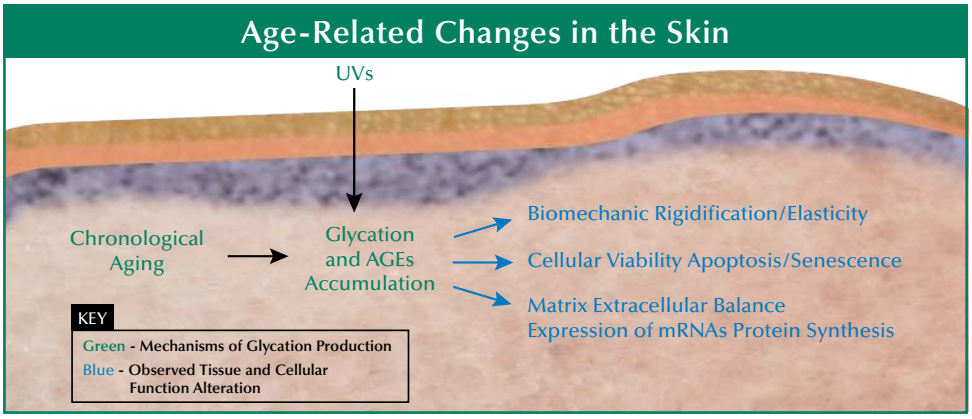
INHIBITING AGES

A number of natural agents have been shown to inhibit glycation and the formation of AGEs. Silymarin (milk thistle) is emerging as an antiglycation agent, with animal studies showing silymarin added to the diet can decrease tissue AGE accumulation, collagen cross-linking, and plasma concentrations of glycated albumin.[19] In a randomized, double-blind trial of diabetic patients, 200 mg of silymarin taken orally three times per day before meals significantly lowered glycosylated hemoglobin (HbA_1C) whereas the placebo group experienced an increase in HbA_1C.[20] Other benefits found with silymarin included reduced cholesterol, LDL-cholesterol, and triglyceride levels as well as a decrease in liver transaminases. Using a human skin explant model, researchers also have found that topical application of *Silybum marianum* (milk thistle) flower extract lowered expression of the AGE CML and stimulated fibrillin-1 expression.[18]

Pyridoxamine and benfotiamine are two other AGE inhibitors. In primary human peritoneal mesothelial cells, benfotiamine decreased the expression of AGEs and their receptor.[21] A similar effect was seen in a rat model of dialysis, where rodents treated with benfotiamine demonstrated reduced expression of AGEs.[23] In a human study, 13 diabetic subjects were fed a high-AGE test meal after being given 1,050 mg/day of benfotiamine for three days.[22] Benfotiamine significantly reduced negative effects normally seen after high-AGE feeding such as impaired flow-mediated dilatation.

Benfotiamine's AGE-lowering mechanism of action is thought to originate from its ability to elevate the levels of intracellular thiamine diphosphate, a cofactor needed for activating transketolase, an enzyme that orchestrates AGE precursors.[23] By blocking the activation of transketolase, benfotiamine reduces tissue level of AGEs.

Pyridoxamine is another antiglycating agent that has been shown to work at the early, intermediate, and late stages of glycation in vitro.[24] Pyridoxamine, a member of the vitamin B_6 family, is converted into pyridoxal-5-phosphate (P5P), the biologically active form of vitamin B_6 in mammals. In skin collagen of streptozotocin-induced diabetic rats, pyridoxamine blocked CML-induced cross-linking by 25%, although it had no effect on the AGE pentosidine.[25] In a study of type 1 and 2 diabetics with diabetic nephropathy, pyridoxamine at doses from 50 mg to 250 mg twice daily suppressed formation of CML and CEL AGEs.[26]



AGEs have also lowered levels of hyaluronic acid (HA) synthesis by up to 49% during in vitro studies.[27] HA is critical to maintaining skin elasticity and its decline may explain a mechanism by which AGE formation results in stiffening of skin. AGEs also trigger the expression of proinflammatory cytokines via NF-kappaB-dependent pathways. In cell culture, high-molecular weight HA suppresses the AGE-related activation of NF-kappaB and the NF-kappaB-regulated cytokines interleukin-1alpha, interleukin-6, and tumor necrosis factor-alpha.[28] The decline in HA triggered by AGEs may produce a vicious cycle whereby AGEs cause a drop in HA levels, resulting in even more AGE-related damage.

It also has been proposed that accumulation of AGEs in the skin could block vitamin D synthesis, and the inflammation and oxidative stress that occurs during vitamin D deficiency could exacerbate the production of AGEs and their toxic effects in smokers.[29]

A LIFETIME OF PHOTODAMAGE

Skin cancer incidence has risen annually and an estimated 3.5 million new cases of cutaneous squamous cell carcinomas (SCC) and basal cell carcinomas (BCC) are diagnosed each year.[30] The incidence of nonmelanoma skin cancer increases with age.[30] Invasive melanoma, which is responsible for most skin cancer deaths, is expected to affect an estimated 73,870 people in the U.S. in 2015.[31] Photodamage from ultraviolet radiation is considered to be a primary cause of skin cancer.[32] Ultraviolet radiation exerts a host of damaging effects including a negative impact on the expression of key genes of extracellular matrix components.[33]

Skin carcinogenesis is not a swift process. It occurs over extended periods through the accumulation of molecular and biochemical alterations in target keratinocytes triggered by UV exposure. The first stage of carcinogenesis (initiation) occurs when UV radiation promotes DNA damage.[34,35] This activates DNA repair processes and reduces the number of mutations that occur after UV exposure, but these repair processes are not able to completely eradicate the mutations in part because ultraviolet radiation damages proteins involved in DNA repair as well as proteins and lipids involved in cellular signaling.[36]

Persisting mutations in the *p53* and *PTCH1* genes result in development of mutant cells that can ultimately become skin cancers.[35] These precancerous cells act as targets for processes that occur during the second stage of skin carcinogenesis (promotion). In this stage, ongoing exposure to UV radiation causes biochemical events such as UV-triggered generation of reactive oxygen intermediates,[34] which push some mutant cells into becoming premalignant actinic keratosis.[34] The reactive oxygen intermediates produced during the promotion stage activate signal transduction pathways that result in the synthesis of a host of proteins, including the enzyme cyclooxygenase-2 (COX-2), which in turn produces prostaglandin E_2.[37] Prostaglandin E_2 triggers a number of events known to spur the development of skin cancer including inflammation, cellular proliferation, and immunosuppression.[37,38] Elevated levels of prostaglandin E_2 have been found in squamous and basal cell cancers and may be associated with an increased tendency for the cancer to metastasize and exhibit invasive behavior.[39]

Immunosuppression is an important aspect of skin cancer development.[30,40] In animal models, suppressed immunity caused by UV radiation encourages tumor growth and

development.[30] Decreased immunity triggered by UV radiation is a risk factor for skin cancer in humans as well. Immunosuppressed organ transplant recipients are more susceptible to cutaneous SCCs and BCCs.[41]

Research indicates that UV radiation suppresses immunity in part by depleting the skin of dendritic cells and enhancing the production of the cytokine interleukin-10.[30] UV radiation also alters responses by mast cells, which results in activation of T and B regulatory cells. This suppresses immunity in the protein products of genes that underwent a UV-related mutation, thus halting the protective function of the immune response and leaving mutated cells undestroyed and able to progress to skin cancer.[36]

Agents that can enhance DNA repair machinery are therefore desirable to protect the skin from photodamage. Sunscreens are not a perfect solution since the most widely used sunscreens contain chemicals with estrogenic activity such as benzophenone-3 (Bp-3), homosalate (HMS), 4-methyl-benzylidene camphor (4-MBC), octyl-methoxycinnamate (OMC), and octyl-dimethyl-PABA (OD-PABA), which are known to disrupt hormones as evidenced by their ability to enhance proliferation of MCF-7 breast cancer cells and increase uterine weight when administered in the feed of rats.[42] In women, higher concentrations of 2,4-dihydroxybenzophenone (2,4OH-BP), a common ingredient in sunscreens which has high estrogenic activity, was associated with an increased risk of developing endometriosis.[43]

As the skin ages and thins, chemicals may be more likely to penetrate the skin,[44] suggesting that the aged may be even more at risk from the hormone-disrupting chemicals in sunscreens. Yet, for patients who remain outdoors for long periods, avoiding sunscreens altogether may not be an option. A good source for determining which sunscreens have a low toxicity is the Environmental Working Group (www.ewg.org).

Both topically and orally, a number of botanicals and nutrients have been shown to protect the skin against photodamage.

OMEGA-3 FATTY ACIDS

Omega-3 polyunsaturated fatty acids (PUFAs) have exerted a number of photoprotective effects in vitro, in vivo, and in human studies. Consumption of omega-3 polyunsaturated fatty acids (PUFAs) significantly reduces PGE_2 levels in the skin compared to omega-6 fatty acids.[45] In mice exposed to UV radiation, omega-3 fatty acids suppress T-cell mediated delayed hypersensitivity.[46] PGE_2 levels also decline in human epidermal keratinocytes exposed to UVB radiation and treated with the omega-3 PUFA eicosapentaenoic acid (EPA).[47] Furthermore, a recent randomized, controlled trial found that oral omega-3 PUFAs reduce UV-related suppression of immune response in human skin.[48] In cultured human melanoma cells, docosahexaenoic acid (DHA) and EPA inhibited cell proliferation, although DHA appeared to have a stronger inhibitory effect.[49] One review investigating PUFA's effect on skin cancer in humans found that individuals with relatively high EPA concentrations and omega-3/-6 ratio had a reduced risk of SCC tumors.[50] In the same review, greater total omega-6, linoleic acid, and linolenic acid concentrations were associated with a decrease in BCC tumor risk. The correlation was strongest in individuals with a skin cancer history.[50]

GREEN TEA

Green tea has exerted photoprotective properties in a number of animal and human studies when used either topically or orally. In hairless mice given green tea in drinking water, the mean time to tumor development after exposure to ultraviolet radiation was extended.[51] Other studies found that the green tea component epigallocatechin-3-gallate or green tea polyphenols had a similar photoprotective effect when applied topically.[52,53] Additional evidence indicated topical administration of green tea polyphenols ameliorated sunburn after UVB exposure in mice and reversed the suppression in immunity that occurs after ultraviolet radiation exposure.[54]

Green tea extracts applied to the skin of humans before UV exposure resulted in a dose-dependent elimination of the erythema response.[55] Green tea both reduces inflammation and inhibits the formation of sunburn cells. Green tea polyphenols block the DNA damage caused by UV radiation, the production of reactive oxygen intermediates, and UVA-induced erythema.[56]

LYCOPENE

A potent antioxidant carotenoid, lycopene has demonstrated photoprotective properties in both animal and human studies. Topically applied lycopene exhibited photoprotective actions in mouse models.[57] In a randomized, controlled trial, 20 women ate 55 grams of tomato paste containing 16 grams of lycopene in olive oil or olive oil alone daily for 12 weeks. The researchers exposed the subjects to ultraviolet radiation. In the subjects eating a high-lycopene tomato paste, UV-induced skin erythema was markedly reduced as was matrix metalloproteinase-1, a biochemical marker of extracellular matrix damage, and mitochondrial DNA 3895bp, a marker of UV-triggered DNA damage.[58]

MELATONIN

Melatonin has garnered some interest for its possible role in preventing photodamage to skin. The human skin and skin cells are involved in melatonin synthesis and melatonin is a potent antioxidant thought to have photoprotective properties.[59] In one review of the medical literature, researchers examined human studies that investigated melatonin's possible role in protecting against UV-induced skin erythema and cellular damage.[60] The reviewers identified four human studies. These studies found that topical melatonin was able to protect against UV-induced skin damage when applied before exposure, but not after exposure. The reviewers noted that melatonin's mechanism of action involved acting directly as an antioxidant and indirectly by influencing gene expression and stabilizing DNA.

Furthermore, skin cancer patients have been found to have lower levels of melatonin.[61] One group of researchers compared 24-hour 6-sulfatoxymelatonin levels in 70 patients with skin cancer and 70 healthy controls. Of the cancer patients, 55 had basal cell carcinoma and 15 had squamous cell carcinoma. The patients without cancer had a mean level of 24-hour urine 6-sulfatoxymelatonin markedly higher compared to the cancer group.[61] The researchers concluded, "It seems that a low level of 24-hour urinary 6-sulfatoxymelatonin renders human beings prone to skin cancer. This association, however, requires further investigation."

SILYMARIN

In both in vitro and mouse models, silymarin has demonstrated an ability to protect photodamaged skin when used topically or orally. In a hairless mouse model, silymarin applied topically blocked UVB-induced skin carcinogenesis. It reduced the percent of mice with tumors as well as tumor multiplicity and tumor size compared to mice not treated with silymarin.[62] Silymarin had a pronounced effect against all the stages of UV-induced carcinogenesis including tumor initiation and promotion.[62] Topically applied silibinin, a primary component of silymarin, also shielded against photocarcinogenesis in hairless mice.[63] When administered orally through the diet, both silymarin and silibinin inhibited photocarcinogenesis by suppressing tumor multiplicity and tumor volume. However, tumor incidence was only moderately reduced. Furthermore, silymarin or silibinin elevated the latency period of tumor appearance when mice were subjected to UVB radiation. Silymarin also significantly

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inhibited UVB-caused sunburn and induction of COX.[64]

LOW-FAT DIET

It has long been known that a high-fat diet made experimental animals more susceptible to UV-induced skin cancers. More than 50 years after the first animal studies in 1939 showed this to be the case, researchers began investigating the effects of a low-fat diet on skin carcinogenesis in humans. In one randomized, controlled clinical trial, subjects with nonmelanoma skin cancer who were put on a diet where fat intake was 20% of total calories consumed developed significantly less new actinic keratosis and nonmelanoma skin cancers compared with those in the control group eating a diet where fat intake was between 37% and 40% of total calories consumed.[65]

In a two-year dietary intervention trial of 101 skin cancer patients, nonmelanoma skin cancer occurrence in the low-fat diet group declined after the first eight months of the study and reached statistical significance by the last eight months.[66] Patients consuming a low-fat diet had markedly fewer cancers in the last eight months compared with control subjects. In addition, the number of patients on the low-fat diet developing skin cancer significantly declined in the last eight months compared with the first eight months. No significant changes occurred in the control group. More recently, researchers have found that high dietary fat intake is associated with an increased risk of SCC in subjects with a history of skin cancer but not BCC.[67]

It is important to urge any patient on a low-fat diet to also avoid consumption of high-fructose corn syrup and an excessive amount of refined carbohydrates, which can all increase exposure to AGEs[16] and likely counteract any beneficial effect of a low-fat diet.

INCREASED ABILITY OF TOXINS TO PENETRATE THE SKIN

The skin is our first line of defense against harmful substances. Yet, with age the thickness of the dermis and epidermis is reduced, allowing more toxic substances to elude the skin's barrier defenses, predisposing older adults to enhanced absorption of pesticides, herbicides, and environmental toxins and their release into systemic circulation.[44,68] Environmental toxins such as organophosphorus and carbamate insecticides and BPA are known to be easily absorbed through intact and unbroken skin of healthy, younger adults[44,69] and the reduction in skin integrity that occurs with age may accelerate the rate and amount of absorption of toxins through the skin.[44]

CLINICAL APPLICATIONS

Aging Skin

Here is a suggested regimen for improving the appearance and the health of our patients' skin:

- Test vitamin D levels. If deficient, recommend at least 2,000 IU per day. Follow up with another test within three months after the patient has begun supplementation to make certain his/her vitamin D levels are 55 nmol/L to 75 nmol/L.
- To inhibit AGE formation in the skin, recommend a diet low in sugar and refined carbohydrates combined with the following dietary supplements:
  - Silymarin (milk thistle) both oral and topical
  - Pyridoxamine
  - Benfotiamine
  - Topical and oral HA (to replenish stores depleted by AGEs)
  - Vitamin D_3 (Depending on results of test as noted above)
- To protect the skin against photodamage, recommend a sunscreen that contains nontoxic ingredients. The Environmental Working Group (www.ewg.org) maintains a list of nontoxic sunscreens.
- Recommend the following dietary supplements (or sunscreens containing these ingredients):
  - Green Tea
  - Lycopene
  - Silymarin
  - Melatonin
- A low-fat diet may decrease the risk of nonmelanoma skin cancers but caution patients eating a low-fat diet to also avoid AGE-containing foods such as refined carbohydrates and high-fructose corn syrup.
- To improve the appearance of aging skin recommend:
  - Topical HA
  - A diet high in vegetables, olive oil, legumes, and vitamin C and low in sugar and refined carbohydrates

IMPROVING THE APPEARANCE OF AGING SKIN

The unseen changes that are taking place beneath the skin's surface, such as skin carcinogenesis, are out of sight, out of mind to many of our patients. They are often most concerned with eliminating wrinkles and enhancing the suppleness of the skin. Consequently, at the same time we are recommending strategies to protect against photodamage and AGE accumulation, we can also recommend hyaluronic-acid based moisturizers. Topical HA has been shown to improve the appearance of the skin. In one study, 76 women between 30 and 60 years of age who had clinical signs of periocular wrinkles applied topical formulations of HA in varying molecular weights to the skin around one eye for 60 days. The women applied a control cream around the other eye. HA treatment resulted in significantly improved skin hydration and overall elasticity compared to placebo. Wrinkle depth also significantly improved in the women using HA molecular weights of 130 kDa and 50 kDa compared to the placebo-treated area.[70]

Furthermore, two groups of researchers have found that diet can influence the rate of skin aging. Purba et al used a food frequency questionnaire to determine dietary intake and then examined skin wrinkling in subjects. The researchers found that higher intakes of vegetables, olive oil, and legumes and lower intakes of butter, margarine, dairy, and sugar products may result in less skin wrinkling.[71]

Cosgrove et al investigated the nutrient intake of women 40 to 74 years old using a 24-hour dietary recall and then clinically examined the skin. The subjects who ate the most vitamin C were less likely to have a wrinkled appearance or skin dryness. The women who ate the most linoleic acid were less likely to have skin dryness or atrophy. Higher fat and carbohydrate intakes were associated with an increased likelihood of a wrinkled appearance and skin atrophy.[72]

CONCLUSION

The aging skin is subject to a lot of alterations caused by insults such as accumulation of AGEs and exposure to ultraviolet radiation. Cutaneous thinning with age also allows for an increased number of toxins to pass through the weakening skin barrier. A variety of botanicals and other natural substances can help reduce AGE accumulation and DNA damage, thereby restoring the skin's integrity.

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Were the New York Attorney General's Demands on Supplement Retailers Justified?

By Shana Rheault, MS

Many of you have probably heard by now that the New York attorney general (NYAG) has demanded that four dietary supplement retailers—Walmart®, Walgreens®, Target®, and GNC®—remove herbal supplement products from store shelves.[1] The NYAG's demands came after his office hired a researcher to conduct DNA barcode testing of herbal products, which concluded that the majority (79%) of herbal supplements purchased from the four retailers did not contain the ingredients they claimed to contain. The NYAG alleged that DNA bar code testing found that many of the products were also adulterated containing a variety of undeclared botanical ingredients.

The NYAG purchased and DNA barcode tested six kinds of herbal supplements from each retailer. His office bought the "Herbal Plus" brand from GNC®, the "Up & Up" brand from Target®, the "Finest Nutrition" brand from Walgreens®, and the "Spring Valley" brand from Walmart®. The herbal supplements tested included *Ginkgo biloba*, St. John's wort, ginseng, garlic, *Echinacea*, Valerian root, and saw palmetto. The DNA testing found that only 21% of the products tested contained DNA from the botanicals listed on the labels. DNA from substances not listed on the labels such as rice, beans, pine, asparagus, primrose, alfalfa/clover, spruce, saw palmetto, Echinacea, wheat, citrus, garlic, and houseplants was also found. These substances were not disclosed on the label making the products adulterated and mislabeled if the test results are accurate.

On February 23, 2015, the NYAG widened the investigation to include other nutritional supplement retailers. He has requested that Nature's Way®, NBTY™, Nutraceutical, and Pharmavite® submit what will likely become hundreds of thousands of pages of documents regarding products they sell in New York state.

The NYAG allegations certainly raise questions about the quality of retail nutritional supplements in general and herbal supplements in particular purchased at large retail chains. But many nutritional supplement industry trade organizations such as the Council For Responsible Nutrition and Alliance for Natural Health USA have criticized the New York attorney general for inappropriately using DNA barcode testing to authenticate contents of the dietary supplements. These organizations claim that DNA barcode testing, which examines the sequences from a standard DNA region in order to identify a species, may not be an accurate measurement of ingredients found in herbal supplements. We were curious to know if this is an accurate claim or if this was just a defense used to protect these companies. After reading a white paper[2] released by industry DNA barcode testing experts as well as reviewing relevant scientific papers,[3,4] here is what we have concluded.

The majority of research on DNA barcoding has investigated its use in substances that are fresh or living, but almost no research exists on its use for botanical extracts in dietary supplements.

WHAT IS DNA BARCODE TESTING?

The concept of DNA barcoding is based on the fact that genomes contain highly conserved regions of both coding and non-coding DNA that during the evolution vary to a highly minor degree within a species. Conserved sequences suggested to be useful in DNA barcoding include cytoplasmic mitochondrial DNA (e.g. *cox*1) for animals, chloroplast DNA (e.g. *rbcL, trnL-F, matK, ndhF,* and *atpB*) for plants, and nuclear DNA (ITS, and housekeeping genes e.g. GAPDH) for plants. Prerequisites for accurate DNA barcode testing include intact cellular material and preserved strands of DNA in the sample to be tested. DNA barcode testing of animals has proved to be less problematic than barcode testing for plants hence testing of plants should be done in laboratories experienced with plant DNA barcode testing. Strict controls are necessary to prevent sample contamination from ambient DNA. Finally, identification is based on comparison to DNA results in standardized databases. At present, publicly available, validated DNA sequence databases do not exist for many botanicals including *Echinacea* and St. John's wort. As a consequence, ambiguous results and misidentifications may occur.

WHAT ARE THE LIMITS OF THE NYAG DNA BARCODE TESTING?

Four industry experts, including Brent D. Mishler, PhD, a professor in the Department of Integrative Biology at the University of California, Berkeley, and Paula N. Brown, PhD, Director of the Natural Health and Food Products Research Group at the British Columbia Institute of Technology, recently released a white paper that specified all the inherent problems of using DNA barcode testing for herbal supplements and why the attorney general's use of this type of testing may have been inappropriate. We believe that the arguments made in this paper are scientifically valid.

The white paper authors noted that the FDA has validated DNA barcode testing as being especially suited to identifying "fresh or living tissue from distinct species such as cows and pigs, or for fish such as tuna or snapper."[2] DNA barcoding has been successfully used in plant materials to distinguish major plant groups such as grasses and pine trees, but not yet to determine exact species.[2]

The majority of research on DNA barcoding has investigated its use in substances that are fresh or living, but almost no research exists on its use for botanical extracts in dietary supplements.[2] That's because extraction methods may remove plant DNA entirely or significantly degrade the DNA sequences needed for accurate testing. So although DNA barcode testing, when done properly, may be able to authenticate many whole herbal preparations, using such technology to identify and verify botanical extracts, which contain no or poor quality DNA, is inappropriate.[2]

Furthermore, the scientist hired to perform the study has no experience in authenticating botanicals using DNA—his work in the past has involved DNA analysis of reptiles, which is clearly less challenging.[5] As noted earlier, there is a major difference between performing DNA barcode testing in animals and its use in botanicals. The laboratory the researcher used also does not appear to be certified for testing in compliance with FDA current good laboratory practices (GLP) for nonclinical laboratories or other standards for quality.

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The fact that unexpected contaminants were found in the dietary supplements also has experts wondering if contamination occurred while the samples were being tested in the laboratory. While rice and wheat could be used as fillers in some dietary supplements, and if used should be disclosed on the label as required by FDA mandated current good manufacturing practices (cGMP), the finding of unusual contaminants such as asparagus, "houseplants," and garlic are not expected adulterants. The fact these adulterants were found in independent samples derived from different suppliers leaves open the possibility of contamination that occurred environmentally and/or between samples in the laboratory. That's why, for accurate results, most researchers would have not only implemented procedures to avoid and eliminate contamination, but also would have retested samples that produced unexpected results in order to avoid false positives.

Even Dr. Pieter Cohen, an assistant professor of medicine at Harvard Medical School, who is the author of a number of studies on adulterated supplements, told *Live Science* he is skeptical of the results of the attorney general's investigation.[6] Cohen pointed out that single-ingredient products like the ones the attorney general had tested are much more likely to contain the ingredient listed on the label compared to formulations. In his interview with *Live Science*, Cohen said he would have expected about 90% of the supplements tested in the attorney general's study to contain the ingredient on the label. "If I had this kind of surprising, counterintuitive results, I would do additional tests," said Cohen.

The American Botanical Council agrees.[7] It criticized the attorney general for using one testing technology from only one laboratory. The DNA barcode test results should have been confirmed with "microscopic analysis and other validated chemical testing," the American Botanical Council said in a recent statement.

NOT THE FIRST TIME THIS HAS HAPPENED

The attorney general-sponsored DNA barcode study was not the first negative study using DNA barcoding to test herbal supplements. On October 11, 2013, a paper titled, "DNA barcoding detects contamination and substitution in North American herbal products" was published in *BMC Medicine*.[8] In the study, researchers used DNA barcoding to test herbal products. The study found that "most of the herbal products tested were of poor quality, including considerable product substitution, contamination and use of fillers," and that "some of the contaminants we found pose serious health risks to consumers."

However, the American Botanical Council heavily criticized the *BMC Medicine* study, which had some of the same flaws as the more recent attorney general sponsored study. The American Botanical Council does not believe the *BMC Medicine* study was properly executed and asked for the article to be retracted, corrected, revised, and resubmitted.[9] Among the problems in the *BMC Medicine* paper was the authors' disregard for the fact that many herbal substances used in dietary supplements are not simply dried powdered plant material. Instead, they are often either extracts or extracts spray-dried onto a carrier such as rice powder. Because the study authors did not provide label facts for any of the herbal products tested, it was impossible for the American Botanical Council to verify the study authors' claims that the fillers they found in the supplements were not mentioned on the labels.

DNA BARCODING NOT AN APPROPRIATE TEST FOR HERBAL EXTRACTS

It remains unclear whether herbal supplements from large retailers are mislabeled, adulterated, and do not supply the statement herbal ingredient as alleged by the NYAG. GNC has been particularly aggressive in defending its products and testing them using validated methods. The other retailers have been remarkably silent. What is clear is that DNA barcode testing is challenging for botanical supplements and entirely inappropriate for botanical extracts. Based on testing methods, the NYAG's claims may have no merit. It is also clear that consumers should purchase their herbal supplements from manufacturers that are independently certified as following cGMP with their mandated identity and purity testing requirements.

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DID YOU KNOW?

- Over 70 years of a human's life, aortic elastic fibers experience more than three billion extension and recoil cycles without being repaired or replaced and are therefore susceptible to damage, leading to increased arterial stiffness and a reduced lifespan.
- **Exercise may increase vitamin D levels due to a short-lived rise in parathyroid hormone.**
- A mutation in the methylenetetrahydrofolate reductase (MTHFR) gene, a gene which is involved in metabolizing folate and homocysteine (HCY), may increase the risk of erectile dysfunction.
- **Benzodiazepine drugs, which are commonly prescribed for anxiety, may increase the risk of dementia.**
- Berberine given to women with polycystic ovarian syndrome (PCOS) prior to in vitro fertilization (IVF) increases the chance of becoming pregnant. In one study, berberine resulted in more live births and fewer side effects compared to the drug metformin.
- **An hour-long nap improves memory.**
- Oral melatonin supplementation improves sleep quality much better than an eye mask and earplugs in subjects sleeping in a noisy, light-illuminated area designed to mimic the intensive care unit (ICU).
- **Women with gestational diabetes are seven times more likely to suffer from obstructive sleep apnea compared to pregnant women without gestational diabetes.**

Advances in the Management of Urogenital Infections and Vulvovaginal Atrophy

By Marina MacDonald, PhD

OVERVIEW

Urogenital infections are a major reason that women visit their family physician. Globally, an estimated one billion women have bladder or vaginal infections each year.[1] The epidemiological association between the vaginal microbiota and urogenital infections, and between urogenital infections and obstetric pathologies, suggests the need to better manage and prevent these conditions. The development of antibiotic resistance in *E. coli* also drives the search for alternative remedies for UTIs. In this article I highlight scientific advances in the management of urogenital infections and vulvovaginal atrophy.

BACTERIAL VAGINOSIS

Bacterial vaginosis (BV) affects up to 30% of all reproductive-age women. Vaginal itching, discomfort, and discharge are often the only symptoms, and many women with BV are asymptomatic.[2,3] BV increases the risk for sexually transmitted diseases, pelvic inflammatory disease, and obstetric complications.[4-6] Bacterial ascent from the vaginal tract is the primary cause of intrauterine infection, which in turn is a leading cause of premature birth and small birth weight infants.[5,7-9]

The healthy vulvovaginal microbiota is dominated by *Lactobacillus* species.[10-12] Commensal *Lactobacillus* species protect against the invasion or overgrowth of opportunistic microbes by producing bacteriocins, hydrogen peroxide, and lactic acid.[13-17] BV is characterized by a decrease in *Lactobacillus* populations and an increase in *G. vaginalis*, *Atopobium vaginae*, and other gram-negative anaerobes.[18-21] *G. vaginalis* forms biofilms, which can persist after treatment and can migrate to the endometrium.[3,22] The depletion of vaginal lactobacilli increases the risk for sexually transmitted diseases.[23-25]

Metronidazole is an effective treatment for BV.[26] However, BV-associated microbes often reappear, leading to disease recurrence in 30% to 40% of BV cases.[27,28] Oral or vaginal probiotics containing certain *Lactobacillus* species have the potential to improve antibiotic efficacy, and to prevent recurrences of BV.[16,27,29-32] *Lactobacillus* species that have shown promising effects include *L. acidophilus, L. rhamnosus*, and a patented proprietary mixture of *L. rhamnosus* GR-1 and *L. reuteri* RC-14, which improved the resolution of BV as compared with a placebo.[16,27,30,33,34] In another controlled study, the vaginal administration of *L. rhamnosus, L acidophilus*, and *Streptococcus thermophilus* decreased the recurrence rate of BV throughout an 11-month follow-up period.[35] Generally, oral probiotics showing efficacy have utilized doses of at least 10^9 colony forming units (CFU)/day.

Probiotic microorganisms that are ingested are believed to ascend to the vaginal tract after they are excreted from the rectum.[36] The daily administration of *Lactobacillus* (*L. fermentum, L. plantarum,* and *L. gasseri*) results in the predominance of the strains in vaginal and rectal samples within 30 to 60 days.[37] Studies using shorter courses of administration, of two weeks or less, may not produce lasting effects.[38] Larger controlled trials will be needed to identify optimal compositions and regimens.[39]

Topical vitamin C and other acidifying agents can help to maintain a low vaginal pH, thereby compensating for low levels of *Lactobacillus* associated with BV.[40,41] In a randomized, double-blind, placebo-controlled trial, the regular use of 250 mg ascorbic acid vaginal tablets after successful treatment of BV reduced the risk of recurrence from 32% to 16%.[41] In another study, probiotics (*L. brevis, L. salivarius* subsp. *salicinius* and *L. plantarum*) were combined with acidifying excipients including vitamin C.[32] The probiotic mixture was found to be better than acidification alone in preventing BV in healthy subjects.[32]

VULVOVAGINAL CANDIDIASIS

VVC is the second most common cause of vaginal infections and it is diagnosed in up to 40% of women with vaginal complaints.[42-45] Approximately 75% of all women will experience an episode of acute VVC in their lifetime, with another 5% to 10% developing recurrent VVC.[44,45] The risk for VVC increases with oral contraceptive usage, pregnancy, uncontrolled diabetes, and long-term antibiotic treatment.[42,43,45,46]

Most cases of VVC are due to an overgrowth of *Candida albicans* (*C. albicans*), which also can colonize 20% to 60% of asymptomatic women.[10,42] The management of VVC typically involves the administration of oral or topical antifungal agents.[47] Recurrent VVC requires initially high doses of antifungal agents for two weeks followed by long-term weekly or monthly therapy.[47]

The role of dysbiosis in the development of VVC is currently a topic of debate.[48] Although a loss of *Lactobacillus* is not a prominent feature,[49] the vaginal microbiota are suspected to play a role in resistance to infection.[10,50] Changes in vaginal fungal diversity have been observed in association with recurrent VVC.[51,52] Various *Lactobacillus* species, including *L. rhamnosus* and *L. reuteri*, have antifungal activity and can compete with *Candida* species for binding to epithelial cells.[13,45,53-56] Several studies support the use of probiotics as an adjunct to conventional antifungal treatments.[13,16,57,58] Probiotics also have been shown to be effective in preventing enteric infections with *Candida* in the setting of the prenatal ICU.[59]

Because of the high rate of recurrence of VVC, there is a need for new approaches. Berberine is a plant alkaloid with a long history of use in traditional medicine. In vitro studies showed that berberine has antifungal activity against *C. albicans* and *C. glabrata* and that it exerts synergistic effects with fluconazole.[60,61] Cranberry extracts also have strong activity against *C. albicans* biofilms in vitro.[62]

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TAKEAWAY POINTS

- Urinary tract infections, bacterial vaginosis, and vulvovaginal candidiasis are the most common infectious disorders faced by women.
- As an adjunct to antibiotic and antifungal treatment, probiotics containing *Lactobacillus* may help to ameliorate infections and prevent recurrences.
- Cranberry extracts, berberine, and D-mannose have the potential to address the pathophysiology of urinary tract infections.
- Vitamin C shows promise for reducing recurrences of bacterial vaginosis.
- As potential alternatives to estriol, hyaluronic acid and soy isoflavones may ameliorate vaginal dryness in women with vulvovaginal atrophy.

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VULVOVAGINAL ATROPHY

Vulvovaginal atrophy (VVA), also known as atrophic vaginitis, is a common condition associated with decreased estrogen levels. In postmenopausal women, the prevalence of VVA is close to 50%. The vaginal epithelium becomes thinner and loses its elasticity, and there is a marked decrease in secretions, resulting in vulvovaginal dryness.[63] Estriol can reverse the mucosal changes associated with VVA, while vaginal moisturizers and lubricants provide symptomatic relief.[63,64]

VVA is associated with diminished levels of *Lactobacillus*.[65-68] In a placebo-controlled trial, the vaginal administration of a proprietary mixture of *Lactobacillus acidophilus* with low-dose estriol was superior to a placebo with respect to epithelial changes associated with VVA.[69] A vaginal gel containing soy isoflavones has been shown to relieve symptoms of VVA.[70] Hyaluronic acid (HA) vaginal tablets may ameliorate vaginal atrophy and dryness nearly as well as estriol.[71-74] A preliminary study suggests that low-molecular-weight HA may be administered orally.[75]

One clinical trial suggests that orally administered D-mannose (2 g/day for six months) may help to prevent recurrent UTIs.

URINARY TRACT INFECTIONS

Approximately half of all women will experience a urinary tract infection (UTI) at some point in their lives.[76,77] At least 80% of UTIs are caused by uropathogenic *E. coli*. The standard treatment for UTI is antibiotic therapy with trimethoprim-sulfamethoxazole (TMP-SMX) or with nitrofurantoin. Even after treatment, 25% of patients will experience a recurrence within six to 12 months.[76] TMP-SMX, which is used for the prophylaxis of recurrent UTIs (rUTIs), often results in the emergence of antibiotic resistance.

Depletion of vaginal lactobacilli increases the risk for UTI, which suggests that repletion with probiotics may be beneficial. A double-blind, placebo-controlled trial of a *L. crispatus* intravaginal suppository showed that it reduced rUTIs in premenopausal women as compared with women who received a placebo.[78] Although further studies are needed, increasing evidence suggests that use of *Lactobacillus* probiotics holds promise.[76]

Natural products, particularly cranberries, have been used extensively to prevent recurrence of UTIs. Studies have been done to compare the effectiveness of cranberry tablets (500 mg twice daily) or *Lactobacillus* (*L. rhamnosus* GR-1 and *L. reuteri* RC-14) to the effectiveness of TMP-SMX.[79,80] Although the antibiotics were superior to probiotics or cranberry at preventing rUTIs, antibiotic resistance developed in 80% to 95% of recurrences, suggesting that the natural alternatives are worthy of consideration.[81,82] In a study of two cases of antibiotic-resistant UTIs, a regimen based on probiotics (*L. rhamnosus, L. acidophilus, S. thermophilus, B. bifidum,* and *L. bulgaricus*) and cranberry extract (700 mg/day) was administered together with several other natural ingredients such as garlic.[83] The natural remedy resulted in the relief of symptoms and prevention of recurrence for more than 12 months.[83]

The bioactive substances in cranberries are proanthocyanidins, which can block *E. coli* adherence to epithelial cells.[84] Clinical studies have utilized a variety of cranberry preparations with unknown concentrations of proanthocyanidins, producing heterogeneous results.[85] Thus, a 2012 Cochrane review suggested that cranberry juice is less effective than previously indicated for UTIs,[86] while a 2012 meta-analysis concludes that cranberry products provide a protective effect.[87] Two well-controlled randomized trials showed that cranberry products reduced the incidence of rUTIs as compared with control interventions.[87-89] Further studies are needed to assess the optimal formulation, dosage, and bioactivity of cranberry products for UTIs.

Uropathogenic *E. coli* invades epithelial cells by binding to mannosylated receptors on epithelial cells. One clinical trial suggests that orally administered D-mannose (2 g/day for six months) may help to prevent rUTIs.[90] In a study of 308 women with a history of rUTIs, 98 patients (32%) had a recurrence within six months; 62 recurrences were in the control group, 15 in the D-mannose group, and 21 in the nitrofurantoin group.[90] This study extends work that began 30 years ago, suggesting that D-mannose may be useful for the prevention of UTIs.[91] D-mannose is a simple sugar found in many fruits, and it also occurs naturally in the human body. Synthetic mannose derivatives (mannosides) also have the potential to block the adhesion of *E.coli* to bladder epithelium and prevent infection, and these derivatives are being pursued for therapeutic applications.[77, 92]

CLINICAL APPLICATIONS
Women's Health

Here is a suggested approach to be used in female patients suffering from urogenital infections or vulvovaginal atrophy:

- When treating urogenital infections, keep in mind antibacterial and antifungal treatments address the symptomology but do not address the dysbiosis associated with many infections.
- Oral or vaginal probiotics containing *Lactobacillus* can potentially be used to restore the normal flora and reduce recurrences of infections.
- Certain natural products can promote a healthy vaginal pH (vitamin C), ameliorate atrophic vaginitis (hyaluronic acid, soy isoflavones) and prevent adhesion of opportunistic pathogens to urogenital epithelial cells (berberine, cranberry, D-mannose). Combinations of probiotics and natural products may be effective.[93]
- Based on a promising clinical trial, D-mannose may be useful for the prevention of recurrent UTIs.

CONCLUSION

Urogenital infections often are associated with a disruption of the normal healthy microbiota, allowing opportunistic microbial and fungal organisms to proliferate. *Lactobacillus* is thought to antagonize the growth of organisms associated with vaginosis, vaginitis, and UTIs, including *Gardnerella, Candida*, and *E. coli*, respectively. Therefore, the regular use of *Lactobacillus*-containing probiotics may be a reasonable strategy to prevent urogenital infections, and to aid in the restoration of the microbiota after antibiotic treatment. Berberine, cranberry, vitamin C, and D-mannose may be helpful in the management of some infections as indicated in this review. Also, hyaluronic acid has the potential to improve the symptoms of VVA.

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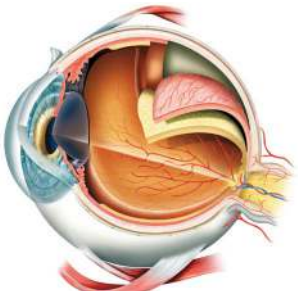
The full list of references is available upon request.

KOREAN GINSENG IMPROVES DRY EYES ASSOCIATED WITH GLAUCOMA



In January 2015, researchers reported that supplementation with Korean red ginseng improved symptoms of dry eye associated with glaucoma treatment. The American Academy of Ophthalmology estimates that 3.2 million women and 1.68 million men age 50 and over are affected by dry eye syndrome. Furthermore, over 2.2 million Americans have glaucoma.

Researchers evaluated 49 subjects with glaucoma and treated with antiglaucoma eye drops resulting in dry eye symptoms. The subjects received 3 grams per day Korean red ginseng or placebo daily for eight weeks. The investigators assessed tear film stability, fluorescein corneal staining for corneal damage, conjunctival hyperemia (blood flow), tear production, meibomian gland dysfunction, and dry eye symptoms using the Ocular Surface Disease Index questionnaire at the beginning of the study and again after the supplementation period.

All of the subjects had dry eye signs and symptoms at the beginning of the study. Supplementation with Korean red ginseng significantly improved the tear film stability and total Ocular Surface Disease Index score compared to placebo.

The study authors stated, "Korean Red Ginseng supplementation may provide an additional treatment option for dry eye and patients with glaucoma using antiglaucoma eye drops."

REFERENCE:
Bae HW, et al. J Ginseng Res. 2015;39:7-13.

PROBIOTIC INFLUENCES INSULIN RESISTANCE AFTER OVEREATING

A study published in February 2015 indicates that probiotic supplementation supports insulin sensitivity after overeating. Low insulin sensitivity is known as insulin resistance, which is a condition in which larger amounts of insulin is required to keep blood glucose stable.

The investigators randomly assigned 17 healthy subjects to receive a fermented milk drink with the probiotic *Lactobacillus casei* Shirota twice daily for four weeks or no supplementation as the control group. The researchers instructed the subjects to maintain their normal diet for the first three weeks of the study. During the fourth week of the study, the subjects ingested a high-fat (65% of energy), high-energy (50% increase in energy intake) diet. The researchers assessed insulin sensitivity using the oral glucose tolerance test before and after the week where the subjects ate the high-fat and high-energy diet.

The investigators showed that body mass index increased in the control group by 0.6 kg and increased in the probiotic group by 0.3 kg. In the control group, fasting plasma glucose levels increased from 5.3 mmol/L prior to the over-feeding week to 5.6 mmol/L after the week of over-feeding. The control group also had a 10% increase of glucose area under the curve (AUC) and a decrease in whole-body insulin sensitivity by 27% after the week of over-feeding. In the probiotic group, normal insulin sensitivity was maintained before and after the week of over-feeding.

The researchers concluded, "These results suggest that probiotic supplementation may be useful in the prevention of diet-induced metabolic diseases such as type 2 diabetes."

REFERENCE:
Hulston CJ, et al. Br J Nutr. 2015;113:596-602.

ATOPIC DERMATITIS IMPROVES WITH PROBIOTIC SUPPLEMENTATION

Probiotic supplementation improves symptoms of atopic dermatitis (eczema), a study epublished in January 2015 reported. It is estimated that 9% to 30% of Americans have atopic dermatitis.

The subjects included 220 children aged one to 18 years with moderate-to-severe atopic dermatitis. The researchers randomly assigned the subjects to receive 1) *Lactobacillus paracasei*, 2) *Lactobacillus fermentum*, 3) *Lactobacillus paracasei* plus *Lactobacillus fermentum*, or 4) placebo for three months.

The investigators assessed changes in scores on the Scoring Atopic Dermatitis (SCORAD), Children's Dermatology Life Quality Index (CDLQI), and Family Dermatology Life Quality Index (FDLQI). The researchers also evaluated skin prick tests, levels of immuno-globulin (Ig)E associated with allergic conditions, and cytokines including interferon-gamma, interleukin (IL)-4, transforming growth factor (TGF)-beta, and tumor necrosis factor (TNF)-alpha. Additionally, the researchers measured 8-Oxo-2-deoxyguanosine (8-OHdG), a marker of oxidative stress, and urine eosinophilic protein X, a marker of eosinophil degranulation, which is a type of white blood cell involved in allergic reactions.

The researchers determined that the *Lactobacillus paracasei, Lactobacillus fermentum,* and *Lactobacillus paracasei* plus *Lactobacillus fermentum* groups had lower SCORAD scores compared to the placebo group and this remained even at four months after discontinuing the probiotics. Similarly, the investigators showed that FDLQI and CDLQI scores were lower in all three probiotic groups compared to the placebo group. Although not statistically significant, the researchers also found that IgE, TNF-alpha, urine eosinophilic protein X, and 8-OHdG levels decreased, whereas IFN-gamma and TGF-beta increased in the probiotic groups. Additional analysis showed that SCORAD scores significantly decreased after probiotic supplementation (especially in children younger than age 12), with breastfeeding greater than six months, and with mite sensitization.

The researchers concluded, "Supplementation of a probiotic mixture of *Lactobacillus paracasei* and *Lactobacillus fermentum* is associated with clinical improvement in children with atopic dermatitis."

REFERENCE:
Wang IJ, et al. Clin Exp Allergy. 2015 Jan 20. [Epub ahead of print.]

ALA AND EPA PROMOTES WEIGHT LOSS

Researchers reported in February 2015 that supplementation with alpha-lipoic acid and eicosapentaenoic acid (EPA) supports weight loss in overweight or obese women. Approximately two-thirds of American adults are overweight with more than one-third obese. Of middle-aged adults age 40 to 59, an estimated 40% are obese. Interestingly, over the past 35 years, obesity rates have more than doubled. The average American is more than 24 pounds heavier today than in 1960.

Investigators randomly assigned 97 overweight or obese women to receive: 1) 1.3 grams per day EPA, 2) 0.3 grams per day alpha-lipoic acid, or 3) EPA plus alpha-lipoic acid daily for 10 weeks. The subjects followed an energy-restricted diet of 30% less than total energy expenditure. At the beginning of the study and again after the intervention period, the researchers evaluated body

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weight, anthropometric measurements, body composition, resting energy expenditure, blood pressure, serum glucose, and insulin and lipid profiles. The investigators also measured serum levels of the hormones leptin, which is involved in appetite suppression, and ghrelin, which stimulates appetite.

The groups receiving alpha-lipoic acid had significantly higher body weight loss. EPA supplementation significantly attenuated the decrease in leptin levels that occurs during weight loss. Additionally, the researchers determined that body weight loss improved lipid and glucose metabolism but without significant differences between the groups.

The investigators stated, "The intervention suggests that alpha-lipoic acid supplementation alone or in combination with EPA may help to promote body weight loss in healthy overweight/obese women following energy-restricted diets."

REFERENCE:
Huerta AE, et al. Obesity (Silver Spring). 2015;23:313-21.

FISH OIL SUPPLEMENTATION STUDIED FOR COGNITION AND CARDIOVASCULAR FUNCTION

In a study published in January 2015, researchers found that fish oil supplementation improves measures of aortic blood pressure and aortic stiffness. The Centers for Disease Control and Prevention (CDC) state that approximately one in three adults has high blood pressure and another one in three adults has prehypertension.

In this clinical trial, investigators randomly assigned 160 healthy adults 50 to 70 years of age to receive: 1) a multivitamin plus 3 grams of fish oil including 240 mg eicosapentaenoic acid (EPA) and 240 mg docosahexaenoic acid (DHA), 2) a multivitamin plus 6 grams of fish oil including 480 mg EPA and 480 mg DHA, 3) 6 grams of fish oil without a multivitamin, or 4) a placebo. The researchers evaluated cognitive performance, red blood cell fatty acids, brachial (arm) blood pressure, and aortic (central) blood pressure at the beginning of the study and again after six and 16 weeks.

The primary cognitive outcomes were not different between the groups, although increases in the red blood cell omega-3/6 ratio were associated with improvements in spatial working memory. The group who received 6 grams of fish oil without the multivitamin displayed a significant decrease in aortic pulse pressure and aortic augmentation pressure, measures of aortic blood pressure and aortic stiffness.

The researchers stated, "Fish oil decreased aortic pulse pressure and augmentation pressure. Reductions in aortic blood pressure were not accompanied by consistent improvements in cognition."

REFERENCE:
Pase MP, et al. J Am Coll Nutr. 2015;34:21-31.

MELATONIN RELATED TO POSTMENOPAUSAL WEIGHT GAIN

Melatonin deficiency may play a role in postmenopausal weight gain, researchers recently discovered. Previous research shows that melatonin secretion decreases with advancing age with significant changes in women after age 40.

The subjects included 90 pre- and postmenopausal women. The researchers divided the women into three groups: 1) women without menstrual disorders to serve as the control group, 2) postmenopausal women without change in appetite and body weight, and 3) postmenopausal women with increased appetite and weight gain. The investigators measured serum melatonin, 17-beta-estradiol, the pituitary hormone follicular stimulating hormone (FSH), and urine 6-sulfatoxymelatonin, a melatonin metabolite.

The level of melatonin and estradiol were lower and FSH was higher in the postmenopausal women compared to the control group. As body mass index increased, urine 6-sulfatoxymelatonin excretion decreased. The data also showed that FSH levels correlated with body mass index, particularly in the overweight women.

The investigators concluded, "The obtained results indicate a significant effect of melatonin deficiency on the process of weight gain in postmenopausal women and justify its use in treatment of these disorders."

REFERENCE:
Walecka-Kapica E, et al. Int J Mol Sci. 2015;16:1030-42.

LOW VITAMIN D LEVELS CORRELATE WITH DEPRESSIVE SYMPTOMS

In March 2015, a study reported that lower concentrations of serum vitamin D are associated with increased risk of depressive symptoms. The Anxiety and Depression Association of America states that an estimated 14.8 million American adults have a major depressive disorder in a given year, with an additional 3.3 million adults with Persistent Depressive disorder, which is a more chronic form.

Researchers measured serum 25-hydroxyvitamin D levels in 1,786 healthy subjects between 19 to 69 years of age. They assessed depressive symptoms using the Center for Epidemiologic Studies Depression Scale.

The investigators determined that 92% of the subjects had suboptimal vitamin D levels defined as less than 30 micrograms/L. The researchers found that as serum vitamin D levels decreased, the risk of depressive symptoms increased. More specifically, the researchers showed that the risk of depressive symptoms decreased by 25% among the subjects with serum vitamin D levels of 20 to 29 mcg/L and decreased by 34% among the subjects with a serum vitamin D level of 30 mcg/L or higher compared to the subjects with serum vitamin D levels of less than 20 mcg/L. Further analysis showed that even after controlling the data for leisure-time physical activity and shift work, the subjects with the highest serum 25-hydroxyvitamin D had a 30% decreased risk of depressive symptoms.

The investigators stated, "Results suggest that lower concentrations of circulating vitamin D are associated with increased likelihood of having depressive symptoms among apparently healthy workers."

REFERENCE:
Mizoue T, et al. J. Nutr. 2015;145:541-6.

PARKINSON'S DISEASE ASSOCIATED WITH VITAMIN D DEFICIENCY

Low vitamin D levels are associated with an increased risk of Parkinson's disease, a study epublished in December 2014 reports. The National Parkinson Foundation indicates that 50,000-60,000 new cases of Parkinson's disease are diagnosed in the U.S. each year.

The subjects included 478 individuals with Parkinson's disease and 431 control subjects. The investigators measured levels of total 25-hydroxyvitamin D, 25-hydroxyvitamin D_2 (which is primarily from diet and supplements), and 25-hydroxyvitamin D_3 (primarily derived from sunlight exposure and vitamin D_3 supplements). Vitamin D insufficiency was defined as a total 25-hydroxyvitamin D level

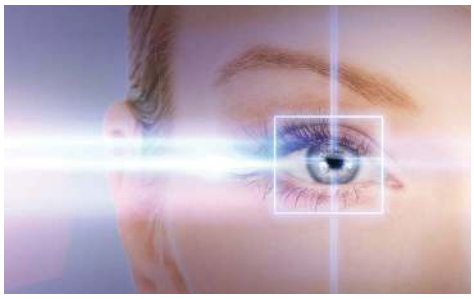
of less than 30 ng/mL and vitamin D deficiency was defined as a total 25-hydroxyvitamin D level of less than 20 ng/mL.

Vitamin D insufficiency was associated with 2.1 times the likelihood of developing Parkinson's disease and vitamin D deficiency was associated with 2.6 times the odds of Parkinson's disease. The data also showed that as 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 levels decreased, the risk of Parkinson's disease increased.

The researchers stated, "Our data confirm the association between vitamin D deficiency and Parkinson's disease, and for the first time demonstrate an inverse association of 25-hydroxyvitamin D_2 with Parkinson's disease. Given that 25-hydroxyvitamin D_2 concentration is independent of sunlight exposure, this new finding suggests that the inverse association between vitamin D levels and Parkinson's disease is not simply attributable to lack of sunlight exposure in Parkinson's disease patients with impaired mobility."

REFERENCE:
Wang L, et al. Mov Disord. 2014 Dec 27. [Epub ahead of print.]

BILBERRY EXTRACT DECREASES MARKERS OF DIABETIC RETINOPATHY



In a study published in March 2015, researchers found that bilberry (*Vaccinium myrtillus*) supplementation reduces markers of diabetic retinopathy in experimental models. The National Eye Institute states that diabetic retinopathy is caused by changes in the blood vessels in the retina and is the leading cause of blindness in the U.S. They estimate that 40% to 45% of Americans diagnosed with diabetes have some stage of diabetic retinopathy.

In this study, diabetic rats received 100 mg/kg bilberry extract for six weeks. The scientists assessed blood glucose levels and body weight, as well as blood flow in the eye using fluorescein-dextran angiography. The researchers also evaluated markers of diabetic retinopathy including retinal vascular endothelial growth factor expression and degradation of zonula occludens-1, occludin and claudin-5, which are proteins that comprise tight junctions between cells.

Diabetic rats supplemented with bilberry had significantly reduced fluorescein leakage during the fluorescein-dextran angiography. Bilberry supplementation also decreased the markers of diabetic retinopathy including retinal vascular endothelial growth factor expression and degradation of zonula occludens-1, occludin and claudin-5. Bilberry supplementation did not affect blood glucose levels and body weight.

The scientists stated, "In conclusion, *V. myrtillus* extract may prevent or delay the onset of early diabetic retinopathy. These findings have important implications for prevention of diabetic retinopathy using a dietary bilberry supplement."

REFERENCE:
Kim J, et al. Int J Food Sci Nutr. 2015;66:236-42.

COCOA FLAVANOLS INFLUENCE COGNITION, BLOOD PRESSURE, AND INSULIN METABOLISM

Cocoa flavanol intake improves age-related cognitive dysfunction, insulin resistance, and blood pressure in elderly individuals, scientists found in a study published in the March 2015 *American Journal of Clinical Nutrition*. An estimated 10% to 20% of individuals over age 65 have mild cognitive impairment and are at increased risk of developing dementia.

Researchers randomly assigned 90 elderly subjects without evidence of cognitive dysfunction to receive a drink containing 1) 993 mg cocoa flavanols daily (high-flavanol group), 2) 520 mg cocoa flavanols daily (intermediate-flavanol group), or 3) 48 mg cocoa flavanols (low-flavanol group) daily for eight weeks. The investigators assessed cognitive function using the Mini-Mental State Examination, the Trail Making Test A and B, and the Verbal Fluency Test at the beginning of the study and again after the eight-week supplementation period. The researchers also evaluated insulin resistance, blood pressure, and lipid peroxidation.

The researchers did not find changes in the Mini-Mental State Examination scores between the three groups. However, they did show that there were significant changes in the high-flavanol and intermediate-flavanol groups on the time required to complete the Trail Making Test A and B compared to the low-flavanol group. The Verbal Fluency Test scores also improved in all three groups, but were significantly greater in the high-flavanol group compared to the intermediate- and low-flavanol groups. Additionally, compared to the low-flavanol group, the high-flavanol and intermediate-flavanol groups had significant improvement in insulin resistance, blood pressure, and lipid peroxidation.

The researchers concluded, "This dietary intervention study provides evidence that regular cocoa flavanols consumption can reduce some measures of age-related cognitive dysfunction, possibly through an improvement in insulin sensitivity. These data suggest that the habitual intake of flavanols can support healthy cognitive function with age."

REFERENCE:
Mastroiacovo D, et al. Am J Clin Nutr. 2015;101:538-48.

GINKGO IMPACTS INFLAMMATION AND INSULIN RESISTANCE IN METABOLIC SYNDROME

Researchers reported in a study published in December 2014 that *Ginkgo biloba* improved blood sugar metabolism and reduced inflammation in individuals with the metabolic syndrome. The American Heart Association states that approximately 34% of adults in the U.S. have metabolic syndrome, increasing their risk for cardiovascular disease (CVD), diabetes, and stroke.

Eleven subjects with metabolic syndrome received *Ginkgo biloba* daily for two months. Researchers measured inflammatory markers including C-reactive protein (hs-CRP) and interleukin (IL)-6. The investigators also assessed insulin resistance (HOMA-IR) and nanoplaque formation.

Ginkgo supplementation reduced hs-CRP by 44.4% and insulin resistance by 15.3% and resulted in beneficial changes in arteriosclerotic, inflammatory, and oxidative stress biomarkers. Additionally, IL-6 decreased by 12.9% and nanoplaque formation decreased by 14.3%.

The study authors stated, "According to a large clinical trial elucidating the importance of insulin resistance and low-grade systemic inflammation for cardiovascular disease and overall mortality risk, these data might indicate a CVD/total mortality risk reduction."

REFERENCE:
Siegel G, et al. Atherosclerosis. 2014;237:584-8.

PRACTITIONER NEWSLETTER
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EXPLORING ETIOLOGIES FOR ALZHEIMER'S DISEASE, DIABETES, AND CARDIOVASCULAR CONCERNS.

America faces a deadly triad of killers: diabetes, heart disease, and Alzheimer's disease. This informative presentation will share new and innovative ways for the busy clinician to address common risk factors for each of these conditions, helping ensure better clinical outcomes and greater patient compliance. Chris D. Meletis, ND, will explore the links between these diseases, and how addressing the underlying pathophysiology can allow patients to break the vicious cycle of disease and ill-health.

Learning Objectives:

- Gain a deeper appreciation of the common clinical markers that are shared by Alzheimer's disease, diabetes, and heart disease.
- Enhanced understanding of cutting edge clinical approaches and "Common Thread" prescribing for multiple health conditions as well as how to achieve increased patient compliance and consistent clinical outcomes.
- Acquire ways to inspire patients to understand the mechanisms that are contributing to these disease states at the cellular level.

PRESENTER
Chris D. Meletis, ND

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