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#### Distinguished Colleagues,

The American Academy of Anti-Aging Medicine (A4M) welcomes you to Las Vegas for the Winter 2016 session of the 24<sup>th</sup> Annual World Congress on Anti-Aging Medicine.

This interactive and dynamic 4-day event will lead you to engage in collaborative discussions, network with a host of forward-thinking medical professionals, and be an integral part of the research and information presented by leaders in preventative medicine. This conference represents an elite platform of medical education and knowledge, where healthcare professionals from an array of specialties are redefining and reshaping medicine. Throughout this conference, you will be provided with new skills and fresh ideas regarding clinical protocols and practices, coupled with the ability to implement them in your practice-and, most importantly, improve patient care and maximize your own potential.

We use the terms Anti-Aging Medicine, Healthy Aging, and Personalized Medicine synonymously. This medical approach utilizes cutting-edge scientific research and personalized, predictive care in order to prevent and reverse the multitude of effects that stem from age-related diseases. We have found that when healthcare professionals directly treat and interact with their patients-not solely the surface-level symptoms-the opportunities for growth and discovery are endless.

With your participation and involvement, we look forward to continuing to expand and become more widely accessible. Your attendance at this event will help spur the transformation of medicine's future, and we thank you for being a fundamental part of the evolution of the healthcare industry.

With warm regards,



Ronald Klatz, MD, DO President, A4M

In R. Kin



Robert Goldman, MD, PhD, DO, FAASP Chairman, A4M

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- 1. An official picture ID is required to pick up name badge.
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#### Thursday, December 8th - 6:15pm

Achieving Hormonal Balance in Younger Women PMS, PCOS, Infertility Presented by: Pamela W. Smith, MD, MPH, MS

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Compounds Used in Sexual Medicine

Presented by: Jennifer Landa MD, FMNM, ABAARM

New and Innovative Formulas for the Skin and Hair

Presented by: Rick Rhoads, PharmD

Reversing the Aging Process

Presented by: Thierry Hertoghe, MD

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#### Friday, December 9th - 6:00pm

Latest Research Utilizing TA-65MD® in Clinical Practice

Presented by: Ron Rothenberg, MD



Part 1: How to Successfully Choose and Implement the Best Medical Spa Setting for 2017. Be Ahead of Your Competition!

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Part 2: Dr. Joe Schwarcz PhD from Mc Gill University and Manon Pilon Present: Facts on Organic or Non-Organic Skin Care



How to Select Your Brand, How To Build Your Own Brand for Your Medical Spa

Presented by: Manon Pilon Author and founder of Derme &CO and Joe Schwarcz, PhD

**Erectile Dysfunction:** 

**Compounded Treatment Strategies** 

Presented by: Anthony Campbell, PharmD, and Keneth Orbeck, MD

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Becoming A Better Diagnostician:

A New Perspective On Illness And Wellness

Presented by: Silvia Binder, ND, PhD, John Cline, MD, James Denninghoff, MD

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a better way to make you better

Heavy Metals & Head Trauma:

Optics and Treatment for Hormone Deficiency

Presented by: Edwin Lee, MD, FACE and Mark L Gordon, MD

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#### Saturday, December 10<sup>th</sup> - 6:00pm

Hormone Dosing Protocol with Dr. Pamela Smith and PCCA

Presented by: Pamela W. Smith, MD, MPH, MS

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Make More, Work Smarter, Better Outcomes

Presented by: James LaValle, RPh, CCN, Bassel Haidar, CEO Metabolic Intelligence, LLC and Andrew Heyman, MD, MHSA, FMNM, ABAARM Sponsored by:



Using Telomere Measurement In Anti-Aging Medicine

Presented by: Mark Rosenberg, MD., Bill Andrews, PhD, and Stephen Matlin, CEO



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### PRODUCT SHOWCASE (NON-CME)

#### Friday, December 9th

Location: Exhibit Hall



#### Saturday, December 10<sup>th</sup>

Presented by: David Tonkin. MD

Sponsored by: Mitochondria, inflammation, ER Stress and MitoQ 11:30 AM - A Research Update Presented by: Greg Macpherson Bpharm ANZCP, CEO MitoQ Ltd WWW.MITOQ.COM Sarcotropin: A Medical Food For Body Sponsored by: 12:00 рм Compositional Changes Related to Aging MedausPharmacy Presented by: Richard Walker, PhD, RPh The Latest in Women's Sexual Health & Sponsored by:

12:30 рм Feminine Rejuvenation Presented by: Shelena Lalji, MD

Sponsored by: Work Smarter, Not Harder: 4:30 PM 7 Figure Income with 5 Months Vacation a Year Presented by: Edward C. Kondrot, MD, MDH

Sponsored by Advanced Nutritional Protocols Based Upon **MethylGenetic** 5:00 рм Genetic SNPs, Labs and Symptoms Nutrition Analysis™ Presented by: Robert Miller, ND www.dnasupplementation.com



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Sunday, December 11th at 12:00pm Your entry form can be found in your attendee bag or at booth #5079





















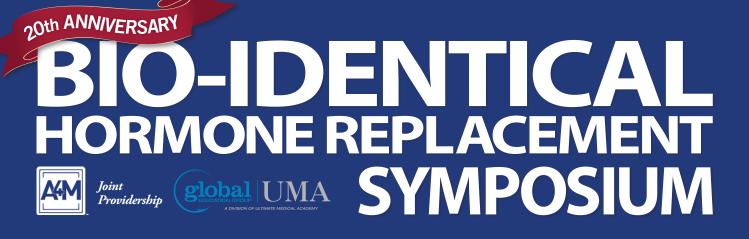




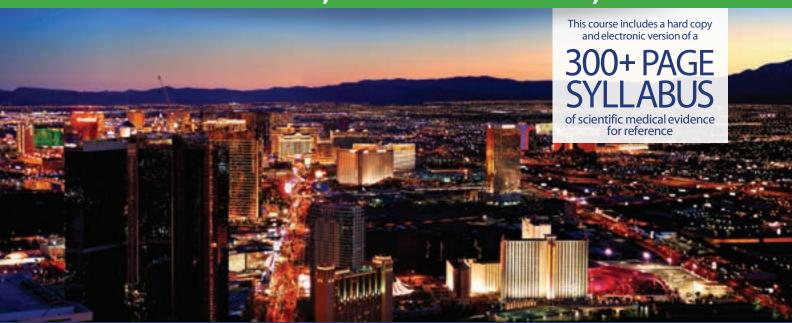








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#### WELCOME RECEPTION

Friday, December 9

4:00pm - 6:00pm Exhibit hall

#### HAPPY HOUR

Saturday, December 10

4:00pm - 6:00pm Exhibit hall

# A Human Milk Prebiotic as a Novel Nutritional Therapy for Adults with Gut Dysbiosis and Dysfunction

By JENNIFER RYAN, ND, Research Fellow



The following article is not endorsed and/or supported by The American Academy of Anti-Aging Medicine.

The purposes of this publication do not imply endorsement and/or support of any author, company or theme related to this article.

#### **The Gut Microbiome**

he human body hosts trillions of microorganisms, known collectively as the "microbiome." The majority of the microbiome lives in the gastrointestinal (GI) tract, predominantly in the colon. Gut flora have many essential roles including providing protection against pathogens, the digestion and absorption of nutrients, and metabolic and immunologic programing.<sup>1-3</sup> The early establishment of healthy gut flora impacts short- and long-term health outcomes. 1-3 Negative influences on the establishment of healthy gut flora and factors that decrease microbial diversity can have lifelong detrimental impacts. For example, antibiotics may cause dysbiosis (alteration of the intestinal microbiome), contributing to the pathogenesis of several diseases and conditions. Children exposed to antibiotics in infancy are more likely to develop atopic disease, food allergy, early childhood obesity, and several types of gut dysfunction including irritable bowel syndrome (IBS), Crohn's disease, ulcerative colitis, and celiac disease. 4-9in particular among children, is the 'Western lifestyle' or 'hygiene' hypothesis. As early childhood infections are assumed to hold a protective effect on the development of asthma and allergies, the use of antibiotics at that sensitive age may lead to an increased risk of asthma and allergy. OB-JECTIVE: The aim of this study is to investigate the association between the use of antibiotics in the first year of life and the subsequent development of asthma and allergic disorders. METHODS: In a population-based sample of 7-and-8-yearold children questionnaire and skin prick test data were collected from 1206 and 675 subjects, respectively. Prevalence rates of asthma, allergic disorders and skin test positivity were compared between children with and without early life use of antibiotics, taking into account other possible risk factors including early respiratory infections. The effect of genetic predisposition was investigated by stratified analyses of children with and without parental hay fever. RESULTS: The use of antibiotics during the first year of life was significantly associated with asthma (OR = 1.7, 95% CI 1.0-3.1 Carefully selected prebiotics represent novel nutritional therapies that may be utilized in the clinical treatment of gut dysfunction and dysbiosis.

#### **Prebiotics in Human Milk**

Prebiotics are complex carbohydrates that are resistant to digestion in the human GI tract; however, many of the organisms that make up the gut microbiome are able to digest them. These substrates beneficially affect the human host through their selective metabolism in the intestinal tract.<sup>10</sup> Human breast milk is rich in unique prebiotics that are known as "human milk oligosaccharides" or "HMOs." After lactose and lipids, HMOs are the third most abundant solid component of human milk.11 Milk oligosaccharides are absent from or occur in lesser amounts in the milk of other species. 12 Acting as prebiotics, HMOs are the primary substrate for Bifidobacterium spp., encouraging colonization by specific species of this beneficial genus in breast-feeding infants. 13 The most abundant HMO, 2'fucosyllactose (2'FL), has many beneficial health effects and functions (Box 1). In the infant colon, 2'FL acts like a soluble prebiotic fiber. 12

#### Box 1. Functions of the Human Milk Oligosaccharide (HMO) 2'fucosyllactose (2'FL)

- Supports the growth of beneficial microbiota including Bifidobacterium spp. 12-14
- Induces the production of short chain fatty acids (SCFAs) including butyrate.<sup>14</sup>
  - SCFAs are energy substrates for colonic epithelial cells
  - ♦ Butyrate has many functions (Figure 1)<sup>15</sup>
- Lowers pH through lactate and SCFA production.<sup>12,14</sup>
- Reduces the risk of GI infection by blocking pathogens and their toxins from binding to intestinal epithelial cell surface receptors.<sup>12,16</sup>

  - Specifically acts as an anti-adhesive antimicrobial (Figure 2) to toxins from Campylobacter jejuni, Vibrio cholera, Escherichia coli, as well as Norovirus 12.16-17



**Figure 1. Effects of Butyrate on the Intestine** (Modified from Canani, et al.<sup>15</sup>)

#### Sources of HMOs and 2'fucosyllactose (2'FL)

Although HMOs are relatively abundant in human milk, not all breast-fed infants are exposed to considerable levels of 2'FL; the amount of HMOs and 2'FL in breast milk vary from mother to mother due to genetic variation. <sup>12</sup> Formula fed infants, weaned children, and adults are generally not exposed to dietary sources of 2'FL (Box 2).

Fortunately for these populations, nature identical 2'FL can be produced in large quantity for use in select infant formulas, dietary supplements and medical foods. Since many of the issues that contribute to the pathophysiology of

#### Box 2. Populations Not Exposed to Dietary 2'fucosyllactose (2'FL)

- Most formula-fed infants (only a few infant formulas available in Europe contain added 2'FL)
- Some breastfed infants (20-30% of nursing mothers do not produce 2'FL)<sup>12</sup>
- Adults and weaned children, even if they consume dairy products (2'FL is undetectable in cow's milk<sup>12</sup>)

#### Box 3. Conditions That May Improve from Supplemental 2'fucosyllactose (2'FL)

- Inflammatory bowel disease (IBD) including ulcerative colitis and Crohn's disease
- Irritable bowel syndrome (IBS)
- Short bowel syndrome
- Celiac disease

gastrointestinal dysfunction can be improved by 2'FL (described in Box 1), there are several patient populations that may benefit from supplemental 2'FL (Box 3).

#### Adult Patient Populations that Could Benefit from Supplemental 2'fucosyllactose (2'FL)

Dysbiosis, mucosal inflammation, and compromised gut function contribute to the pathogenesis of IBS and IBD. <sup>18-19</sup> Lower abundance of *Bifidobacterium* spp. in the gut is associated with IBS and IBD. Individuals with IBS have been shown to have significantly lower levels of *Bifidobacterium* spp. compared to healthy controls. <sup>20-21</sup> In patients with IBD, the presence of active disease is associated with decreased levels of *Bifidobacterium* spp. <sup>22</sup> As previously described in Box 1, 2'FL supports the growth of *Bifidobacterium* spp., which use 2'FL as an energy substrate. <sup>12</sup>

Patients with IBS and IBD have lower levels of butyrate-producing microorganisms. <sup>23,24</sup>generating more than 20 million 16S rRNA sequences. In patients with IBS, a significantly lower microbial diversity was associated with a lower relative abundance of butyrate-producing bacteria (P = 0.002; q < 0.06 Butyrate-producing microorganisms have been found to inversely correlate with ulcerative colitis disease activity.<sup>25</sup> A recently published systematic review showed that low levels of butyrate and other SCFAs is considered a functional change observed in patients with Crohn's disease.<sup>26</sup> Butyrate improves intestinal barrier function and reduces methane-producing microorganisms in the human colon, which may reduce abdominal gas.<sup>23</sup>generating more than 20 million 16S rRNA sequences. In patients with IBS, a significantly lower microbial diversity was associated with a lower relative abundance of butyrate-producing bacteria (P = 0.002; q < 0.06 Previous research has shown that 2'FL increases levels of butyrate, likely by encouraging the growth of butyrate-producing gut flora. 14, 27-28

As previously described in Box 1, 2'FL acts as a decoy and anti-adhesive antimicrobial that blocks pathogens from

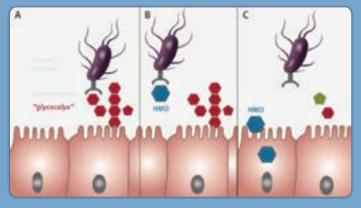


Figure 2. HMOs Including 2'FL Have Anti-Adhesive Effects

adhering to receptors on intestinal epithelial cells. <sup>12,16</sup> It has been proposed that *Campylobacter jejuni* contributes to the pathogenesis of IBD; it is also a leading cause of post-infectious IBS. <sup>29,30</sup> In a mouse model, 2'FL was shown to significantly decrease intestinal colonization with *Campylobacter jejuni*. <sup>31</sup>2-fucosylated carbohydrate moieties, including human milk oligosaccharides (HMOSs 2'FL has also been shown to bind to *Vibrio cholera*, pathogenic *E. coli* exotoxins, as well as *Clostridium difficile* toxin A and toxin B. <sup>16</sup>

#### **UGIR**, a Novel 2'FL-Containing Medical Food for Adults

A unique medical food, UGIR was clinically designed for the nutritional management of gut dysfunction and malabsorption. These issues may be associated with IBS, IBD, short bowel syndrome, and celiac disease. Due to the high prevalence of nutrient deficiencies in individuals with IBD and celiac disease (as a result of nutrient malabsorption and malnutrition), nutritional support is recommended.<sup>32-34</sup> UGIR is consumed as a reconstituted beverage and its key ingredients are listed in **Box 4.** UGIR is currently the *only* available product that contains 2'FL for use in adults.

#### **Box 4. UGIR Key Ingredients (per Serving)**

- 7 grams of Prebiotics
  - ♦ 2 grams 2'fucosyllactose (2'FL)
  - \$\delta\$ 5 grams Isomalto-oligosaccharide (IMO) from tapioca
- 15 grams Opti-Protein
  - ♦ 12 grams pea/rice protein
  - 3 grams branched-chain and essential amino acids (BCAA, EAA)
  - ♦ 500 mg Sustamine® di-peptide glutamine
- Healthy Fats
  - ⟨ 400 mg alpha-linolenic acid (ALA) from flax seeds
- 20+ Vitamins & Minerals

#### **Summary**

In addition to protein, healthy fats, vitamins and minerals, UGIR delivers the prebiotics IMO and 2'FL in a single product. By providing the exclusive ingredient 2'FL, UGIR makes a unique compound (typically available only to nursing infants) accessible to non-pediatric patients with several challenging gastrointestinal conditions. Employing cutting-edge nutritional science, 2'FL and UGIR can restart the gut by restoring a healthy foundation of beneficial microbiota, SCFAs, and optimal intestinal barrier function.

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#### COMPOUNDING FOR HORMONE REPLACEMENT THERAPY

An Overview of Delivery Methods and Considerations

By ANTHONY J. CAMPBELL, PharmD



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ormones. For some, that word causes one to reminisce about days of youthful vitality and vigor. For thers, it only brings a sense of loss or frustration as they cope with aging and the decline of what once was. The concept of replacing hormones that are continually in decline is not new and has been a part of popular culture for several decades. For example, early in the 1940's, the FDA approved estrogen replacement for the treatment of menopause and its related symptoms. For the next 60 years, menopausal women were encouraged to take (non-bioidentical) oral conjugated estrogens and progestin not only for the relief of menopausal symptoms, but for the proposed increase in their overall cardiovascular health and longevity as well. In fact, in was considered malpractice if a physician did not prescribe oral HRT for menopausal women. However, the foundations of that belief were shaken in 1998 after the results of the first significant randomized, placebo-controlled trial of hormone replacement therapy – the *Heart and Estro*gen/Progestin Replacement Study (HERS) – involving 2,800 women suggested an overall lack of benefit for cardiovascular disease prevention. Furthermore, the study revealed that the treatment increased the rate of thromboembolic events and gallbladder disease. Four years later, the foundations of popular belief completely and catastrophically collapsed in 2002 when the Women's Health Initiative (WHI) results were released. This randomized trial in nearly 17,000 women showed net harm from conjugated estrogen and progestin therapy in postmenopausal women, and subsequently advised directly against the practice entirely. Since there was no other widely available alternative, this left patients wondering what they were to do and found themselves in a state much like their grandmothers were in 60 years earlier: suffering with menopausal symptoms with no clear answer or direction how to alleviate them. Fortunately, compounding

pharmacies were able to fill the void by preparing bioidentical products individualized for each patient's specific need. A bioidentical hormone – in contrast to a conjugated estrogen or progestin – is defined as a molecule identical to a hormone produced by the human body (i.e., estradiol, estriol, progesterone, testosterone, etc.). With the ability to be compounded and delivered in various dosage forms – each with their own special considerations, among the most commonly prescribed dosage forms for hormone replacement therapy are topical, oral, and parenteral.

Because the raw chemicals for topical HRT are highly lipophilic, micronized, and have molecular weights between 270 and 315g/mol, hormones are the ideal candidates for transdermal absorption across the skin. Known as percutaneous absorption, the active ingredient(s) pass(es) across the skin and directly into the circulatory system; thereby bypassing liver metabolism's first-pass effect widely known to cause drug degradation and decreased systemic absorption. An important point to keep in mind when prescribing/using topical hormones is that the target of application to the skin should not always be the bloodstream, but rather the adipose tissue(s). Adipose tissue acts as a sort of reservoir for hormones, allowing for a depo-like effect such that the hormone is delivered into the systemic circulation over a period of time. However, in order for percutaneous absorption to be effective, there are several factors that must be carefully considered. The epidermal layer, or specifically the Stratum Cor*neum*, offers the greatest resistance to penetration and serves as the single-most rate-limiting barrier to absorption of the active pharmaceutical ingredient (API). The thickness of this dermal layer varies throughout the body and is reduced when skin is damaged, dehydrated, diseased, irritated, or abraded. Areas such as the heel and palm of the hands have a considerably thicker Stratum Corneum than areas such as forearms,

wrists, or behind the ears; therefore, they would not be ideal locations for topical drug application. Hydrating the *Stratum Corneum* will effectively increase the potential for percutaneous absorption, as will an increase in skin temperature.

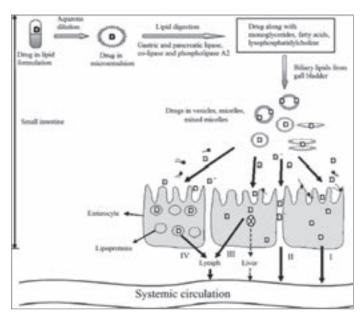
Another important factor to keep in mind is that not all bases will systemically deliver the same amount of API. At times, prescribers and/or patients may prefer the use of a specific base vehicle to be used in their topical HRT products as an alternative to a base for those patients that do not appear to be "absorbing very well." Penetrative bases, such as a PLObase (pluronic-lecithin organogel) or Lipoderm<sup>TM</sup>, for example, are typically reserved for the delivery of API into and through the skin for systemic absorption into the bloodstream. This isn't to say it *cannot* be used for HRT creams, but rather to note that by delivering the hormones directly into the bloodstream, and thereby exposing to potentially extensive first-pass metabolism via the liver, the concentration of the hormone(s) may be required to be altered a bit than when prescribed in traditional non-penetrating creams. That is to say that an HRT cream in a non-penetrating base that is, for example, 0.5mg/mL, would not deliver a systemically equal amount of hormone if used in a penetrating base, such as Lipoderm<sup>TM</sup>. The type of base used is extremely important and an understanding of how each base delivers API's is required. An example of how different bases vary in API absorption can be seen in the following example:

- Comparing the commercial product Estragel® with (now discontinued) Estrasorb®:
  - » The first is a water/alcohol based Carbomer gel, the second is an oil-in-water emulsion.
  - » Estragel<sup>®</sup> uses a daily dose of 0.75mg Estradiol to affect an actual absorbed dose of 0.035mg.
  - » Estrasorb® had an absorbed dose of 0.050mg, but required 8.7mg Estradiol to accomplish that. More than 11x the amount of estradiol found in Estragel.

As easily observed, there is a substantial difference in absorption ability between the two products: They both deliver estradiol, but one is more efficient than the other due to the properties of the base utilized. While nearly any base could potentially be used for topical HRT therapy, there is a difference in related absorption profiles. Maintaining patient consistency with bases is necessary with continued therapy, but if a change is desired, note that a change in API concentration will likely need to be made as well in order to elicit an equal systemic absorption profile. As a general rule, lipophilic drugs (such as hormones) will elicit a greater absorption profile when using a hydrophilic base, as there is a greater diffusion gradient between the (non-lipophilic) base and the (lipophilic) dermal layer, causing an increase in flux away from the base and toward the skin.

Because some may find it difficult or inconvenient to apply a topical cream, orally administered hormones serve as an alternative option. Although it is convenient and many may find it less cumbersome compared to topical administration, orally delivering hormones isn't without its limitations and

inefficiencies. Following oral administration, hormones pass directly from the gut to the liver via the portal vein, giving a high local concentration that greatly affects hepatic metabolism. Though all preparations of a given type of estrogen (e.g., estradiol) may be molecularly identical before their introduction into the human body, estrogens administered orally are modified by the liver before entering the bloodstream and in this process, most is converted to estrone. Likewise, when given in a non-modified capsule, frequent dosing is often required as up to 95% of the dose can be destroyed in the upper GI tract before entering the portal system. Modifying the capsule contents to contain cellulose powders to provide a type of sustained-release can provide less dosing failures and side effects from the peak-and-valley effect of non-modified capsules; however, absorption is erratic and not predictable due to the insolubilized form of the drug. A method of overcoming the extensive metabolism of oral hormones is through providing them in a compounded micro-emulsion of a lipid-based formulation (LBF). By solubilizing the hormone in an LBF, systemic absorption occurs via the lymphatic system; thereby, bypassing GI destruction and first-pass metabolism. A schematic diagram illustrating this is shown below:



**Figure 1:** Schematic diagram of mechanisms of intestinal drug transport from lipid-based formulations.

Adapted from: Kalepu S, Manthina M, Padavala V. Oral lipid-based drug delivery systems – an overview *Acta Pharma Sin B* 2013;3(6):361-372.

- 1. Facilitating transcellular absorption due to increased membrane fluidity
- II. Allowing paracellular transport by opening tight junctions
- III. Increased intracellular concentration & residence time by surfactants
- IV. Lipid stimulation of lipoprotein/chylomicron production

The encapsulation of progesterone in a micro-emulsion lipid-based vehicle is an example of this delivery method. Because the commercially available product of progesterone is provided in a peanut oil emulsion, it can be an allergen to many users. Compounding and encapsulating progesterone in an alternative oil emulsion, such as olive oil, provides a great opportunity to individualize progesterone therapy for patients while providing a dosage form with superior efficacy and absorption.

While estrogens and progesterone are often thought of in regard to HRT for women, testosterone is often thought of in regard to male hormonal replacement therapy. At this time, there is no testosterone pill, patch, or cream approved for women in the U.S. and those made for men have too high a concentration for women's therapeutic use. However, testosterone can be prescribed in a compounded formula and prepared by a pharmacist for women who require it. Testosterone is absorbed from the GI tract with oral administration, but because of extensive first-pass metabolism, oral bioavailability is very poor. Therefore, one of the more common forms of testosterone used by males is an oil-based, intramuscular injection.

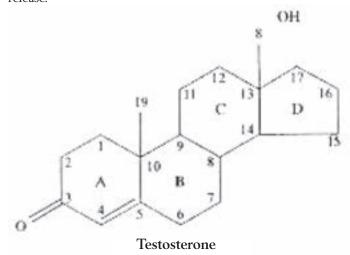
This method of delivering testosterone is highly effective, but comes with a number of cautionary advisories, which will be discussed a bit later. Intramuscular injections of testosterone formulations have been developed that reduce the rate of testosterone secretion, with esters being less polar and slowly absorbed from intramuscular sites. Esters have a duration of action of 2—4 weeks following IM administration. The esters are hydrolyzed to free testosterone, which is subsequently inactivated in the liver. In order to understand why one would want to use a multiple-blend form of testosterone as opposed to a single-ingredient product, there has to be an understanding about the differences in the various esters of testosterone (e.g., Cypionate, Enanthante, Propionate, etc.).

An ester is a chain composed primarily of carbon and hydrogen atoms. This chain is typically attached to the parent steroid hormone – in this case, testosterone – at the 17<sup>th</sup> carbon position (refer to structure shown below). Esterification of an injectable anabolic/androgenic steroid basically accomplishes one thing: It slows the release of the parent steroid from the site of injection. This happens because the ester will significantly lower the water solubility of the steroid, and increase its lipid solubility. This will cause the drug to form a deposit in the muscle tissue, from which it will slowly enter into circulation as it is picked up in small quantities by the blood. Generally, the longer the ester chain, the lower the water solubility and the longer it will take to for the full dosage to reach general circulation.

Esters are physiologically inactive; therefore, in order for the compound to become active, the ester must first be removed. This automatically occurs once the compound has filtered into blood circulation, where enzymes quickly remove (hydrolyze) the ester chain. This will effectively restructure the chemical, enabling the drug to attach to the appropriate receptor. Then, and only then, will the steroid be able to have an effect on muscle tissue. With this in mind, you can understand why considering testosterone cypionate more *potent* than propionate makes little sense, as your muscles are seeing only free testosterone no matter what ester was injected. The only difference is the amount of time it takes for the testosterone to be "free" so it can be used within the body.

For an injectable testosterone product composed of 3 esters, such as Tri-Test (Cypionate/Enanthate/Propionate), the rationale is that the short-chain ester (Propionate) is hydrolyzed quicker for a faster onset, while the longer-chain esters,

being more lipophilic, take longer to completely hydrolyze and become physiologically available; in essence, delayed-release.



Comparison of Testosterone Esters and (Molecular Weight) of Each:

- T. Propionate C22H32O3 (MW = 345)
  - » t ½: approximately 2 days
- T. Enanthate  $C_{26}H_{40}O_{3}$  (MW = 400)
  - » t  $\frac{1}{2}$ : approximately 10 days T. Cypionate  $C_{27}H_{40}O_3$  (MW = 412)
    - »  $t \frac{1}{2}$ : approximately 12 days

Comparatively, testosterone itself has a molecular weight of 288 with  $C_{19}H_{28}O_{2}$ , so it is easy to see that the esters are of much greater size than testosterone alone and that their physiologic release profile is in proportion to their respective molecular sizes.

While one should be intimately familiar with *all* of the possible adverse reactions from injecting an oil-based testosterone product prior to using, among a few of the more common are the localized injection site reactions, such as pruritus, rash, and urticaria. Additionally, swollen/raised and tender protuberances at the injection site may occur (due to the viscous oil not being delivered deep enough into the muscle tissue and/or leaching back out of the muscle and into the subcutaneous space). This type of reaction has potential to occur with each oil-based injection and, while sometimes painful, is self-limiting and will subside as the oil dissipates over the course of several days.

In conclusion, choosing a delivery method for patients requiring HRT is a decision that must be made with consideration given to each patient's unique characteristics, preferences, and abilities in order to provide a dosage form that will contribute to therapeutic compliance and success. Due to the number of physiologic factors for each dosage form that must be carefully considered for proper or complete systemic absorption, providing hormone therapy to patients in need can sometimes be difficult or confusing. However, with the assistance of a knowledgeable compounding pharmacist, formulating a treatment plan and therapeutically effective compound that is unique for a selected patient is both achievable and rewarding.

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## Modulation of Metabolic Detoxification Pathways Using Foods and Food-Derived Components: A Scientific Review with Clinical Application

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Research into human biotransformation and elimination systems continues to evolve. Various clinical and *in vivo* studies have been undertaken to evaluate the effects of foods and food-derived components on the activity of detoxification pathways, including phase I cytochrome P450 enzymes, phase II conjugation enzymes, Nrf2 signaling, and metallothionein. This review summarizes the research in this area to date, highlighting the potential for foods and nutrients to support and/or modulate detoxification functions. Clinical applications to alter detoxification pathway activity and improve patient outcomes are considered, drawing on the growing understanding of the relationship between detoxification functions and different disease states, genetic polymorphisms, and drug-nutrient interactions. Some caution is recommended, however, due to the limitations of current research as well as indications that many nutrients exert biphasic, dose-dependent effects and that genetic polymorphisms may alter outcomes. A whole-foods approach may, therefore, be prudent.

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#### 1. Introduction

Food-based nutrients have been and continue to be investigated for their role in the modulation of metabolic pathways involved in detoxification processes. Several publications to date have leveraged cell, animal, and clinical studies to demonstrate that food-derived components and nutrients can modulate processes of conversion and eventual excretion of toxins from the body [1]. In general, the nature of these findings indicates that specific foods may upregulate or favorably balance metabolic pathways to assist with toxin biotransformation and subsequent elimination [2, 3]. Various whole foods such as cruciferous vegetables [2, 4, 5], berries [6], soy [7], garlic [8, 9], and even spices like turmeric [10, 11] have been suggested to be beneficial and commonly prescribed as part of naturopathic-oriented and functional medicine-based therapies [12, 13].

While these foods are important to note, the science in this active area of inquiry continues to evolve to reveal new findings about food-based nutrients and their effect on health. Thus, the purpose of this review article is to summarize the science to date on the influence of whole foods, with a special focus directed towards phytonutrients and other food-based components, on influencing specific metabolic detoxification pathways, including phase I cytochrome enzymes, phase II conjugation enzymes, antioxidant support systems, and metallothionein upregulation for heavy metal metabolism. Based on this current science, the paper will conclude with clinical recommendations that may be applied in a personalized manner for patients via the discretion of a qualified health professional.

#### 2. The Metabolic Pathways of Detoxification

Discussion of physiological pathways for detoxification has been mainly centered around phase I and phase II enzyme systems. This review will cover phase I cytochrome P450 enzymes as well as phase II enzymes, specifically UDP- glucuronosyl transferases, glutathione S-transferases, amino acid transferases, N-acetyl transferases, and methyltransferases. Note that there are other important classes of phase I enzymes, namely, hydroxylation and reduction, which are not covered in this review. While these important enzymes are pivotal to consider, this review of the effect of food on detoxification will also extend into other pathways, including ways to promote gene expression of antioxidant-related enzymes and of metallothionein, an endogenous protein carrier for heavy metals. Each of these four classes of detoxification-related pathways will be discussed within the context of nutrients.

2.1. Phase I Cytochrome P450 Enzymes. Initially, the "phases" of detoxification were described as functionalization (or phase I), or the addition of oxygen to form a reactive site on the toxic compound, and conjugation (phase II), or the process of adding a water-soluble group to this now reactive site [14, 15]. The "Phase I" cytochrome P450 superfamily of enzymes (CYP450) is generally the first defense employed by the body to biotransform xenobiotics, steroid hormones, and pharmaceuticals. These microsomal membrane-bound, heme-thiolate proteins, located mainly in the liver, but also in enterocytes, kidneys, lung, and even the brain, are responsible for the oxidation, peroxidation, and reduction of several en-

dogenous and exogenous substrates [13, 15, 16]. Specifically, the function of CYP450 enzymes is to add a reactive group such as a hydroxyl, carboxyl, or an amino group through oxidation, reduction, and/or hydrolysis reactions [15]. These initial reactions have the potential to create oxidative damage within cell systems because of the resulting formation of reactive electrophilic species.

It is accepted that any variability in the number of CYP450 enzymes could have benefit(s) and/or consequence(s) for how an individual responds to the effect(s) of (a) toxin(s). Clinical application of the knowledge of these phase I CYP450 enzymes has been primarily addressed within pharmacology to understand the nature of drug interactions, side effects, and interindividual variability in drug metabolism [15]. The ability of an individual to metabolize 90% of currently used drugs will largely depend on the genetic expression of these enzymes [17]. It is established that many of these CYP450 genes are subject to genetic polymorphisms, resulting in an altered expression and function of individual enzymes. Currently, there exist some laboratory tests to identify the presence of these genetic variants. It is conceivable that having knowledge about foods and their individual (phyto)nutrients, especially in the case of dietary supplements and functional foods, could be worthwhile for clinicians to consider for patients who are taking a polypharmacy approach. Furthermore, as nutritional strategies become more personalized, it would seem that this information could be interfaced with a patient's known CYP450 polymorphisms to determine how to best optimize health outcomes.

2.1.1. CYP1 Enzymes. The CYP1A family is involved in metabolizing procarcinogens, hormones, and pharmaceuticals. It is well-known for its role in the carcinogenic bioactivation of polycyclic aromatic hydrocarbons (PAHs), heterocyclic aromatic amines/amides, polychlorinated biphenyls (PCBs), and other environmental toxins [18, 19]. Low CYP1A2 activity, for example, has been linked to higher risk of testicular cancer [20]. However, due to their rapid conversion to highly reactive intermediates, excessive activity of CYP1A enzymes without adequate phase II support may enhance the destructive effects of environmental procarcinogens [21]. Indeed, genetic polymorphisms in this cytochrome family have been suggested as useful markers for predisposition to certain cancers [15]. CYP1 enzymes are also involved in the formation of clinically relevant estrogen metabolites: CYP1A1/1A2 and CYP1B1 catalyze the 2-hydroxylation and 4-hydroxylation of estrogens, respectively [22]. The potential role of 4-hydroxyestradiol in estrogen-related carcinogenesis, via the production of free radicals and related cellular damage [22], has prompted investigation into factors that modulate CYP1 enzymes.

Various foods and phytonutrients alter CYP1 activity (Tables 1(a) and 1(b)). Cruciferous vegetables have been shown, in humans, to act as inducers of CYP1A1 and 1A2, and animal studies also suggest an upregulation of CYP1B1 [4, 23–27]. The inductory effect of crucifers on CYP1A2 seems especially well established. Clinical studies also indicate that resveratrol and resveratrol-containing foods are CYP1A1 enhancers [28]. Conversely, berries and their constituent polyphenol, ellagic acid, may reduce CYP1A1 overactivity [6],

and apiaceous vegetables and quercetin may attenuate excessive CYP1A2 action [24, 29]. Cruciferous vegetables and berries have been suggested as possible modulators of estrogen metabolites: berries for their reducing effect on CYP1A1 [6] and cruciferous vegetables for their stronger induction of CYP1A versus 1B1 enzymes [25–27, 30]. Chrysoeriol, present in rooibos tea and celery, acts selectively to inhibit CYP1B1 *in vitro* [31] and may be especially relevant to patients with CYP1B1 overactivity. However, further research is needed to confirm this finding.

Many foods appear to act as both inducers and inhibitors of CYP1 enzymes, an effect which may be dose dependent or altered by the isolation of bioactive compounds derived from food. Curcumin at 0.1% of the diet has been shown, in animals, to induce CYP1A1, for example, [35], yet a diet of 1% turmeric was inhibitory [46]. Black tea at 54 mL/d induced both CYP1A1 and 1A2 [33], yet 20 mg/kg of theaflavins was inhibitory to CYP1A1 [45]. Soybean intake at 100 mg/kg upregulated CYP1A1 activity [7], yet at 1 g/kg black soybean extract [44] and 200 mg daidzein twice daily [49], its effect was inhibitory. Further research is needed to confirm different dose effects and impact in humans.

Varied effects may also occur from different members of the same food group. Seemingly contradictory to research showing that cruciferous vegetables activate CYP1 enzymes, kale (another member of the cruciferous family) appears to inhibit CYP1A2 (as well as 2C19, 2D6, and 3A4) in animals [51]. The dose used, at 2 g/kg per day, is 15-fold higher than the typical level of human consumption [51], and more research would be required to determine whether lower intake levels would also have a similar effect. The same authors also tested the effects of an equivalent volume of cabbage consumption and found no such inhibitory effect, pointing to the possibility that different cruciferous vegetables may have distinct effects on cytochrome activity.

2.1.2. CYP2A-E Enzymes. The large CYP2 family of enzymes is involved in the metabolism of drugs, xenobiotics, hormones, and other endogenous compounds such as ketones, glycerol, and fatty acids [15, 54]. Some notable polymorphisms occur in the CYP2C and CYP2D subgroups, leading to the classification of patients as "poor metabolizers" of various pharmaceuticals: warfarin and CYP2C9, antiarrhythmia agents, metoprolol and propafenone, and CYP2D6, phenytoin, cyclobarbital, omeprazole, and CYP2C19, for example, [15, 17]. CYP2D polymorphisms may be associated with Parkinson's disease and lung cancer [15]. Clinical evidence exists for the induction of CYP2A6 by quercetin and broccoli [4, 29] (Table 2(a)). In animals, chicory appears to induce CYP2A enzymes [41] and rosemary and garlic may upregulate CYP2B activity [9, 37]. Clinical studies using resveratrol and garden cress indicate CYP2D6 inhibition [28, 55] (Table 2(b)). Ellagic acid, green tea, black tea, and cruciferous vegetables also appear to inhibit various CYP2 enzymes.

CYP2E1 enzymes have also attracted particular interest for their role in various diseases. 2E1 metabolizes nervous system agents such as halothane, isoflurane, chlorzoxazone, and ethanol and bioactivates procarcinogenic nitrosamines and aflatoxin B1 [15, 65]. It produces free radicals regardless

of substrate [15], and CYP2E1 polymorphisms have been associated with altered risk for coronary artery disease [66 and gastric cancer [67]. CYP2E1-induced oxidative stress has also been shown to lead to impaired insulin action via the suppression of GLUT4 expression [68]. Attenuation of 2E1 overactivity may therefore be an important consideration in high-risk patients.

Watercress and garlic are CYP2E1 inhibitors in humans [59, 60]. *In vivo* evidence also suggests that N-acetyl cysteine, ellagic acid, green tea, black tea, dandelion, chrysin, and medium chain triglycerides (MCTs) may downregulate CYP2E1 [33, 43, 54, 61, 63, 64]. MCT oil may specifically attenuate the ethanol-induced upregulation of CYP2E1 and production of mitochondrial 4-hydroxynonenal, a marker of oxidative stress [64].

2.1.3. CYP3A Enzymes. The occurrence of the different CYP3A isoforms is tissue-specific [15]. Rooibos tea, garlic, and fish oil appear to induce the activity of CYP3A, 3A1, and 3A2 [8, 36, 69, 70] (Table 3(a)). Possible inhibitory foods include green tea, black tea, and quercetin [33, 56, 71, 72] (Table 3(b)). The most clinically relevant of the enzymes is CYP3A4, which is expressed mainly in the liver and to a lesser extent in the kidney [13]. Caffeine, testosterone, progesterone, and androstenedione are substrates of the CYP3A4 enzyme system, as are various procarcinogens including PAHs and aflatoxin B1 [15]. To date, however, the principal driver for research on CYP3A4 has been due to its role in the metabolism of over 50 percent of all pharmaceuticals [73]. The potential for drug interaction with this single enzyme, coupled with the wide interindividual differences in enzymatic activity, generates some level of risk in administration of high doses and multiple drugs as well as food-drug and herb-drug interactions. Grapefruit juice is perhaps the most well-known food inhibitor of this enzyme [74], though resveratrol and garden cress, a member of the cruciferous vegetable family, appear to have similar effects in humans, albeit at intakes above what would be expected without high-dose supplementation [28, 55]. Curcumin may upregulate 3A4 activity [11].

Once again, there are indications that a biphasic effect may be seen from dietary bioactive compounds; Davenport and Wargovich (2005) found that shorter-term or lower dosing with garlic organosulfur compounds produced potentially anticarcinogenic effects but that longer-term higher doses (200 mg/kg) of allyl sulfides led to minor hepatic toxicity [8]. One garlic clove contains only 2,500–4,500  $\mu$ g of the allyl sulfide precursor, allicin [76], so the higher dose is much more than would be consumed in a typical human diet. In another example, two components of cruciferous vegetables, sulforaphanes and indole-3-carbinol, inhibited and increased activity, respectively [57, 75], highlighting the potential for human studies using whole foods to clarify the outcome of consumption.

2.1.4. CYP4 Enzymes. Less is known about this family of enzymes, since it is thought to play a smaller role in drug metabolism. It is, however, understood to be a primarily extrahepatic family of cytochromes, inducible by clofibrate and ciprofibrate (hypolipidemic drugs), NSAIDs, prostaglandins, and toxicants such as phthalate esters [15, 77]. The CYP4B1

**Table 1:** (a) Human and in vivo example nutrient inducers of CYP1 enzymes. (b) Human and in vivo example nutrient inhibitors of CYP1 enzymes.

(a)							
Enzyme	Food, beverage, or bioactive compounds Food sources in italics	Type of study	Dosages used and references				
	Cruciferous vegetables	Clinical	500 mg/d indole-3-carbinol (23)				
	Resveratrol Grapes, wine, peanuts, soy, and itadori tea (32)	Clinical	1 g/d resveratrol (28): note high dose used				
	Green tea	In vivo	45 mL/d/rat (avg. 150 g animal weight) green tea (33)				
	Black tea	In vivo	54 mL/d/rat (avg. 150 g animal weight) black tea (33)				
CYP1A1	Curcumin Turmeric, curry powder (34)	In vivo	1,000 mg/kg/d/rat curcumin (35), or about 150 mg per rat per day				
	Soybean	In vivo	100 mg/kg soybean extract (7)				
	Garlic	In vivo	30 to 200 mg/kg garlic oil (36)				
	Fish oil	In vivo	20.5 g/kg fish oil (36): note high dose used				
	Rosemary	In vivo	Diet of 0.5% rosemary extract (37)				
	Astaxanthin Algae, yeast, salmon, trout, krill, shrimp, and crayfish (38)	In vivo	Diets of 0.001-0.03% astaxanthin for 15 days (39)				
CYP1A2	Cruciferous vegetables	Clinical	7 -14 g/kg cruciferous vegetables including frozen broccoli and cat flower, fresh daikon radish sprouts and raw shredded cabbage, and to and green (24) 500 g/d broccoli (4) 250 g/d each of Brussel sprouts and broccoli (25) 500 g/d broccoli (26) 45 mL/d/rat (avg. 150 g animal weight) green tea (33)				
	Green tea	In vivo	Green tea (2.5% w/v) as sole beverage (40)				
	Black tea	In vivo	54 mL/d/rat (avg. 150 g animal weight) black tea (33)				
	Chicory root	In vivo	Diet of 10% dried chicory root (41)				
	Astaxanthin Algae, yeast, salmon, trout, krill, shrimp, and crayfish (38)	In vivo	Diets of 0.001-0.03% astaxanthin for 15 days (39)				
CYP1B1	Curcumin Turmeric, curry powder (34)	In vivo	Diet of 0.1% curcumin (35)				
	Cruciferous vegetables	In vivo	25–250 mg/kg indole-3-carbinol (27)				
		(b)					
Enzyme	Food, beverage, or bioactive compounds Food sources in italics	Type of study	Dosages used and references				
	Black raspberry	In vivo	Diet of 2.5% black raspberry (6)				
	Blueberry	In vivo	Diet of 2.5% blueberry (6)				
CYP1A1	Ellagic acid Berries, pomegranate, grapes, walnuts, and blackcurrants (42)	In vivo	30mg/kg/d ellagic acid (43) 400 ppm ellagic acid (6)				
	Black soybean	In vivo	1 g/kg black soybean seed coat extract (44): <i>note high dose used</i>				
	Black tea	In vivo	20mg/kg theaflavins (45)				
	Turmeric	In vivo	Diet of 1% turmeric (46)				
CYP1A2	Apiaceous vegetables	Clinical	4 g/kg apiaceous vegetables, including frozen carrots and fresh celery, dill, parsley, and parsnips (24)				
	Quercetin Apple, apricot, blueberries, yellow, onion, kale, alfalfa, sprouts, green beans, broccoli, black tea, and chili powder (47, 48)	Clinical	500mg/d quercetin (29)				
	Daidzein Soybean (49)	Clinical	200mg twice daily dosing of daidzein (49)				
	Grapefruit	Clinical	300mL grapefruit juice (50)				
	Kale	In vivo	2 g/kg/d kale, as freeze-dried kale drink (51)				
			100mg/kg agrlio oil (52)				
	Garlic	In vivo	100mg/kg garlic oil (52)				
	Garlic Chamomile	In vivo In vivo	Free access to 2% chamomile tea solution (53)				
	Chamomile	In vivo	Free access to 2% chamomile tea solution (53)				

isoform is involved in the metabolism of MCTs (medium chain triglycerides), as well as the bioactivation of pneumotoxic and carcinogenic compounds [78].

Polymorphisms and overexpression of this subgroup may be associated with bladder cancer [15] and colitis [79]. A report by Ye et al. (2009) which examined the link between colitis and CYP4B1 activity found that the promotion of CYP4B1 activity by caffeic acid (found in caffeine-containing foods) (Table 4) correlated with reduced inflammation and disease activity [79]. Green tea may act to induce CYP4A1, as suggested by animal studies [40]. More research is needed to clearly identify food influences on this enzyme family.

2.2. Phase II Conjugation Enzymes. After a xenobiotic has gone through the process of becoming hydrophilic through reactions overseen by CYP450 enzymes, its reactive site can be conjugated with an endogenous hydrophilic substance. This reaction is often referred to as "phase II detoxification." Conjugation involves the transfer of a number of hydrophilic compounds (via their corresponding enzymes), including glucuronic acid (glucuronyl transferases), sulfate (sulfotransferases), glutathione (glutathione transferases), amino acids (amino acid transferases), an acetyl group (N-acetyl transferases), and a methyl group (N- and O-methyltransferases) [81]. The result of the collective activity of these enzymes is an increase in the hydrophilicity of the metabolite, theoretically leading to enhanced excretion in the bile and/or urine [81]. Similar to the CYP450 enzymes, genetic polymorphisms can have profound influence on the function of these conjugating enzymes [82], with potential implication in the development of several forms of cancer [83].

It is conceivable that modulation of phase II enzymes by food-based bioactive compounds may be advantageous in patients who have altered enzyme activity due to genetic polymorphisms or who have a high toxic burden due to chronic exposure to environmental pollutants, overactive phase I activity, or hormonal imbalance. For example, James et al. (2008) suggest that upregulation of glucuronidation and sulfonation by certain bioactive compounds may be a useful consideration for the elimination of environmental PCBs [19].

2.2.1. UDP-Glucuronosyltransferases. This class of enzymes, comprising multiple proteins and even subfamilies, plays an essential role in enhancing the elimination of biotransformed toxins in urine and feces, as well as metabolizing steroid hormones and bilirubin [84, 85]. Their function is to catalyze the covalent linkage of glucuronic acid from UDP-glucuronic acid to an accepting functional group on the molecule, a process referred to as glucuronidation [86]. Glucuronidation occurs primarily in the liver but can occur in other tissues, such as the small intestine [86, 87]. Bilirubin, specifically, is principally conjugated by UGT1A1 in hepatocytes [88] and then excreted with bile into the intestinal tract. It has been estimated that 40-70% of all medications are subject to glucuronidation reactions in humans, thereby suggesting the significance of this conjugation enzyme family [88]. Since UDP-glucuronosyltransferases (UGTs) also metabolize phytochemicals, alterations in their effects may be seen with genetically downregulated enzyme activity; flavonoids are conjugated with glucuronide and sulfate; therefore,

UGT or sulfotransferase (SULT) polymorphisms may produce variability in phytochemical clearance and efficacy [89].

Clinical and observational studies point to cruciferous vegetables, resveratrol, and citrus as foods and bioactive compounds that induce UGT enzymes [25, 28, 90–92] (Table 5(a)). Animal studies also suggest the potential for other foods and nutrients, including dandelion, rooibos tea, honeybush tea, rosemary, soy, ellagic acid, ferulic acid, curcumin, and astaxanthin, to enhance UGT activity [37, 39, 53, 93–95]. Interestingly, the effect of resveratrol was seen only in individuals with low baseline enzyme levels/activity, suggesting that some phytochemicals may modulate, rather than outright induce, enzymatic activity [28]. In addition, many studies note that effects are variable depending on gender and genotype [85, 90, 92]; for example, women with the UGT1A1 28 polymorphism (7/7) were responsive to citrus intervention, whereas those with other genetic variants were not [92].

Meaningful interpretations of these studies may still be elusive, however: in one combined dietary trial, the consumption of 10 servings per day of a combination of cruciferous vegetables, soy foods, and citrus fruits did not have a significant effect on UGT enzyme activity compared with a diet devoid of fruits and vegetables [85]. The authors hypothesize that these results may be due to their choice of specific foods within those groups or due to Nrf2 activation (discussed in subsequent sections) when fruits and vegetables were avoided.

The effects of UGT activity may also be enhanced by D-glucaric acid by theoretical inhibition of beta-glucuronidase enzymes [100]. Beta-glucuronidase enzymes act to reverse UGT conjugation reactions. D-glucaric acid is found in many fruits, vegetables and legumes (Table 5(b)). When tested in humans, however, a diet supplemented with cruciferous vegetables (2/3 cup broccoli, 1/2 cup cabbage, and 1/2 cup radish sprouts), citrus fruits (1 cup grapefruit juice, 1/2 cup orange juice, 1 cup orange/grapefruit segments, and 1 orange peel), and soy foods was found to have no effect on beta-glucuronidase activity [101] (amounts standardized for 55 kg body weight), indicating that the clinical effects of D-glucaric acid consumption still need further clarification.

In vivo research suggests that polyphenol extracts of certain berries, specifically strawberries and blackcurrant, may inhibit beta-glucuronidase activity in the intestinal lumen; Kosmala et al. (2014) observed this effect using both strawberry pomace water extract and water-alcohol extract containing 5.1% and 17.1% ellagic acid, and 0.2% and 10.9% proanthocyanidins, respectively [100]. Jurgon'ski et al. (2014) found a similar inhibitory effect using a diet of 1.5% blackcurrant extract (total polyphenolic content 66.8 g/100 g extract) [102]. Interestingly, the highest levels of beta-glucuronidase activity were seen in rabbits fed a high fat diet (32% calories from fat, including 10% from lard), without blackcurrant extract supplementation, suggesting that dietary fat may also alter enzyme activity [102].

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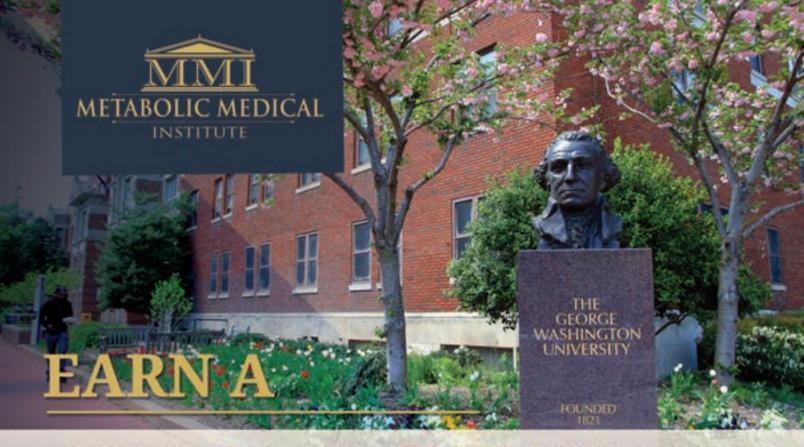
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# THE INTERACTION OF NEUROACTIVE STEROIDS WITH NEUROTRANSMITTTERS DURING THE PERIMENOPAUSAL TRANSITION

By KATE PLACZEK, PhD

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# **Neurological Nature of Perimenopause**

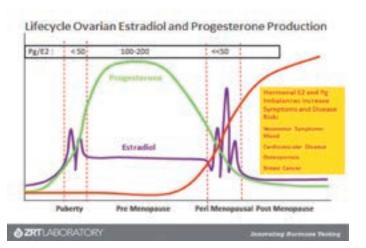
Perimenopause is the dynamic period in a woman's life when the first endocrine, biological, and clinical features of impending menopause appear. It usually begins in the late 40's and ends in the early-to-mid 50's. The constant and predictable fluctuation of hormones throughout each menstrual cycle eventually begins to wind down at perimenopause. Dramatic variability in the hormonal milieu at perimenopause can in some women give rise to unrelenting systemic consequences, such as a dramatic increase in vasomotor symptoms, including hot flashes, night sweats, sleep disturbances and mood lability, and somatic symptoms, such as vaginal dryness.[1]

In clinical terms, perimenopause heralds the beginning of the shutdown of the reproductive system. Although the classical definition focuses mainly on the reproductive system, perimenopause begins in the brain and is fundamentally neurological in nature.[2] The breadth of neurological symptoms associated with perimenopause, such as mood changes, insomnia, decreasing cognitive function, and temperature dysregulation, are a manifestation of the disruption of centrally-regulated mechanisms. The ovarian hormones estradiol and progesterone, which are essential reproductive and neural substrates, are at the heart of this transition.

# Perimenopause and the Ever-Changing Hormones

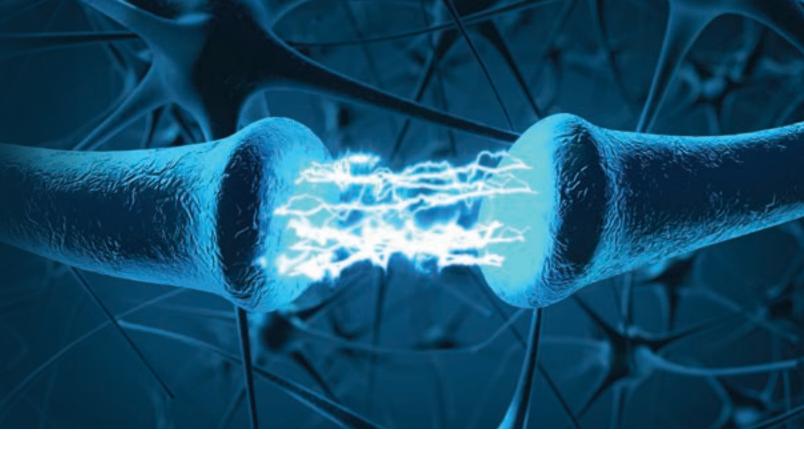
Erratic or anovulatory cycles signal that the steady decline of progesterone levels has been initiated: no corpus luteum – no progesterone. Even without sufficient progesterone, estradiol can be derived from the male hormones that the ovaries and the adrenals produce. Therefore estradiol

levels go on to spike and fall for the duration of perimenopause. Sufficient estrogen is essential to good health, but in perimenopause, when progesterone and estradiol fall out of balance with each other, estrogen dominance arises with a multitude of negative symptoms [3]. The frequency and severity of these symptoms appear to peak in late perimenopause and early post-menopause. The key to understanding perimenopausal symptomatology is to acknowledge that the loss of the delicate balance between estradiol and progesterone has profound neurological consequences.



# The Neuronal Substrates - Estradiol and Progesterone

Operating synergistically with many biological systems, estradiol and progesterone belong to the complex gonadal hormone family that regulates a myriad of physiological



functions, which extend to higher cognitive functions such as mood, motor coordination, and pain sensitivity. [4;5] Estradiol, progesterone and their bioactive metabolites regulate these complex processes via a network of classical nuclear and membrane receptors. The locations of the estrogen and progesterone receptors in the brain coincide with the relevant neuroanatomical regions that regulate executive and affective functions, and vasomotor stability.[6] Furthermore, cholinergic, noradrenergic, serotonergic, and dopaminergic neurons all respond to estradiol, progesterone and their neuroactive metabolites.[7] Fluctuations in ovarian function have a profound impact not only on the components of the hormonal milieu, but on neurotransmitter levels and signaling as well. As women approach menopause, the effects of the ever-changing hormonal flux on the neurotransmitter systems become very apparent.

### The Neuroendocrine Nature of Hot Flashes

Estradiol directly modulates neurotransmitter systems for norepinephrine and serotonin, activates receptors, upregulates neurotransmitter biosynthesis and release, blocks re-uptake and degradation of neurotransmitters and modulates the overall structural morphology of many brain regions. [5;8] In their turn, neurotransmitters (norepinephrine and serotonin) directly regulate the thermoneutral zone – the homeostatic range of body temperature. Compression of the hypothalamic, cortical, and subcortical thermoneutral zones, which follows the drop in levels of estradiol and progesterone at perimenopause, leads to episodic releases of heat in response to slight increases in the core body temperature. [2] As estradiol declines, brain norepinephrine levels increase while serotonin

decreases, inducing a narrowing of the thermoneutral zone. [9] Decreased estradiol consequently results in upregulation of monoamine oxidase-A (MAO-A) levels in the brain, causing more rapid degradation of serotonin and further narrowing of the thermoneutral zone. [10] In neurochemical terms, as estradiol decreases, serotonin turnover is increased. Serotonin is a potent thermoregulatory agent that modulates hot flashes in an opposite manner from norepinephrine. Therefore, putatively reduced serotonin function during perimenopause and an abundance of norepinephrine signaling appear to have vasomotor manifestations in the context of a declining hormonal environment. Higher norepinephrine, precipitated by physical and emotional stressors of impending menopause, further exacerbates hot flashes and night sweats.

### **Hot Flashes Resulting from Compromised Bioenergetics**

Experienced by approximately 80% of women during perimenopause, hot flashes commonly co-occur with other neurological symptoms, such as sleep disturbances, mood changes, and a decline in cognitive function.[2] Characterized by the onset of intense and troublesome rises in body temperature, hot flashes are complex and poorly understood.

Increasing evidence suggests a tight-knit relationship between hot flashes and impaired glucose homeostasis. Glucose levels and the degree of insulin resistance correlate with frequency of hot flashes.[11;12] Together with low progesterone levels, fluctuating levels of estradiol coincide with a decline in brain bioenergetics and shift towards a metabolically compromised phenotype; an effect that can be remedied with hormone replacement therapy (HRT).[13]

In symptomatic female patients receiving HRT during perimenopause, glucose metabolism is preserved in brain regions with estrogen-dependent neurological functions, an effect not observed in symptomatic patients without HRT.[14;15]

# **Depression in Perimenopause**

The compromised metabolic state of perimenopause extends beyond hot flashes. As the estrogen network uncouples from the bioenergetics system, the hypometabolic state may render some women vulnerable to increased risk of mood disorders. Perimenopausal women are four times more likely to report depressive symptoms than premenopausal women. [16] Substantial changes in brain bioenergetics may also be linked to the development of depression – postmenopausal women with depression display selective regional changes in metabolism. Specifically, this manifests as hypometabolism in the pons (part of the brainstem) and hypermetabolism in the frontal gyrus (frontal lobe), compared to postmenopausal women without depression.[17] It is therefore intuitive that fluctuating estradiol in perimenopause, essentially unopposed by the low progesterone, may eventually result in alterations of executive brain

functions, such as mood, sleep and memory. Interestingly, administration of transdermal estradiol normalizes the heightened emotional activation caused by the decreased in serotonergic functioning in menopausal women, presumably working by restoring the compromised bioenergetics.[18] Targeting the serotonergic system directly with SSRIs is also an option to help with the menopausal transition; however, for many women, SSRIs are the less-than-desirable option because of sexual side effects. The studies referenced above further support the magnitude of positive effects of estrogen on cognition and mood via modulation of neurotransmitter

## **What about Progesterone?**

function.[19]

The importance of estradiol to female health and well-being is undeniable. The only nuance is that progesterone is essential to ensure that the effects of estradiol administration are balanced and not overdone. Progesterone, however, is not a passive bystander, but rather a very crucial neuronal messenger both as the parent hormone and in the form of its neuroactive metabolites. Progesterone has direct actions in the brain through progesterone receptors, but its metabolite, allopregnanolone, directly modulates neuronal excitability through its activity as a potent allosteric modulator of the GABA-A

receptor.[20] The GABAergic system is the major inhibitory system in the CNS, or "off switch," and the primary regulator of affect and cognitive functioning.[21] At relatively high levels, seen during the luteal phase, pregnancy, and with progesterone supplementation, allopregnanolone signaling via the GABA-A receptor has calming, anxiolytic, anesthetic and sedative effects.[22;23] Specifically, when sleep problems arise around the time of the menopausal transition, progesterone supplementation often promotes a sense of calm, helps to restore normal sleep patterns, and prominently improves adverse symptomatology.[3;24]

# **The Progesterone Paradox**

Progesterone does not have a perfect record in alleviating symptoms. In fact, in a minority of women (5-10%), progesterone has a paradoxical effect and worsens the symptoms it was meant to treat. The inconsistency has been puzzling for providers and patients alike, but has its origins in the

interaction of allopregnanolone with the GABA-A receptor. Differences in the subunit composition of the GABA-A receptor are now believed to make some women particularly sensitive, in a negative way, to allopregnanolone.

The compositional subunit assembly of the GABA-A receptor at any given time greatly predicts how sensitive the GABAergic system will be to allopregnanolone.[20] In other words, the GABA-A receptor has to mix-and-

match the five subunits in different wavs to adapt to the ever-changing hormonal flux. Some women display differences in GABA-A subunit structure and abnormal patterns of neural activation, which renders them hypersensitive to allopregnanolone.[25-28] To make matters even more complex, negative mood increases, correlated with increasing serum allopregnanolone levels, show a biphasic effect.[29] The lower endogenous concentration of progesterone and allopregnanolone, such as is observed during the luteal phase of a perimenopausal woman, or a premenopausal woman with luteal insufficiency, typically worsens the symptoms. However, a further increase in allopregnanolone with exogenous progesterone supplementation can lead to a decrease in symptom severity.[30;31] Oral progesterone increases the circulating levels of allopregnanolone, which likely accounts for its success in bringing about anxiolysis when used at night before bed.

# **Personalizing HRT**

The influence of HRT on health status varies greatly depending on the specific individual patient, as seen above, and also on

the type of preparation of HRT, its components, and its route of administration. For example, oral progesterone undergoes first-pass metabolism, yielding high levels of allopregnanolone that freely crosses from the bloodstream through the bloodbrain barrier into the brain. Neuroactive steroids are unique in that they can form in the peripheral system and readily enter the brain. With vaginal or topical administration of progesterone, a smaller increase in allopregnanolone occurs in the peripheral circulation; however, progesterone entering the brain is directly converted to allopregnanolone.[32] Personalizing HRT may just be the best strategy for each individual patient to sustain neurological, bioenergetic capacity.

### **Conclusion**

Perimenopause is a developmental change in the evolving landscape of endocrine and neurological transformations. Following completion of the perimenopausal transition, the neurological system reaches a new homeostatic domain. Notably, for some women, intervention is not necessary; however, for others intervention is appropriate and may alleviate distressing symptomatology. HRT serves as the logical choice, given the tremendous impact that estradiol and progesterone elicit on the brain. Timing of the administration of HRT appears to be critical to the effectiveness of the therapeutic approach, with perimenopause providing the optimal window of opportunity for introducing exogenous hormones. Whatever intervention path is decided on by the provider and patient, one of the primary goals should be to focus on sustaining healthy brain function. A balanced brain is the foundation for optimal physical, psychological, and emotional well-being.

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# PERSONALIZED MEDICINE SIMPLIFIED: Powered by Big Data

By ANDREW HEYMAN, MD, MHSA, FMNM, ABAARM JAMES LAVALLE, RPH, CCN, DHM, DHPH

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wo of the most exciting trends in health care are the intersection of Personalized Medicine and the Quantified-Self (QS) movement. The convergence of these two fields represents an opportunity to tailor treatments, diets and lifestyle choices to the uniqueness of the person and support them on their path toward wellness.

The implementation of personalized medicine often falls short due to an overreliance on genetic testing alone, and as many experts now agree, single-nucleotide polymorphism (SNP) assessment by itself is not a reliable predictor of health and future disease without full genomic and metabolomic profiling. Additionally, the ability to collect vast amounts of medical information based on new technologies and online computing requires complex mathematical modeling to derive significance from that data, the application of which has not yet been made clinically available.

Medicine is now at a tipping point where, for the first time, technology, big data and personalized care are beginning to converge in practical ways to address the many challenges and failures in the health care system. Successful solutions will bridge the gap between information and understanding, and they will be sophisticated enough to collect large amounts of information while remaining simple enough to clarify and individualize the results to the patient in an actionable way. Furthermore, the process should integrate

the best scientific evidence and also take into account the person's quality of life and how they are currently feeling.

The Metabolic Code platform is the first cloud-based solution in the field of Integrative Medicine to address the promises and challenges of big data and personalized care simultaneously. Its system offers a complete environment in which clinical decision-making and longitudinal care are synthesized. The Metabolic Code's platform takes it a step further by tracking a person's biochemical signature (labs), activities and lifestyle choices with the goal of optimizing an individual's health and vitality throughout every stage of their life. Additionally, it provides efficiency with operations and inventory management and, as a result, streamlines the patient-centered care model.

The Code seamlessly integrates with various laboratory companies, while aggregating biometric and self-reported data. All of the patient's information is then computed through thousands of evidence-based algorithms to generate a personalized wellness plan that includes nutrition programming, an exercise plan and dietary supplements. At the practitioner's discretion, the report of the personalized lifestyle recommendations with the associated scientific references can be shared with the patient, and a plan of action developed between practitioner and patient (See Figure 1). The patient then learns exactly what is the right thing to do



for their health. The system offers the option to direct-ship supplements to patients through the auto-ordering functionality, and patients have access to vetted clinically proven diets and a complete set of videos of exercise programs for various fitness levels. Over time, the practitioner can use the Code to track patients, assess progress and offer suggestions between office visits.

The genesis of the Metabolic Code derived from the need to develop best practices in Integrative Medicine, deployed through a technological platform to improve clinical outcomes and operational efficiencies while generating reliable revenue streams. The Code is the result of contributions from a wide variety of experts including physicians, pharmacists, dieticians, and a world-class health informatics and technology team.

# **Triads: Integrative Physiology Made Simple**

The Metabolic Code collects a large amount of biometric data, subjective symptoms, blood, urine and salivary lab tests in order to determine where the greatest metabolic challenges exist for an individual. The Metabolic Code algorithms assign values for these data inputs into five key domains, called Triads.

The Triads are composed of three interdependent processes to form a collective sub-unit of important biochemical

interactivity (See Table 1). The resulting Metabolic Code report organizes and sorts the information into a ranking system based upon which Triad is the most imbalanced. It produces a simple and clear set of recommendations on how to improve the function of each Triad.



Triad 1 (Adrenals - Thyroid - Pancreas) encompasses the relationship between 3 important hormones: cortisol, insulin, and thyroid, which reflect the state of stress, glucose

balance, and metabolism. In their most basic function, each is responsible for energy production. When Triad 1 is balanced, a person feels vital and healthy, but when unbalanced, a person feels fatigued and has a higher propensity for being overweight or obese.



**Triad 2 (Gut - Immune - Brain)** is comprised of the digestive tract, immune system, and central nervous system. Together, these intelligent body systems make moment-to-moment decisions

with regard to absorption and assimilation, and set key boundaries physically, immunologically, and mentally. When Triad 2 is normal, a person feels organized and secure within themselves and their environment. When out of balance, this physiologic network becomes disordered and unpredictable.

ENERGY	RESILIENCY	ENDURANCE	DETOXIFICATION	POTENCY	
1	2	3	4	5	
Adrenal glands	GUT (gastrointestinal system or digestive tract)	Cardiovascular	Liver	Testosterone	
Thyroid gland	Immune system	Pulmonary	Lymph	Estrogen	
Pancreas	Brain (Central Nervous System)	Neurovascular	Kidneys	Progesterone	

**TABLE 1: Metabolic Code Triads** 

The key concept of Triad 2 is **resiliency**. An immune system that responds appropriately, proper absorption and utilization of nutrients, and clarity of mind are central to a balance within Triad 2.



Triad 3 (Cardiopulmonary - Neuro - Vascular) includes the cardiopulmonary unit, autonomic nervous system, and vascular tree. This triad reflects the relationship of the mind and heart,

and is mediated by the respiratory cycle. When Triad 3 is in balance, the individual has plenty of metabolic resiliency and strength to meet the challenges of life. The key concept in this Triad is **endurance** and stamina. The delivery of oxygen and the pliability of the vascular tree, along with the sense of neurologic balance, are key constructs of this triad.



Triad 4 (Liver - Lymph - Kidneys) contains the drainage organs of the liver, lymph, and kidneys. These organs form a functional unit for detoxification and elimination, metabolic processing

and removal of toxins, and enzymatic activities. They allow us to thrive in a toxic world. When Triad 4 is in harmony, there is a smooth flow of substrates and emotions, and when out of balance, inflammation, stagnation, and retention ensue.



Triad 5 (Estrogen - Progesterone - Testosterone) includes the reproductive hormones estrogen, progesterone, and testosterone, which play a central role in the reproductive lifecycle.

They confer a sense of **potency**, power, and self-esteem for both men and women.

In addition to system- or organ-based intra-relationships within each Triad, all of the Triads can influence one another. This is known as Triad stacking and explains how different metabolic networks affect each other.

Although the Triads are conceptual in nature, they reflect and express a practical framework of clinical systems biology. Their power is in their ability to focus the practitioner and patient on important aspects or domains of health and take corrective measures to restore harmony and vitality in measurable ways.

### **The Report: Wellness in Action**

Data from the questionnaire, biometrics and blood work are analyzed to produce a report, essentially a snapshot, of the patient's current health status. The report also acts as a roadmap back to vitality by offering specific therapeutic recommendations, further testing, and lifestyle support.

The patient's Triad score is displayed in numerical and graphical representation, categorized by:

# LOW RISK MODERATE RISK HIGH RISK

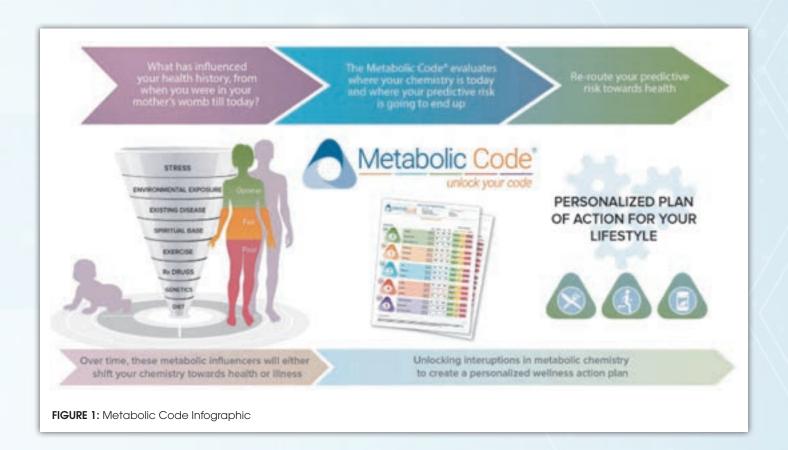
The report is comprehensive, individualized and practical. It includes:

- Scoring system to identify greatest to least Triad imbalances
- Individualized lab and questionnaire ranking
- Explanation of every lab abnormality
- Data stacking between Triads
- Specific dietary supplement recommendations
- Description of Triad organ systems

### **Outcomes, Efficiency and Revenues**

The practice of Integrative Medicine has become more complex in recent years with the availability of new testing methods and advancements in understanding chronic illnesses. Practitioners must develop new competency in information management, which poses time and intellectual challenges in the process of medical care. Technology ensures efficiencies in this regard, simplifies aggregation and analysis of data, and offers reporting to patients of their health status in meaningful ways. The role of technology reduces the barriers of entry in Integrative Medicine in this regard, and supports evidence-based clinical decision-making.

Point-of-care systems also create a common language between practitioner and patient, and bridge the gap between



information and understanding on both sides of the therapeutic relationship. Patients need to understand their current health status, and mounting risks for future disease. Yet most patients forget 80% or more of the details after office visits and need ongoing support to reinforce their self-understanding and plan of care. By offering a comprehensive, patient-centric report, the likelihood of follow-through with healthier behaviors and choices increases, with improved follow-up and adherence to suggested regimens.

While cloud-based technology supports practitioners in the process of care, equally importantly is the opportunity it provides to collect outcome data across large populations. This is the very sort of data currently missing from Integrative Medicine, and usually is the major criticism by conventional practitioners. Unfortunately, it is costly and cumbersome to conduct large randomized control trials, which also do not reflect how Integrative Medicine practitioners actually care for patients.

Additionally, many challenges exist in the collection of medical outcomes data, even more so in the field of health and wellness. Practices generally function independently of the conventional health care system, with little industry oversight and unregulated dietary supplements. By definition, this style of practice is multi-modal, interdisciplinary and usually combines lifestyle recommendations with dietary supplements, traditional healing methods and sometimes, conventional therapy. Point- of-care platforms link the process of care to patient outcomes and reduce barriers to conducting high-quality research.

To that end, the Metabolic Code platform is being used to establish a Practice-Based Research Network (PBRN). Clinical outcomes will be tracked and analyzed, and the analytical process will fine-tune the algorithms and decision trees to refine patient reports and further improve the reliability and validity of clinical recommendations. Comparative analyses will be conducted on treatment approaches, specific dietary supplements and lifestyle recommendations.

In reviewing the underlying science, practical clinical application, and marketplace innovation, The George Washington University has agreed to include the Metabolic Code system for all its students in the master's program in Integrative Medicine. The Code offers an information-rich teaching environment for students that accelerates their understanding of fundamental concepts in Lifestyle Medicine and ensures exposure to real-world tools for future Integrative practitioners.

The George Washington University also views the deployment of the platform as an enormous research opportunity as practices begin to adopt the system. GWU will provide research support and analytics to assess clinical outcomes, improve the algorithms, and produce clinical care guidelines and research papers that will help change the medical industry. The partnership between the Metabolic Code and GWU will ensure a leap forward in Lifestyle Medicine by establishing a health services infrastructure and research agenda to produce best practices in medical wellness.

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# Anti-Aging Interventions – FROM TELOMERE BIOLOGY TO LONGEVITY

By MARK ROSENBERG, MD

The following article is not endorsed and/or supported by The American Academy of Anti-Aging Medicine.
The purposes of this publication do not imply endorsement and/or support of any author, company or theme related to this article.

### INTRODUCTION

uring aging and prolonging life has been in the agenda of scientists for hundreds of years. In 2009 the Nobel Prize in Physiology was awarded for the discovery and introduction of aging's official biomarker, the telomere. Since then, telomere biology has been receiving tremendous attention with hundreds of new studies constantly being published on the implications of telomeres not only with aging itself, but also with a vast number of age-related diseases. It is becoming progressively more apparent that the main research focus is moving from the causes of aging to the discovery of effective interventions that can slow the aging process down or even reverse it. By looking at the most recent publications, one would observe that significant progress has been made on this matter and various interventions are now available to maintain or elongate telomeres and promote longevity. Consequently, the monitoring of telomere length through telomere testing remains fundamental.

# **TELOMERES IN A NUTSCHELL**

Telomeres are the repetitive nucleotide sequences that protect the ends of chromosomes and help maintain genomic stability. These sequences conform to a specific pattern (TTAGGG) and can span less than 4,000 to up to 15,000 base pairs joined together. With every replication cycle (cell division), telomeres get shorter by approximately 20 - 200 base pairs. Once telomeres reach a critically short length, the cell enters a phase of replicative senescence where no further division is possible. As telomere length approaches this critical

point, the probability for DNA damage and telomere dysfunction is much greater. To say that senescent cells undergo apoptosis and die would be a common misconception. Instead it is recognized that senescent cells can secrete factors that can influence age-related diseases.<sup>7</sup>

Telomerase is a ribonucleoprotein that adds *de novo* telomere repeat sequences to the end of telomeres, lengthening them. Therefore, telomerase can help decrease the telomere attrition rate or even reverse it. Unfortunately, typically only germ cells and stem cells display meaningful telomerase activity, in contrast to other somatic cells that express telomerase at very low levels, if at all. This helps explain why there is a need to develop anti-aging therapies that not only protect telomeres, but also potentially elongate them.

### THE VALUE OF TELOMERE TESTING

Genomic instability and telomere dysfunction are of critical importance for aging both at a cellular level<sup>3-4</sup> and beyond. There is an abundance of studies demonstrating that failure in telomere maintenance is strongly associated with premature aging phenotypes and age-related diseases like inflammation, cardiac disease, cancer and neurodegeneration.<sup>5-6</sup> Additionally, it has been published that telomere length can be used to stratify risk in cardiovascular diseases<sup>48</sup> as well as provide prognostic information

on mortality risk in type 2 diabetes  $^{49}$  and many types of cancers,  $^{50-53}$ 

The above highlight the significance of incorporating telomere testing in the regular examination of patients but also of healthy individuals. This is not only important for diagnostic and prognostic reasons discussed earlier, but also for continuous monitoring of an individual's progress after the initiation of an anti-aging intervention.

It is important to mention that for a more comprehensive understanding of one's telomere status, a number of data is required. This includes the number and percentage of critically short telomeres, the median rather than the mean and the full histogram of telomere length distribution. The only technology available that can offer such a comprehensive approach is q-FISH (Quantitative Fluorescence *in situ* Hybridization), which is uniquely able to measure telomeres at the individual chromosomal level inside the nuclei of cells. Currently, this technology is commercially available through one CLIA certified laboratory, which possesses a proprietary technology called Telomere Analysis Technology® (TAT®).

### A GROWING ARSENAL OF ANTI-AGING INTERVENTIONS

Below we will discuss different possible interventions with published results that are proven to have positive effects on telomere protection and maintenance.

### CALORIC RESTRICTION AND WEIGHT LOSS

Caloric restriction has been long known to increase longevity and delay aging. 80 years ago, a study at Cornell University demonstrated that when caloric restriction was applied in rats (30% less food), the result was a 30% increase in mean and maximum lifespan. Until recently there was no clear demonstration of how and whether caloric restriction can affect telomeres. However, in two studies that took place in 2014 and 2016, the telomere lengths of 74 and 42 obese adults respectively were measured before and after weight loss interventions. In both studies it was observed that the telomere lengths of the participants were longer after the intervention. In the second study it was also noted that the

lengthening was more significant in participants with shortest telomeres at baseline. Equally, in another study assessing the relationship between telomere length and adiposity in

309 individuals, it was concluded that those with higher total and abdominal adiposity were characterized by lower telomere length, 10 confirming that obesity accelerates aging. 44

# LIFESTYLE INTERVENTIONS

Physical activity has always been considered a significant part of a healthy lifestyle. Late studies have demonstrated that among other variables exercise directly benefits telomeres. A study taking into account leucocyte telomere length in a population of 6,503 individuals showed that movement-based behaviors are associated with reduced odds of being in the lowest 30%. <sup>11</sup> Moreover, in two other studies, running-specific physical activity <sup>12</sup> as well as ultra-

endurance aerobic exercise<sup>13</sup> were shown to be linked to longer telomere lengths, further emphasizing the significance of exercise in longevity.

The dangers behind high *alcohol consumption* have been well documented and include morbidity and mortality. <sup>14</sup> Reducing alcohol consumption not only can improve overall health, but also significantly reduce telomere attrition and promote healthy aging. Currently, there are various studies demonstrating the negative effect of alcohol on telomeres. Telomere length in alcohol abusers is shown to be approximately 50% that of social drinkers. <sup>15</sup> Likewise, even minor alcohol consumption in midlife has been found to be associated to shorter telomere length in old age. <sup>16</sup>

Another lifestyle aspect that can greatly affect telomere biology is *smoking*. There is aggregated evidence that smoking is linked to telomere length and greater shortening prospectively is observed in smokers compared to individuals who do not smoke. <sup>17-19</sup>

In support to the amassed data that suggest that stress and depression is harmful to telomeres, a very good addition to one's habits for telomere protection is *meditation*. Individuals that practice meditation regularly have been shown to display much longer telomere lengths than healthy non-practicing individuals.<sup>20</sup> This probably can be explained due to the experiential avoidance of negative emotions that can be achieved through meditation and more generally, techniques to manage stress.

# INTERVENTIONS THROUGH DIET, NUTRITION AND SUPPLEMENTS

There are various nutrients whose consumption has been proven to positively correlate with telomere length. These nutrients can be incorporated in one's diet through different types of foods and through favorable dietary patterns. However, all of them are also commercially available as supplements and comprise effective solutions for telomere maintenance and healthy aging. It is also worth discussing existing compounds that not only protect, but may also elongate telomeres through the activation of the telomerase enzyme.

# Antioxidants

Antioxidants constitute one such solution. Biological and physiological aging are affected by the rate of wholebody free radical production and therefore a contributing factor to human degenerative diseases of aging is the oxidant-antioxidant imbalance.<sup>21</sup> Cells that enter the phase of replicative senescence produce high amounts of reactive oxygen species,<sup>22</sup> resulting in general and telomere-specific DNA damage.<sup>23</sup> Damaged DNA in human cells demonstrated accelerated telomere attrition and premature replicative senescence.<sup>24</sup> Moreover, in a human study on premenopausal women, those with the highest degree of chronic oxidative stress were found to have shorter telomeres equivalent to one decade of biological aging.<sup>25</sup> On the other hand, total circulating antioxidant capacity is positively correlated with telomere maintenance through reduction in systemic oxidative stress.<sup>26-27</sup>

There is compelling evidence on various antioxidant solutions that have clearly demonstrated their telo-protective

# Using telomere measurement in anti-aging medicine

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Dr. Bill Andrews Dr. Mark Rosenber



# ROUND TABLE

**Dr. Mark Rosenberg**, M.D., President of The Institute for Healthy Aging

**Dr. William H. Andrews, Ph.D.,** President & CEO, Sierra Sciences

Stephen Matlin, CEO, Life Length

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effects. Carotenoids like alpha-carotene, beta-cryptoxanthin<sup>28</sup> and especially  $\beta$ -carotene<sup>29</sup> have been proven to be associated with longer telomere length in humans. Similar correlations are displayed in individuals with high serum concentrations of the carotenoids lutein and zeaxanthin.<sup>30</sup>

Owing to their antioxidant properties, the intake of individual vitamins or multivitamin products can similarly be very beneficial for telomere biology. Vitamins A, E, C and folic acid can reduce oxidative stress and protect telomeres as shown by various studies.<sup>31-32</sup>

Many publications also support the direct telomeres benefits of polyphenols found in green tea<sup>33</sup> and coffee beans,<sup>34</sup> due to their antioxidant potential. Another nutrient, Coenzyme Q intake prevented accelerated aging in an experiment on animals.<sup>35</sup> It is worth mentioning that various minerals like magnesium<sup>31</sup> or potassium<sup>32</sup> can also have significant health benefits regarding telomere protection.

## Fatty acids

Various epidemiological studies exist that explored the link between intake of specific fatty acids and telomere length. In a longitudinal study conducted within a period of 5 years, the rate of telomere attrition was significantly lower in those patients with baseline blood levels of marine omega-3 fatty acids, <sup>36</sup> proposing their beneficial role in telomere maintenance. Similarly, in another study on elderly individuals with mild cognitive impairment, reduced telomere shortening was observed in those participants receiving DHA supplementation.<sup>37</sup>

### Dietary Patterns

In support of the suggestive evidence that dietary antioxidants and other micronutrients relate to longer telomere length, reports from different studies propose the additive and synergistic interactions of nutrients and phytochemicals.<sup>38</sup> Therefore, certain dietary patterns and combinations might prove much more beneficial than the consumption of their components individually.

A dietary pattern, very popular for its health benefits is the Mediterranean diet.<sup>39</sup> The routine consumption of olive oil, fruits, vegetables, nuts and whole grains deliver an abundant supply of fiber and antioxidants that have telomere protective potential, as explained above. There are three published studies that demonstrate the advantageous effect of the Mediterranean diet. In the first study, adherence to this diet maintained endothelial telomere length and reduced oxidative stress in elderly subjects.<sup>40</sup> In the second and third studies, subjects following this diet had longer telomeres than the relevant control groups.<sup>41-42</sup>

# Telomerase Activators

There is a noteworthy effort from researchers and companies worldwide to develop compounds that can activate telomerase, the enzyme that elongate telomeres only in specific cells in our body. Such a telomerase-activating compound could help directly elongate telomeres in cells where telomerase is normally not present, slowing down aging and



potentially reversing it. There are many studies currently ongoing that could reveal relevant treatments with great potential in the future. However, conclusive data on certain products already exists and it is worth mentioning some of the most representative examples.

TA-65: In a randomized, double-blind and placebo-controlled study, TA-65, which is based on an Astragalus extract, appeared to lengthen telomeres of healthy individuals over a one-year period.  $^{45}$ 

Danazol: Recently Danazol, a synthetic steroid widely used for the treatment of endometriosis, was used to treat individuals with telomere diseases. The study concluded that Danazol led to telomere elongation, suggesting its potential use in antiaging therapies.<sup>46</sup>

Statins: In a cross-sectional study conducted in 230 subjects treated with different statin treatments, it was observed that statins can affect telomere erosion through modulation of telomerase activity.<sup>47</sup>

# **SUMMARY**

There is no doubt that telomere length and telomere maintenance are directly implicated with the process of aging itself. A higher rate of telomere attrition can accelerate aging and broaden the gap between one's chronological and biological age. A short telomere length, and a higher percentage of short telomeres in one's body, certainly increases the probability of an age-related disease onset. Today, there are numerous interventions that can help reduce telomere attrition. These include weight loss, antioxidant nutrients, exercise, moderate alcohol consumption and increasingly, supplementation with telomerase activators. However, before embarking on any program, it is essential to establish and then monitor annually a patient's telomere length for prognostic and diagnostic reasons, and incorporate telomere testing in the regular required medical examinations. This becomes even more crucial for patients that follow an anti-aging intervention where progress should be closely observed.

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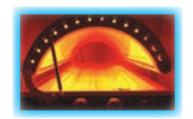
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# IONODERME INFUSION SYSTEM-AFFINITY

A novel system for direct Intrafollicular infusion of liquid solutions containing active ingredients.

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Skin provides the largest interface between the human body and the external environment and at the same time serves as an effective barrier. It prevents excessive loss of water from the body and likewise blocks entry of most topically applied substances, except for ones which are lipid-soluble and of low molecular weight. Under physiological conditions the intact stratum corneum significantly impedes penetration of large hydrophilic, charged molecules and particulate substances through the skin.

A number of strategies are currently employed to overcome the barrier function of the skin to enhance penetration of therapeutic compounds. Substance delivery through the hair follicles has gained a lot of interest in the field of skin penetration research as the follicles represent an important pathway for topically applied substances. They function as a reservoir and also as portals of entry to viable skin. In addition, they represent invaginations of the epidermis, which significantly increase the skin surface area available for penetration. It has been shown experimentally that when follicular openings are selectively blocked during intrafollicular infusion, a subsequent decrease of concentration of the active compounds is measured in the skin. Calculation of total ducts inner surface is difficult, since it varies with age, sex, locale, etc., but it is estimated as being ten times greater than the total size of the epidermis. Therefore, the presence of large numbers of hair follicles significantly increases the penetration rates of topically applied compounds. [1-3]

Hair follicles also serve as a reservoir, in which active substances are protected from shedding, and are retained for prolonged time periods, enhancing their penetration to and through the skin cells. Furthermore, due to the follicular ducts' orientation down through the skin, the pores can serve as a channel leading to the depth of the dermis. Deposition of active compounds in the depth of the follicle may target specific cell groups there, such as follicular stem cells, important for skin rebuilding and regeneration. [4]

Following the understanding of the possible advantages of transfollicular penetration, different therapeutic approaches have been developed over the last several years. One of them is direct intrafollicular infusion of liquid solutions containing active ingredients. [5]

To achieve intrafollicular infusion, the system must fulfill the following requirements:

- 1. The liquid delivery source has to be directly attached to the follicular orifice. If not, due to duct resistance, liquid flow will flood the skin surface instead of being delivered into the duct.
- 2. The diameter of the flow source apparatus has to be smaller than that of the duct orifice. If not, the flow impact will deform the surrounding tissue to create a "check valve" having a closing effect on the duct orifice.

3. To create a short-term linkage between the duct pores and supplying nozzles to fill up the follicular reservoir, constant motion of the liquid delivery hand piece over the skin is required.

# **IONODERME INFUSION SYSTEM-AFFINITY**

The Ionoderme Infusion System-Affinity was recently developed to accomplish intrafollicular delivery of active substances. The "heart" of the system consists of plastic tips, having two micro-nozzles each of 50µm diameter, attached to the rotating delivery apparatus. As a result, this novel design produces rotating micro-jets of solution (Fig. 1).

Installed on the ergonomic hand piece, the tip comes in direct contact with the skin surface. Then, a negative pressure is created linking the skin surface with the rotating nozzle openings. At this moment, solution begins to flow through the nozzles under moderate pressure. While the operator moves the hand piece slowly over the treatment area, each time a contact occurs between an open pore orifice and a micro-nozzle emitting a micro-jet of solution, follicular space is filled with the solution. (Fig.2). Negative pressure (vacuum) helps to support skin and tip contact during treatment. It also recovers wasted solution, keeping the treatment area dry.

The infused solution is now "locked" in the follicular duct, walled by only two layers of epithelial cells in the depth of the dermis, with a larger potential absorption area and longer contact time. This magnifies the biological activity of the active ingredients in the solution.

The procedure is easily carried out, with the only skill needed by the operator being the ability to keep gentle contact between the treatment tip and the skin surface. Hard pressure will deform the surrounding skin and duct structure and may completely close it.

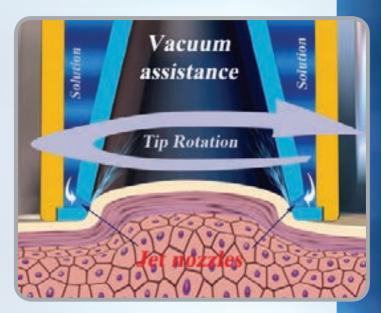


Fig. 1: Rotating micro-jets of solution are delivered through two micro-nozzles, each of 50µm diameter

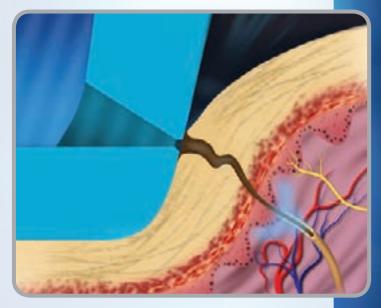


Fig. 2: Each time a contact occurs between the pore orifice and the jet nozzle, follicular space is filled with solution

Active ingredients, including bleaching substances, antioxidants, vitamins and any other compounds targeting deeper layers of the skin, but so far blocked by the natural biological defense systems, can be delivered more effectively by the Ionoderme Infusion System-Affinity. Not only is penetration of these ingredients more effective through the follicular walls, but the longer contact time between the solution in the follicular reservoir and the skin cells, increases the partition coefficient of absorption (similar to the patch technique).

Other groups of therapeutic substances that can be delivered by Ionoderme Infusion System-Affinity include peeling agents such as alpha or beta hydroxy acids, trichloroacetic acid, and more. Because of the more efficient absorption, lower concentrations of the peeling agents produce more significant effects. In the case of peeling substances, while lower parts of the follicular duct are "attacked", the upper parts remain relatively spared. In addition, the focal mode of penetration, centered exclusively on the follicles, creates fractional damage to the skin with faster regeneration.

To accommodate various treatment targets and treatment zones, Ionoderme Infusion System-Affinity's working parameters can be changed to regulate the amount of infused solution and the depth of its delivery.

# TREATMENT PROTOCOL

Treatment protocol includes a series of 4-6 weekly or bi-weekly treatments. In the maintenance phase, treatments can be spaced to accommodate the patient's wishes. While during the treatment light discomfort can be sensed (due to motion of the hand piece under negative pressure), the post-procedural course is eventless.



Fig.3: 46-year-old patient with acne scars after 2 sessions of lonoderme Infusion System-Affinity treatment

The results are usually already visible just a few days after the first procedure. Because the intrafollicular effect is three-dimensional, the changes to the quality of the skin take place throughout its thickness, producing the aesthetic result (Fig.3-4).

Due to the high safety of the system, Ionoderme Infusion System-Affinity provides an unmatched opportunity to treat non-facial skin, such as the neck, décolleté and all other folliclebearing areas.



Fig. 4: 42-year-old patient with melasma after one treatment session with the Ionoderme Infusion System-Affinity.

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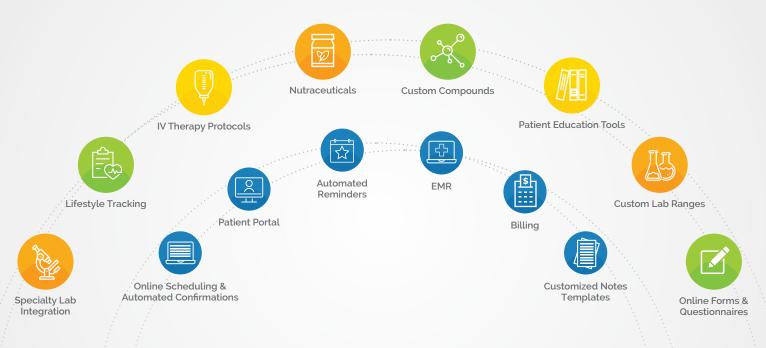
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ne of the most common conditions that family physicians and other primary care providers face in clinical practice is degenerative joint disease (DJD), more commonly known as osteoarthritis (OA).¹ DJD accounts for one of the greatest causes of disability, loss of productivity, suffering, health decline and social isolation in our society.² The general approach to diagnosing and treating DJD has, surprisingly, not changed a great deal over the past several decades. Apart from total joint replacement, treatment options are palliative at best, with symptom control playing a prominent role.³ Yet, simple approaches utilizing diet, lifestyle and nutrition have been developed that make a profound difference in calming down inflammation not only in joints, but throughout the whole body.⁴

"Osteoarthritis is a disorder involving movable joints characterized by cell stress and extracellular matrix degradation initiated by micro and macro injury that activates maladaptive repair responses including pro-inflammatory pathways of innate immunity. The disease manifests first as a molecular derangement (abnormal joint tissue metabolism) followed by anatomic, and/or physiologic derangements (characterized by cartilage degradation, bone remodeling, osteophyte formation, joint inflammation and loss of normal joint function), that can culminate in illness."<sup>5</sup>

The prevalence of OA of the knee, hip or hand is estimated to be 20-30% of adults,<sup>6</sup> with an estimated lifetime risk of developing knee OA of 40% in men and 47% in women.<sup>7</sup> Risk factors for OA include person-specific factors, such as age, sex, obesity, genetics and race/ethnicity; as well

as joint-specific factors related to abnormal loading of the joints, such as history of injury, level of activity, occupation, leg-length inequality, strength, and joint alignment/flexibility. The main joints affected by OA include the knees, hips, interphalangeal joints, thumb base, first metatarsal-phalangeal joints and spinal facet joints. People who suffer with OA typically develop initial symptoms in middle age, with acceleration of symptoms after 50 years of age. Common symptoms include usage-related joint pain, morning or inactivity-related stiffness and movement restriction; with rest/night pain occurring with severe OA (Oarsi Primer Disease Diagnosis). Common signs of OA include crepitus, joint enlargement, reduced range of motion and joint line tenderness; with muscle weakness, atrophy and joint deformity in severe OA. Joint effusions may or may not be present. 9

The diagnosis of OA remains clinical and may be made without radiographic or laboratory investigations. OA is also a diagnosis of exclusion and the physician should rule out other forms of arthritis such as rheumatoid arthritis, psoriatic arthritis, septic arthritis, crystal arthropathies (which include gout), and so on. The diagnosis of the severity of OA is based on quality-of-life questionnaires, physical examination and radiography. A commonly used quality-of-life questionnaire is the Western Ontario McMaster Index (WOMAC), with higher scores indicating greater severity of OA. In general, there is poor correlation between symptoms, disability and structural changes. Two large epidemiological studies – the Framingham Osteoarthritis Study and the Osteoarthritis Initiative – demonstrated that

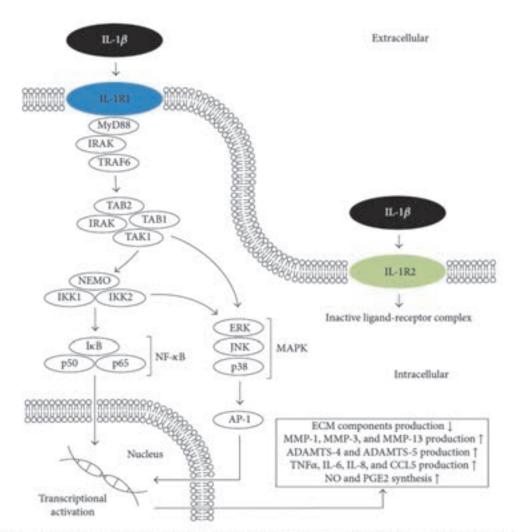


FIGURE 2: IL-1 $\beta$  associated intracellular signaling pathways and downstream cellular targets and effects. IL-1R1: interleukin-1 receptor, type 1; IL-1R2: interleukin-1 receptor, type 2; MyD88: myeloid differentiation primary response gene (88); IRAK: interleukin-1 receptor-associated kinase; TRAF6: TNF receptor-associated factor 6; TAK1: also known as mitogen-activated protein kinase kinase kinase 7 (MAP3K7); TAB1: also known as mitogen-activated protein kinase kinase kinase 7 interacting protein 1 (MAP3K7IP1); TAB2: also known as mitogen-activated protein kinase kinase 7 interacting protein 2 (MAP3K7IP2); p50, p65: subunits of proteins forming NF- $\kappa$ B; I $\kappa$ B: (inhibitor of  $\kappa$ B) an endogenous complex of proteins inhibiting the activation of NF- $\kappa$ B; IKK1,2/NEMO: NF- $\kappa$ B inhibitor kinase 1,2 (I $\kappa$ B kinase 1,2)/NF- $\kappa$ B kinase inhibitor (NF- $\kappa$ B essential modulator); ERK: extracellular-signal-regulated kinase; JNK: c-Jun N-terminal kinase; p38: p38 mitogen-activated protein kinases; MAPK: mitogen-activated protein kinases; AP-1: activator protein 1.

hip pain was not present most of the time when there was radiographic evidence of hip OA. Furthermore, many of the participants with painful hips did not have radiographic evidence of hip OA.<sup>15</sup>

There is ongoing research attempting to find biomarkers useful in the diagnosis of osteoarthritis. These biomarkers fall into 2 classes: biomarkers of joint tissue turnover and biomarkers of inflammatory status, which include cytokines, chemokines and cell type markers important in the pathology of OA. Unfortunately, there has been little clinical validation of these biomarkers and there remains a large, unmet medical need to identify, test, validate and qualify biomarkers for clinical use. 17

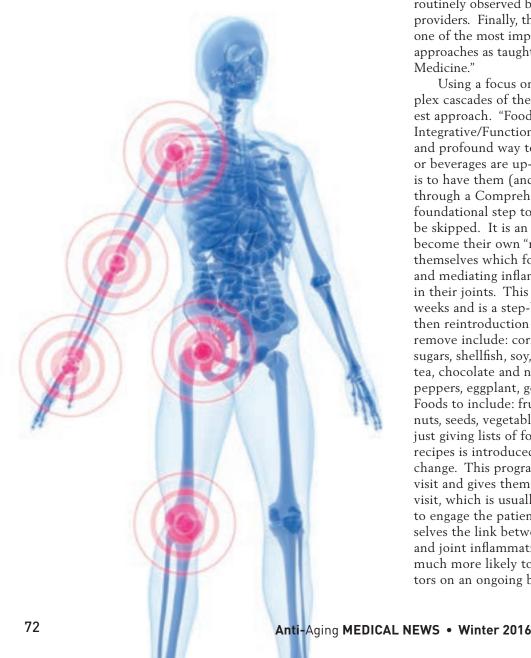
Osteoarthritis results when there is an imbalance between the mechanical forces within a joint and the ability of the articular cartilage to withstand those forces. All the tissues in a joint – cartilage, bone, synovium, ligaments and

adipose tissue – are involved in the osteoarthritic process. Once the tolerance of the articular cartilage to mechanical forces is exceeded, inflammatory mediators are released from chondrocytes and the synovium, which may result in the progressive loss of cartilage. Synovial macrophage activation results in the upregulation of NFkB, with many cytokines being abundantly expressed and released. The feed-forward, inflammatory cascade of cellular responses to injury results in inflammatory cell infiltration, which leads to erosion and fibrillation of the articular cartilage, fibrosis, subchondral bone sclerosis, synovial hyperplasia and osteophyte formation. The problem arises when the catabolic inflammatory processes surpass the matrix anabolic/synthetic activities, leading to the progressive destruction of the articular cartilage in osteoarthritic joints. The problem arises when the catabolic activities, leading to the progressive destruction of the articular cartilage in osteoarthritic joints.

Interleukin-1 (IL-1) was the first cytokine discovered in the 1980s.<sup>21</sup> It has long been considered the most potent

catabolic cytokine. It coordinates systemic host defense responses to pathogens or various injuries. In the joint, it is released by synovial macrophages,  $^{22}$  as well as by chondrocytes.  $^{23}$  It results in the down-regulation of chondrocyte type II and proteoglycan synthesis. It also stimulates and enhances the release of chondrocyte-mediated cartilage destructive enzymes including matrix metalloproteinases and A Disintegrin And Metalloproteinase with Thrombospondin Motifs (ADAMTS), which is a family of peptidases. IL-1 has two known isoforms – IL-1 $\alpha$  and IL-1 $\beta$  – and both bind to the IL-1R1 (IL-Receptor type 1). IL-1 $\alpha$  is released intracellularly upon cell death and IL-1 $\beta$  is considered to act as an extracellular cytokine (Figure 2).  $^{21}$ 

Cells that are activated by IL-1 produce and release small amounts of the IL-1 inhibitor IL-1Ra (receptor antagonist), which has a down-regulating effect on the IL-1 inflammatory cascade.<sup>24</sup> It is understood that high levels of IL-1Ra are needed to effectively balance the impact of IL-1.<sup>25</sup> There is growing evidence that the introduction of IL-1Ra into joints results in significant down-regulation of the inflammatory cascade.<sup>26</sup>



Since OA of the knee is the most common presentation of osteoarthritis in clinical practice, the management will focus on this joint. Consensus-based treatment guidelines were developed by the Osteoarthritis Research Society International (OARSI) and provide the current, evidence-based approach for family physicians and primary care providers in the management of knee OA.<sup>27</sup> Looking carefully at these guidelines, it should be noted that there is no listing of intra-articular injections of hyaluronic acid. This treatment approach remains controversial, with proponents on either side. A recent systematic review concluded that "meta-analysis of only the double-blinded, sham-controlled trials with at least 60 patients did not show clinically important differences of hyaluronic acid treatment over placebo."<sup>28</sup>

Furthermore, based on the current understanding of the pathophysiology of osteoarthritis, none of the recommended treatments effectively address the upregulated inflammatory cascades and are considered palliative. The problem with oral anti-inflammatory-type agents is the significant and systemic adverse effects that occur and are routinely observed by family physicians and primary care providers. Finally, these guidelines make no mention of one of the most important and foundational treatment approaches as taught by Hippocrates: "Let Food Be Thy Medicine."

Using a focus on food to modulate the highly complex cascades of the inflammatory orchestra is the wisest approach. "Food first" is a foundational tenet of an Integrative/Functional approach to medicine. A simple and profound way to help patients discover which foods or beverages are up-regulating the inflammatory cascades, is to have them (and preferably those they live with) go through a Comprehensive Elimination Diet.<sup>29</sup> This is a foundational step towards health recovery that should not be skipped. It is an important tool that allows patients to become their own "medical detectives" and to discover for themselves which foods and/or beverages are triggering and mediating inflammation in general and, specifically, in their joints. This approach takes approximately 4-6 weeks and is a step-by-step, methodical elimination and then reintroduction of various food families. Foods to remove include: corn, dairy, eggs, gluten, simple processed sugars, shellfish, soy, beef, pork, processed meats, coffee, tea, chocolate and nightshades (tomato, white potato, bell peppers, eggplant, gogi berries, ashwaganda and tobacco). Foods to include: fruits, healthy oils, lean meats, legumes, nuts, seeds, vegetables and non-gluten grains. Instead of just giving lists of foods to patients, a new set of tasty recipes is introduced, making it easier to implement change. This program is given to the patient on the first visit and gives them "homework" to do prior to the second visit, which is usually 6 weeks later. This program helps to engage the patient and, when they discover for themselves the link between consumption of foods/beverages and joint inflammation, they become empowered and are much more likely to eliminate dietary triggers and mediators on an ongoing basis.

Besides eliminating dietary triggers and mediators of inflammation, it is also important to introduce foods, herbs and spices that have inflammation-modulating properties. Extra virgin olive oil has been shown to significantly reduce joint edema as well as cartilage destruction by down-regulation of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$  and IL-17.<sup>30</sup> Piperine from black pepper has a down-regulating effect on IL-1 $\beta$  in human osteoarthritis chondrocytes.<sup>31</sup> Piperine also increases the bioavailability of curcumin.<sup>32</sup> Cordyceps, a genus of ascomycete fungi, has a similar effect on down-regulating IL-1 $\beta$ .<sup>33</sup>

The spice with the most profound impact on inflammatory modulation is turmeric, which has been used in Ayurvedic medicine for over 4,000 years. It is the rhizome of the plant Curcuma longa and contains over 20 different active compounds, with the most prevalent being the curcuminoids.34 Curcumin modifies over 80 molecular targets involved in the inflammatory orchestra.<sup>35</sup> A recent randomized controlled trial<sup>36</sup> studied the effects of taking 1 g per day of an oral curcumin formulation (curcumin plus phosphatidylcholine for better absorption) over 8 months. The 50 patients in the treatment group had a >50% decrease in WOMAC score and a threefold increase in treadmill walking distance as compared to the control group. Inflammatory biomarkers such as serum IL-1B, IL-6, soluble CD-40 ligand, soluble VCAM-1 and ESR were significantly decreased in the treatment group.

Avocado and soybean oils are often used in the manufacturing of soap. The unsaponifiable fraction of these oils is called avocado/soybean unsaponifiable or ASU.

ASU contains a number of inflammation-modulating phytochemicals such as phytosterols. Human clinical trials have been published showing improvement of OA in the test subjects as compared to the controls.<sup>37</sup>

Boswellia, also known as Frankincense, is a group of resins from the Boswellia Serrata tree. One of these resins, acetyl-keto-beta-boswellic acid, inhibits the lipooxygenase pathway and thus decreases inflammation. Nine clinical trials have been published demonstrating some benefit for OA.<sup>38</sup> Boswellia is often combined with other plant extracts such as curcumin for synergistic effect in OA.

A common antecedent, trigger and mediator for OA is ongoing obesity, which is an important modifiable factor when it comes to addressing OA.<sup>39,40</sup> Other mediators of inflammation could include overuse or misuse of joints, ongoing biotoxin exposure from overgrowths of certain organisms in the microbiome, ongoing exposure to other toxic influences such as heavy metals, chemicals and so on. When formulating a treatment approach for OA, it is important to think in terms of an anti-inflammatory lifestyle.

#### **Leading-Edge Approaches for OA**

There is great concern that, in the not-too-distant future, the need and demand by the aging, general population will outstrip the availability of total joint replacements. As well, better approaches than palliation need to be developed that are safe and effective. To this end, ways to modulate the inflammatory cascades have been

in development over the past few decades. The focus in the past has been to block the inflammatory pathways in order to decrease the catabolism within joints. Currently, the focus also includes looking at ways to cause tissue to regenerate, increasing the anabolic events within joints. The following are a few of the leading edge approaches that are now being utilized.

### IL-1Ra (Interleukin-1 Receptor Antagonist protein)

IL-1Ra was first discovered in 1986 and, as previously mentioned, cells such as the chondrocytes, which produce and release IL-1B, also release small amounts of IL-1Ra. Other cells such as mononuclear cells and macrophages in the blood also produce and release cytokines as well as IL-1Ra.<sup>41</sup> Strategies to inhibit the biological activities of IL-1β have been developed. A recombinant IL-1Ra, known as Anakinra, has demonstrated the effective blockade of the IL-1 inflammatory cascade. A double-blind, placebo-controlled study (n = 170) concluded that it was safe to use in humans, but was not associated with improvements in OA symptoms as compared to placebo.<sup>42</sup> In 1994 it was discovered that if peripheral whole blood is drawn into a syringe containing glass beads coated with CrSO4 to initiate monocyte activation, incubated at 37.0°C for 24 hours and then centrifuged, the resulting serum is selectively enriched with the anti-inflammatory cytokines IL-1Ra, IL-4 and IL-10.43 Subsequent research has shown that not only is the autologous conditioned serum (ACS) rich in these anti-inflammatory cytokines, but also contains over 35 other factors including fibroblast growth factor b (FGFb), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), IGF-1, platelet-derived growth factor (PDGF) and transforming growth factor  $\beta$  (TGF  $\beta$ ) – as well as a number of the pro-inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$ . <sup>44</sup> The net effect is anti-inflammatory when the ACS is injected into osteoarthritic joints. This has been demonstrated in animal and human clinical trials with levels 1 and 2 scientific evidence - knees, 45 hips, 46 TMJ. 47 A recent prospective, observational study demonstrated the therapeutic power of collaboration using ACS and physiotherapy.<sup>48</sup>

### Mesenchymal Stem Cells (MSCs)

A promising approach to cartilage regeneration in joints is the use of MSCs. MSCs occur in numerous tissues including bone marrow and adipose tissue.<sup>49</sup> MSCs have the ability to differentiate into bone, cartilage, muscle, as well as adipose tissue.<sup>50</sup> There is a growing scientific literature demonstrating that MSCs can successfully regenerate cartilage in animals and humans.<sup>51</sup> Adipose tissue-derived stem cells (ADSCs) in the form of stromal vascular fraction (SVF) contain stem cells that can differentiate into cartilage, bone, muscle and adipose tissue, similar to MSCs.<sup>52</sup> Recent studies have demonstrated that ADSCs can regenerate cartilage in the osteoarthritic knees of human patients.<sup>53</sup> Even more promising is the use of MSCs from bone marrow aspirate concentrate (BMAC), as it is an approved procedure by the FDA. BMAC, besides being

a source of MSCs, also contains various growth factors including PDGF, TGF-b, as well as bone morphogenetic protein (BMP)-2 and BMP-7, which are known to have both anabolic and anti-inflammatory effects. A recently published review of the BMAC literature concluded that overall, the outcomes reported with the use of BMAC for the treatment of early knee osteoarthritis are good to excellent. However, this field is in its infancy and the level of scientific evidence in the studies published varied from grades 2-4. There is a need for large, randomized controlled trials to evaluate the efficacy of BMAC for the treatment of knee pathologies.

### **Case Study**

A 64-year-old, married woman presented with a many-year history of progressive osteoarthritis affecting both knees (right > left), the lower back, neck, and small joints of her hands. She also had one kidney. Over the years, the pain and stiffness in her joints led to an inability to cook and do housework, loss of sleep, fatigue, anxiety and inability to golf, garden and line dance – which she was passionate about. She was careful about her diet and had previously gone through the comprehensive elimination diet. She also avoided gluten and nightshade foods; and since she had one kidney, she was careful to avoid medications such as NSAIDS and other analgesics. She had tried a number of oral herbal remedies with little improvement. On examination, she appeared tired, moved carefully and walked with a right-sided limp, was unable to flex her fingers more than 50%, and demonstrated decreased range of motion in her cervical and lumbar spine regions. Her right knee had a mild-moderate effusion and was warm to the touch, with tenderness over the joint lines and lacking full flexion. All standard laboratory work was within normal limits including CRP. X-rays showed moderate changes of osteoarthritis in the medial compartment of the right knee, as well as many of the PIP and DIP joints in both hands. Her initial WOMAC score was 44/96 or 45%. She met with the Family Nurse Practitioner in my clinic for three 1-hour educational sessions, reviewing the functional medicine anti-inflammatory lifestyle. She was assessed by an excellent physiotherapist for structural and functional problems that she had developed over the years as a result of the OA. She was then coached and supervised on specific flexibility and strengthening exercises by the physiotherapist. She was placed on oral curcumin twice daily with food and began twice-weekly intravenous curcumin treatments over 3 weeks. A total of five Autologous Conditioned Serum injections to the right knee were given over 3 weeks and tolerated well. An important component of the treatment program was ONDAMED® (German electromagnetic, focused electromagnetic field device) over 3 weeks. Following her third ACS injection, she reported the ability to line dance for 2 hours with no triggering of inflammation in her knees. The following 2 days she played 18 holes of golf each day, again without triggering inflammation. She had regained full function of her hands and had no further back and neck pain. Over

the subsequent weeks she noted that she was able to sleep deeply, had increased energy, was able to do housework, cooking and gardening. Importantly, her chronic anxiety also resolved. At the end of the 3-week program, her WO-MAC score had dropped to 11/96 or 11%; and one month later, the WOMAC score had dropped to 6/96 or 6%. At the 3-month follow-up she was clinically doing very well and the WOMAC score had dropped to 0/96, and this was maintained at the eighteen-month follow-up. This case demonstrates the importance of a multimodal approach combining the functional medicine anti-inflammatory lifestyle, Autologous Conditioned Serum joint injections, judicious use of curcumin, ONDAMED® and supervised exercise.

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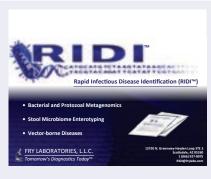
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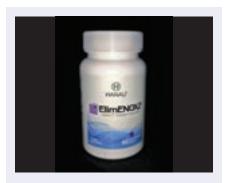


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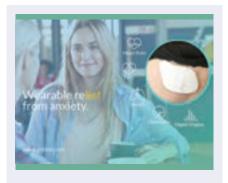
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# THE POTENTIAL BENEFITS OF "4-SPOT URINE TESTING" FOR HPA AXIS ASSESSMENT



By CARRIE JONES, ND and MARK NEWMAN, MS

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he health of the entire hypothalamic-pituitary-adrenal (HPA) axis is critically important for maintaining proper health primarily because of cortisol's role in the body. Cortisol is known as a glucocorticosteroid because of its effects on blood sugar regulation (gluco), it is made in the cortex of the adrenal glands (cortico) and of course, it acts as a potent steroid hormone with effects that are far reaching systemically.

Released primarily in response to perceived low blood sugar in order to directly stimulate glucuoneogenesis and indirectly, glycogenolysis, cortisol helps to counter inflammation through certain cytokines in the immune system and increases to prepare the body for "fight or flight" as part of the normal, healthy stress response. Like all things, balance is key, as high levels can result in inflammatory tissue damage, bone loss, blood sugar dysregulation, memory loss via the hippocampus and insomnia. Low levels may worsen depression and fatigue, result in more inflammation throughout the body, reduce gastric-acid secretion in the stomach and affect proper blood sugar regulation.

While there are well-known adrenal gland conditions such as Addison's and Cushing's disease, other conditions and symptoms seem to fall on a broadly categorized HPA axis dysfunction spectrum. This is where proper testing comes into play, as someone may have low cortisol production, causing symptoms such as fatigue and a weakened immune system; however their results are not low enough to diagnose Addison's disease or warrant cortisol replacement. Alternatively, someone may have high cortisol production causing symptoms like weight gain or insomnia, yet does not fall into the Cushing's disease category.

How is HPA axis dysfunction best tested in order to properly identify problems and treatment when there are many parts to evaluating cortisol throughout the day, such as total production, cortisol clearance, free cortisol values, and the diurnal pattern?

By the middle of the end of the 1950's there were reasonable solutions to both Addison's and Cushing's diseases. About the same time, although in relative independence, Hans Sale and others were dramatically increasing our understanding of the stress response. Until the 1980's lab testing for cortisol was limited primarily to serum cortisol and 24-hour urine testing of free cortisol. In 1981 salivary cortisol testing was first reported. In 2006 Jerjes showed similar patterns of free cortisol in saliva and urine using "spot" urine collections serially throughout the day. They also looked at the metabolites of cortisol. Their research showed that while free cortisol is lower in patients with chronic fatigue syndrome, levels of cortisol metabolites are not. They showed equivalent results when looking at cortisol's end metabolites in one study. In a separate study, they showed slightly increased metabolites of cortisol in patients with CFS. If free cortisol results are lower and cortisol metabolites are equivalent or higher in patients with CFS, cortisol metabolism/clearance may be up-regulated. Increased clearance of cortisol may ultimately lead to hypocortisol conditions even if the patient's cortisol production is "normal."

### The Importance of Cortisol Clearance - Using Anorexia as a Case Study

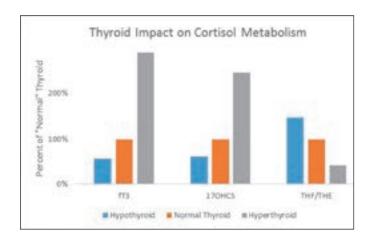
Elevations in hormone levels are often assumed to be due to increased hormonal production. In some scenarios, however, elevations may be due to decreased metabolism or clearance of a hormone. Such is the case with elevated free cortisol (and DHEA) in anorexia nervosa. In 2012 Oskis, et al., published that patients with anorexia nervosa may have "hypersecretion of both cortisol and DHEA." They concluded this after finding higher levels of both hormones in the saliva of patients with anorexia. However, in 2011 levels of cortisol and DHEA *metabolites* were published, showing dramatically *lower* levels in patients with anorexia. High levels of free hormone and reduced levels of metabolites imply that the elevation in free hormones may be caused by slow metabolism/clearance of these hormones.

More than 30 years before the two studies referenced above, Boyar showed that cortisol's metabolic clearance rate was indeed twice as slow in anorexic patients. This study also showed that levels of T3 were more than 2.5 times lower in the anorexia patients (more on the role of hypothyroidism later).

As we characterize anorexia as a condition with respect to cortisol, higher levels of free cortisol (which may lead patients to be more at risk for depression and other conditions seen in hypercortisolism) and lower levels of cortisol metabolites are both relevant observations. Knowing both the free and metabolized hormone levels gives insight into situations where clearance/metabolism rates may be abnormal.

Common conditions where cortisol clearance rates may be abnormal:

Hypo/Hyperthyroidism – In a 1993 publication, Taniyama published data correlating thyroid status with the excretion rates of cortisol metabolites (also confirmed by Hoshiro, 2006). They measured 17-hydroxycorticosteroid (total of cortisol metabolites, 17-OHCS) levels in urine, along with the ratio of cortisol metabolites (THF) to cortisone metabolites (THE). As you can see in the graph below, excretion of cortisol metabolites (17OHCS) generally parallels thyroid status. Patients with higher thyroid levels also show a preference for cortisone metabolites, meaning cortisol tends to spend more time in its inactive form. Conversely, a hypothyroid patient tends to hold on to their cortisol. It is not as readily deactivated to cortisone, nor is it cleared as rapidly as 17OHCS cortisol end products.



data from Taniyama, 2003

Obesity - Weight gain has been associated with higher cortisol since the proper characterization of Cushing's syndrome in the 1930's by Harvey Cushing. Patients with Cushing's disease typically do not show the usual diurnal pattern of cortisol (particularly free cortisol). They exhibit elevated levels throughout the day and are not suppressed by Dexamethazone. These

# What is the advantage of gaining insight into the clearance rates of cortisol?

Elevated free hormone levels may be driven by increased production or decreased clearance, but the best intervention may be different depending on which cause is the primary driver behind the abnormality.

patients also tend to gain weight, especially abdominally. In spite of this correlation, studies have not shown a positive relationship between BMI and free cortisol in either urine or saliva. Cortisol metabolites, however, have been shown to be very strongly correlated to BMI. The above data implies that cortisol production (best approximated by the total of cortisol and cortisone metabolites) is increased in obesity and that cortisol clearance may be up-regulated as well. Adipose tissue also contains higher levels of 11bHSD1, the enzyme responsible for increasing the conversion of the inactive cortisone into cortisol. In many individuals, the activation of this enzyme results in excess cortisol locally, resulting in more adipose gain. Measuring the systemic preference for cortisol metabolites versus cortisone metabolites helps to understand the enzymatic activity individually. This further supports the concept that elevated cortisol is a risk factor for abdominal weight gain. However, it is important to look at cortisol production and clearance, not just free cortisol alone.

### **Lab Tests Available for Cortisol**

There are several options traditionally used for cortisol testing, all with their pros and cons (see box). Serum cortisol requires a blood draw, making it difficult to "collect" throughout the day and assess the patient's cortisol pattern. Due to the nature of the collection, serum results are a mix of bound and unbound cortisol in the bloodstream, making it extremely difficult to distinguish between the two in one lump sum number. Salivary testing is done by spitting into small tubes at set points throughout the day. While this method does evaluate the daily pattern, it does not include the metabolites of cortisol. Neither serum nor salivary testing has the ability to give insight into the clearance of cortisol. 24-hour urine testing involves the collection of urine in a large container for an entire day. While not entirely pleasant and often inconvenient, this method does report both free and metabolized cortisol; however, it is unable to graph free cortisol through the day.

It is worth noting that some urine labs test "total" cortisol and not "free" cortisol. There is little support in the literature for the measurement of "total" cortisol as measured in urine. The newest model of testing involves a 4-spot dried

	Adrenal Hormones				Reproductive Hormones	
	Free Cortisol	Diurnal Cortisol Pattern	Cortisol Metabolites	DHEA/ DHEAS	Reproductive Hormones	Reproductive Hormones Metabolites
4-Spot Urine						
Saliva	4				-	
24-Hr Urine						
Serum						

urine testing for comprehensive hormones whereby patients urinate on small strips of filter paper at set points through the day. The samples dry and are sent back to the lab. This method is able to evaluate the daily free cortisol throughout the day, plus report free and metabolized cortisol with a simple collection process that is easily transportable.

Health care as a whole is becoming more integrative, functional and personalized as practitioners are encountering more patients who seem to have increasingly challenging health conditions. Complex cases involving autoimmunity, resistant weight loss, genetic mutations, environmental exposures and nutrient deficiencies have become the norm, requiring advanced education, skills, and testing. At the heart of it all sits the hypothalamus-pituitary-adrenal axis trying to manage blood sugar, orchestrate the immune system, and handle the onslaught of stress on a daily basis.

Without a proper understanding of the big picture, practitioners may be treating patients for a free cortisol level that only tells half the story. HPA axis dysfunction involves more than just the adrenal glands, as the brain, liver and kidneys make-up the rest of the axis from start to finish. Evaluating cortisol production and clearance in addition to the free cortisol levels provides an additional level of information to guide the health care provider in their personalized treatment approach.

In order to get this information, providers can either order saliva in combination with 24-urine testing or use a new model of testing – spot urine testing. This new model uses dried urine collections at four specific points in the day. Patients can avoid spitting into a tube or carrying around a 24-hour jug and the dried urine strips are easily mailed. With this method, cortisol testing becomes uniquely comprehensive including both the diurnal cortisol pattern graphed throughout the day, plus the metabolites to better approximate overall output of adrenal cortisol production. As an added bonus, the reproductive hormones and their metabolites can be tested as well, lending even more insight for the provider and patient.

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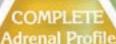
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# The Role of the Endothelial Glycocalyx in the Pathogenesis of Atherosclerosis:

# A NEW FRONTIER IN CARDIOVASCULAR HEALTH

By DERRICK DESILVA JR, MD; JEFFREY GLADDEN, MD, FACC; CHEN CHEN, PhD; JON WARD, MA

The following article is not endorsed and/or supported by The American Academy of Anti-Aging Medicine.

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uch recent work on the pathogenesis of atherosclerosis has focused on the "response to injury theory." In brief, the theory holds that atherosclerosis may be understood as an inflammatory response to insults occurring to the endothelium.¹

When the endothelium is healthy, atherosclerosis does not occur. When the endothelium is damaged, it produces surface-adhesion molecules, causing monocytes and t-lymphocytes to stick to its surface, which then penetrate the endothelium into intima. As low-density lipoprotein (LDL) particles follow the path, they enter the intima and begin to oxidize. This sets the stage for foam cell formation and plaque development. The resulting plaque then builds up and, when internally inflamed or externally eroded, can be a threat to rupture its contents into the arterial flow, potentially triggering a blood clot that if large enough or not lysed quickly enough, can occlude the artery to various degrees, causing anything from mild to devastating downstream ischemia. Indeed, it is the clotting in response to plaque disruption, not the plaque accumulation in the arteries, per se, that poses the real threat. 75% of heart attacks occur at arteries that are less than 50% blocked, while mild plaquing escapes traditional stress tests 80% of the time.

Needless to say, this means that merely measuring the serum levels of LDL and HDL is inadequate to assess event risk. To interrupt the cycle, clinicians need to be primarily concerned with the health of the endothelium.

The injury-response theory is gaining widespread acceptance, but it begs an important question: What causes the

injury to the endothelium in the first place? Multiple candidates have been cited such as:

- Direct trauma causing physical injury
- Turbulence in the blood flow, for example at artery bifurcations
- Excessive blood glucose levels
- Circulation of free radicals
- TMAO (trimethylamine-N-oxide)
- Higher than normal concentrations of LDL or VLDL
- High blood pressure
- Circulating toxins
- Deterioration of the NO system

All of these explanations have value, but they miss a critical factor in endothelial health that merits more attention than it has received in the current literature. That factor is the endothelial glycocalyx.

### The Endothelial Glycocalyx

Popular accounts of the endothelium inaccurately describe it as "the inner lining" of the blood vessels. Here's Wikipedia:

"Endothelium is a type of epithelium that lines the interior surface of blood vessels and lymphatic vessels, forming an interface between circulating blood or lymph in the lumen and the rest of the vessel wall."

What's missing from these accounts is the glycocalyx, a slippery-smooth gel coating of the endothelium that positions



an additional layer between the endothelium and the circulating blood. This is the true interface. In other words, it's the endothelial glycocalyx — not the endothelial cells themselves — that has (or should have) direct contact with the circulating fluids and particles.

When we help doctors explain the endothelial glycocalyx to patients, we sometimes liken it to the non-stick surface of a frying pan. The analogy is useful because it highlights the protective function of this important structure. A healthy glycocalyx ensures that LDL particles "slip by" without contacting the endothelium. Conversely, when the endothelial glycocalyx is compromised (which happens very easily), the endothelium becomes susceptible to injury impairment and LDL penetration.

Important clinical implications follow. To prevent atherosclerosis, we must protect the endothelium from injury and preserve its vital functions. To protect endothelial function, we must support its *existing natural protection*, the endothelial glycocalyx. In layman's terms: If you want to stop food sticking to your saucepan, take care of the non-stick coating! If you want the infrastructure of the endothelium to work, you need to protect its surface.

### A Closer Look at the Glycocalyx

The endothelial glycocalyx is a thin gel-like layer that coats the entire luminal side of the vascular endothelium. It is a meshwork mainly of glycoproteins, proteoglycans and glycosaminoglycans at a thickness of approximately 1  $\mu$ m magnitude. Syndecans and glypicans are the core proteins

of heparan sulfate (a glycosaminoglycan) proteoglycans bound to endothelial cells identified in the glycocalyx. Glycoproteins such as selectins and integrins are also anchored on endothelial cells, while some other soluble proteins and proteoglycans simply dock in glycocalyx.<sup>5</sup>

Extensive research has revealed the importance of glycocalyx-mediated endothelial function in vascular and microvascular health.

For example, the endothelial glycocalyx:

- Regulates vascular permeability and fluid balance due to the large size and negative charge of glycosaminoglycans.<sup>6-7</sup>
- Provides a physical barrier against inadvertent adhesion of platelets and leukocytes to the vascular wall.<sup>8</sup>
- Regulates coagulation as many of mediators of coagulation pathway are buried inside the glycocalyx under normal physiological conditions.<sup>5</sup>

Most intriguingly, the glycocalyx is found to be a mechano-sensor and -transducer of the shear-force inside blood vessels.<sup>3</sup> The signal is believed to be transduced to endothelial nitric oxide synthase (eNOS) via heparan sulfate in the glycocalyx to either up- or down-regulate the synthesis of nitric oxide (NO) in response to the blood flow.<sup>9-10</sup>

Figure 1 illustrates the chemical structure of the endothelial glycocalyx and its signal transduction to eNOS and subsequently sGC (soluble guanynyl cyclase) to induce smooth muscle relaxation via shear stress.

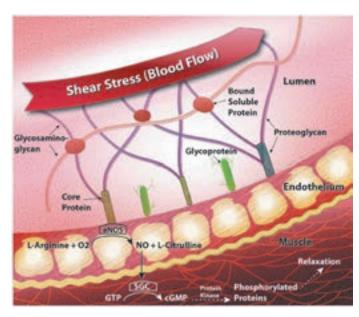


Figure 1. Structure of the endothelial glycocalyx and its activation of vascular muscle relaxation via NO in response to increased shear force.

### Damage to the Endothelial Glycocalyx

The endothelial glycocalyx is a delicate structure and can be damaged by several common mechanisms involved in the pathogenesis of atherosclerosis. These include high blood glucose, 11 oxidative stress, 12 and inflammation. 4 It is known that high-sugar diets, cigarette smoking, stress, and aging can all degrade the glycocalyx.

Hyperglycemia is a major cause for disruption of the endothelial glycocalyx. <sup>11,13</sup> In fact individuals with hyperglycemia and diabetes are known to have less endothelial glycocalyx. <sup>14</sup> Such a change may explain the endothelial dysfunction and increased microvascular permeability that lead to major complications in the diabetic population. <sup>15-16</sup>

There are several other disease conditions identified so far to be associated with a compromised endothelial glycocalyx:

- Coronary heart disease<sup>17</sup>
- Renal diseases<sup>18</sup>
- Lacunar stroke (a small vessel disease)<sup>19</sup>
- Severe trauma<sup>20</sup>

These electron-microscope images show the deterioration of the endothelial glycocalyx:









### **Clinical Interventions**

Given the vital role the endothelial glycocalyx plays in the pathology of many vascular and micro-vascular related diseases, it has naturally become a target for pharmaceutical intervention. <sup>21-22</sup> However, glycocalyx drug development is still in its infancy and no substantial progress has been made to date. <sup>23</sup>

A dietary supplement has been tested and shown to have measurable benefits for a compromised glycocalyx in healthy subjects. Brand-named Arterosil, its primary ingredient is rhamnan sulfate derived from rare marine algae. Rhamnan sulfate has a similar chemical structure to heparan sulfate found abundantly in the human endothelial glycocalyx, and may exert its bioactivity by regenerating the glycocalyx.

In an early clinical trial, the positive impact on the glycocalyx was established by measuring recovery of RHI (reactive hyperemia index) in 20 healthy human subjects following a high-sugar, high-fat meal. Results were compared with and without consumption of ArterosilHP. The study confirmed a significant improvement in glycocalyx RHI recovery with the supplement.

Important safety data were obtained for complete metabolic panel (CMP), thyroid stimulating hormone (TSH), complete blood count (CBC), and partial thromboplastin time (PTT) from the trial. No significant changes were observed for any of these tests after 4 weeks of ArterosilHP supplementation. There was also no serious adverse event reported during the study and the product was well tolerated by all subjects.

### The Glycocalyx and Arterial Elasticity: A New Frontier

The clinical significance of arterial elasticity is well established: Central arterial stiffness has been shown to be an independent predictor of cardiovascular morbidity and mortality. While the prognostic value of this measure is widely accepted, the causes of arterial stiffness are still subject to debate. Some research suggests that the issue is not limited to the larger arteries themselves, but may extend to the microvascular system. Other studies indicate the role of endothelial function in determining the degree of arterial elasticity.

One useful contribution to this debate may prove to be a new focus on the endothelial glycocalyx. Because the glycocalyx serves to protect the integrity of the endothelium, and hence of the arterial wall, it stands to reason that a healthy glycocalyx might be associated with good arterial elasticity.

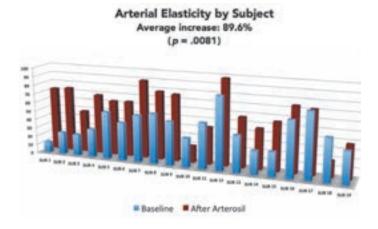
We conducted a pilot study to test patients for arterial elasticity — among other markers — before and after the consumption of ArterosilHP.

Nineteen healthy human subjects (11 females, age 22 to 64 and 8 males, age 30 to 60) were randomly recruited for the single-blinded clinical study, which was conducted at an independent cardiology center on the Baylor Medical Campus in Plano, Texas. Their vascular health condition was evaluated utilizing MaxPulse, an FDA approved Class II device (The Cardio Group, 6440 N. Central Expressway, Suite 100, Dallas, TX 75206). The MaxPulse utilizes accelerated plethysmography technology, with data being gathered by way of a finger probe. This technology, also known as pulse wave analysis, includes multiple factors including wave type, arterial elasticity, eccentric constriction and remaining blood volume valuations.

In this study, the baseline reading was taken at approximately 2 hours (+/- 30 minutes) post consumption of a breakfast of the subjects' choice. Immediately after the baseline reading, one capsule of ArterosilHP was swallowed. A post-dose reading was taken every 30 minutes for 3 hours, for a total of 7 readings (baseline, 30 min, 60 min, 90 min, 120 min, 150 min & 180 min +/- 5 minutes). The patients

were kept in a quiet, ambient environment. No food or liquid (other than small amounts of water as needed) was consumed during the 3 hour testing period.

The results are summarized in the table below:



In sum, 78.9% of subjects experienced an increase in arterial elasticity. The average percentage increase in arterial elasticity was 89.6% (p = .0081). The mean time to maximum increase was 118 minutes. There was concurrent improvement in remaining blood volume and eccentric contraction.

In this preliminary study, we were able to demonstrate that ArterosilHP improves arterial elasticity in healthy human subjects. It is likely this acute beneficial effect is a result of improved glycocalyx and its mediated endothelial functions. These new data are in agreement with our previous findings that ArterosilHP helps regenerate the endothelial glycocalyx and restore compromised endothelial functions. Clearly, there is a need for further studies to validate these early results.

We know that arterial stiffness indicates adverse changes of blood vessel structure and function, and poses a significant threat to patients' cardiovascular health.<sup>27</sup> If it transpires that rebuilding the glycocalyx has a rapid and positive impact on arterial elasticity, this could suggest a valuable clinical intervention, both for patients presenting disease conditions and for those seeking preventative care.

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ne of the greatest paradigm shifts in medicine over the past few decades has been the unfolding discoveries revealing the metabolic influence of the human microbiome, especially that which resides within the gastrointestinal tract. Indeed, it is difficult to find a medical discipline that is not actively investigating the potential role played by the gut microbiome in human health and disease. This explosion of knowledge has been welcome news for many healthcare providers, although keeping up with the published research, changing nomenclature and therapeutic ramification of this information has been a difficult task. Along with this new fascination for all things microbiome related comes the natural inclination to "assess" and "fix" this complex ecosystem as one might do with other systems of the body. Here is where we need to slow down and ask whether we know enough about what is "normal," what is "fixable," and what may just be an adaptation to a person's lifestyle inputs.

### **Dysbiosis: Imbalance Within the Microbiome**

Most integrative and functional medicine clinicians are familiar with the term dysbiosis. Essentially, it describes any significant imbalance in the gut microbial ecosystem, especially one that leads to a negative host response. This includes either overgrowth or depletion of a particular commensal species, family or phylum of bacteria, or a geographic dislocation of one or more species (i.e., colon bacteria colonizing the small intestines). While an infection by a pathogenic microbe such as *Salmonella* is not usually called "dysbiosis," the

opportunistic overgrowth of bacteria like *Clostridium difficile* or a yeast like *Candida albicans* is often directly related to an infection or an antibiotic-induced alteration in the gut microbiota (i.e., dysbiosis-induced). Ironically, with the advanced technologies now available to help the clinician analyze specific changes in the gut microbiota, this term has almost become too generic for the research setting, and clinicians should be aware that more specific terms might be used to define specific microbiome-host dysfunctions. Still, the notion that a disturbance in the gut microbial ecosystem — dysbiosis — may be a major trigger in a wide range of gastrointestinal and systemic disorders is an important factor often missed by clinicians uninformed by these recent discoveries.

However, as the technology used to analyze an individual's gut microbiota advances, we are finding more and more subtle differences in the amounts and ratios of species; some of which are associated with various chronic disease conditions. Properly understood, many of these subtle changes are microbial adaptations to the person's lifestyle and metabolic signals; although others might deem them "dysbiosis." Here are a few things to consider when trying to understand the stability, adaptability and the alterability of the human gut microbiome.

#### How Stable is the Adult Microbiome over Time?

Before discussing the many factors that can alter the gut microbiome, it is important to establish the relative stability of a person's microbiota. This is especially important for the clinician using fecal samples to analyze a person's microbiota, and whether this analysis represents only a transient snapshot or are semi-permanent features of the patient's core microbiome. Recently, several studies have attempted to answer this question by analyzing the microbiome (via fecal samples) of the same individuals over time, often tracking dietary or other changes along the way. In general, they have discovered that a fairly large core microbiome (~40 species of bacteria) remains stable in an individual over at least one year.¹ In fact, a landmark study published in *Science* (2013) using fecal samples from 37 individuals showed that a person's "core" gut microbiota is remarkably stable for greater than five years (they speculate for decades).² They also showed that the core species found in close family members are very similar, and that species within the Bacteroidetes and Actinobacteria phyla are more stable than those in other phyla (e.g., Firmicutes).

One of the most comprehensive analyses of the core microbiome of adults was published recently by Falony, et al., called the Flemish Gut Flora Project (FGFP). The study was originally designed to investigate the global core microbiome and to study the impact of host and environmental factors on microbiota variation within an average, healthy Western European population in Flanders, Belgium.<sup>3</sup> Of the 503 metadata variables that emerged, 69 factors were shown to correlate significantly with overall microbiome community variation. The association between BMI and microbiome composition was found to be small, but significant in effect size. Of all the covariates detected, 63% were driven by medication (mostly the use of antibiotics and laxatives), making the use of any medication the largest predefined explanatory variable tested in this study. However, when breaking down the covariates into smaller categories, stool consistency as measured by the Bristol Stool Scale (BSS) emerged as the top single non-redundant microbiome covariate in the FGFP metadata. BSS score reflects transit time, water availability, and potential niche differentiation within the colon ecosystem. For 12 of the 20 FGFP core genera, core abundance was shown to increase with looser stools. The authors note medication use and BSS scores have been largely ignored in microbiome studies, and should be considered in future studies based on the strong findings in the FGFP cohort.

The connection between stool consistency and microbiota abundance/diversity has been further analyzed, Vandeputte, et al. (2016) found stool consistency (as measured by BSS) was strongly associated with fecal microbial richness.<sup>4</sup> Species richness was shown to significantly decline as stool firmness declined (p=0.0007), reaching its minimum in those with diarrhea. The group also found enterotypes were distributed over BSS scores, the Prevotella enterotype was more abundant in looser stools (p=0.019) and the Ruminococcaceae-Bacteroides (RB) enterotype completely dominated firmer samples (p=0.019), perhaps suggesting that enterotypes may be a surrogate marker for bowel transit time since transit time may select for bacteria with certain traits. For instance, individuals with a short transit time may have greater amounts of fast-growing bacterial species, while those with slow transit times may instead select bacteria with greater adherence to host tissue.

What this and other data seem to reveal is that while a core microbiome (structure and species) is part of development, and is formed as part of an individual's early inoculation and environmental exposure, subtle and important changes in the variable commensal microbiome can occur without fundamentally altering the long-term stability of the core species (at least as determined by analysis of fecal microbiota). This means differences in fecal microbiota (beyond the core species enumeration and ratio) may reflect adaptations to environmental or pathophysiological changes that can be meaningfully followed in clinical research or in clinical application. Lastly, there is also a significant portion of the microbiota that is transient; most comes from our food (including probiotics), water, air, and environment. These microorganisms rarely persist beyond a week or two, but they can be important modulators of both the core and variable microbiome, and greatly influence host physiology.

Therefore, sampling and analyzing fecal microbiota as a routine part of a yearly physical exam may be a way to note subtle (or radical) changes in the microbiome consistent with noted changes in a patient's overall health. We predict microbiome testing will soon be a routine practice for annual exams, like standard blood tests, and will be part of the health assessment of most subjects.



**Three Microbiomes in One.** This figure illustrates the relative abundance of bacterial species within the core, variable and transient microbiomes in adults. This allows for a balance between metabolic stability and adaptability.

#### Is There an Ideal Microbiome?

This is a very tricky question. If the question implies a basic set of microbial species that is ideal for any human subject living anywhere, then the answer is "no." However, we believe there is likely a *suitable* microbiome for each person; one that is properly adapted to their particular geography, genetics, diet and environment. In fact, if we are correct in understanding the microbiome as the ultimate adaptable organ system, the microbiota within the GI tract (and therefore in the fecal sample) should change with age, with seasonal dietary changes and with health status. Comparing the microbiome of Western subjects consuming monotonous and poor diets to the microbiome of hunter-gatherers eating seasonally shows dramatic differences. While some would describe the microbiota of these hunter-gatherers as ideal, it probably would be poorly sustained and suited to benefit the Western subject eating a standard Western diet.

As we shall see, general changes in the fecal microbiota are indicative of certain gastrointestinal disorders (when compared to "healthy" control individuals). Some of these alterations reflect wholesale changes at the phylum-level, while

others are subtle changes in one or two species. Most of the time, however, the first analysis of a patient's microbiome is performed after a dysfunctional change has already occurred (often the first encounter with the patient) and therefore clinicians do not usually have access to a patient's former ("baseline") core microbiome for comparison. Nonetheless, analyzing gut microbiota (via fecal microbiota testing) is often helpful in both diagnosing a complex GI-related issue, as well as for confirming therapeutic impact (via follow-up testing).

100

### Genetic Dysbiosis: When Immune Cells Misinterpret the Microbiota

When an imbalance in the number and types of commensal microbes develops in the gut, the immune system is activated to respond in a variety of ways. New research now suggests that in some patients, the immune system can misinterpret a "normal" microbiome as "dysbiotic" due to genetic polymorphisms in a variety of immune receptors tasked with interpreting the gut microbial environment.<sup>5</sup> Pattern recognition receptors are an important component of both the innate and adaptive immune response, and are an important mechanism for helping the host immune system interface with the gut microbiome. However, similar to an overreaction to a benign food protein that causes an immune response in some individuals (based on genetics), certain commensal organisms may trigger an inappropriate response when the host immune system misinterprets their molecular pattern as "pathogenic" (e.g., inflammation, auto-immune cross-reactivity, etc.).

Likewise, inappropriate receptor expression caused by changes in genetics (gene/protein sequence) or genomics (altered expression pattern of one or more receptor) can result in dysfunctional immune regulation of the commensal organisms within the gut (e.g., reduced levels of sIgA to target pathobionts). This lowers the precision of the immune surveillance within the GI tract, allowing for an imbalance in the commensal organisms; creating a host-derived genetics-induced dysbiosis. Clinicians attempting to help correct a patient's gut dysbiosis must understand that host factors, including genetics, may profoundly affect how they respond to therapies intended to re-balance the gut microbiome (via diet, probiotics, prebiotics, etc.). Therefore, some recommendations or products may work better or quicker (or not at all) in some patients as compared to others.

#### **EDITOR'S NOTE:**

This article is a modified excerpt of Dr. Guilliams' new book *Gastrointestinal Health: A Lifestyle and Nutrient Approach*, designed to help clinicians navigate the principles and protocols of the functional and metabolic medicine approach to the complex topic of GI health.

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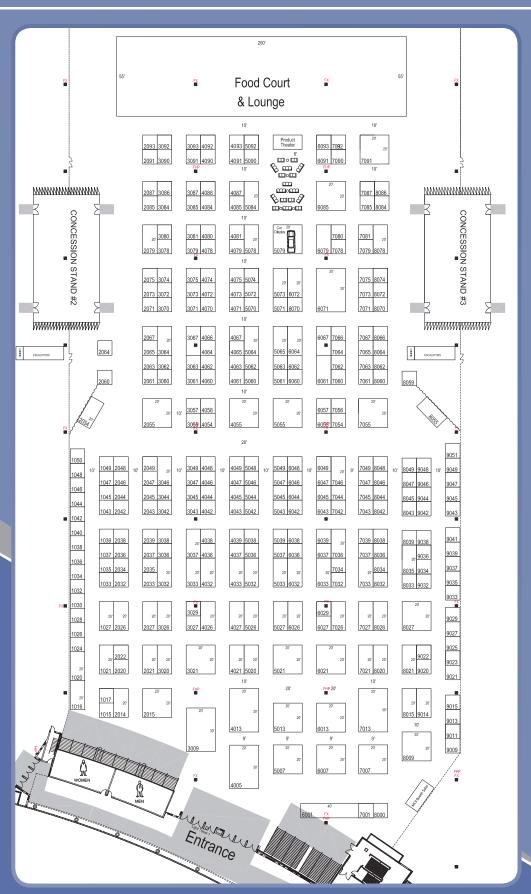
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### 24<sup>th</sup> Annual World Congress on Anti-Aging Medicine

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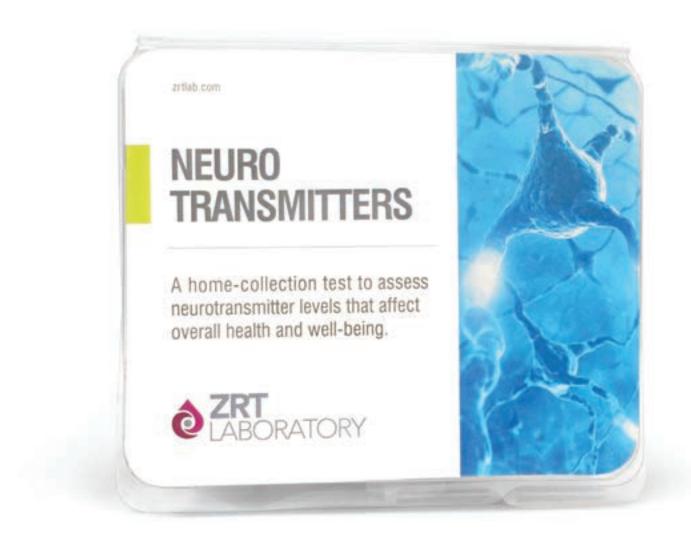
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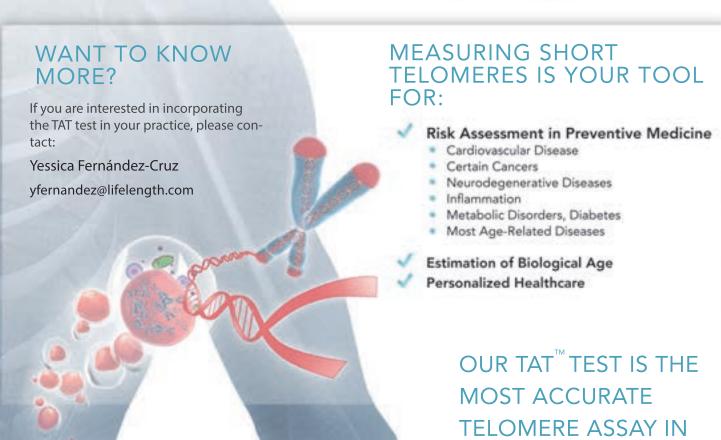
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**Ph**: 847-222-9546 • **F**: 847-222-9547

Email: Info@pyroluriatesting.com Website: www.kryptopyrrole.com

DHA Laboratory is a third generation, family owned and operated laboratory performing unique biochemical and nutrient based blood, urine, and hair testing. Located in Mount Prospect Illinois, DHA was founded by Ellen Hanson in 2003 to support the treatment model of the original Pfeiffer Treatment Center. Ellen started work to validate the kryptopyrrole quantitative urine procedure in the late 1970's, and has since worked with the top clinicians worldwide to provide reliable biochemical assays.



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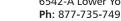
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Friday December 9th 11:30 AM "Introduction to the Pharmacology of Peptides"



AND



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healthycell® is company dedicated to cellular health that formulates multi-nutrient dietary supplement products. Its core philosophy is making sure its nutrition products contain only the highest quality independently screened ingredients and scientifically proven effect. healthycell® empowers health-conscious people to optimize their health to enjoy more productive lives. All life is made of cells…every organ, every tissue. The mission of healthycell® is to maintain the health of these cellular building blocks.



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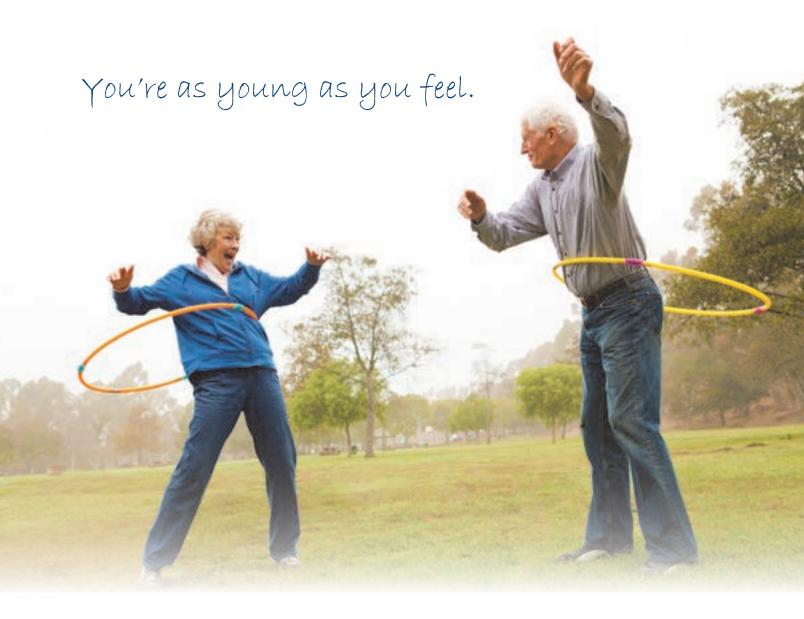
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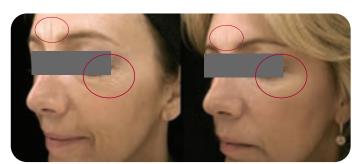
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# 2017 EDUCATIONAL SCHEDULE

#### **JANUARY 26-28**

Boca Raton Marriott | Boca Raton, FL

- > A4M Protocols Workshop (Non-CME) (Jan. 27-28)
- > MMI Module XVI-B Cardio
- > MMI Module XX-B Triad

#### MARCH 2-4

The Cosmopolitan | Las Vegas, NV

- > A4M BHRT Symposium
- > MMI Module II Cardio
- > MMI Module VI Toxicology
- > MMI Module XIV-D Weight Management

#### **MARCH 2017**

TBD

- > A4M Pellet Workshop
- > A4M Advanced Symposium

#### **APRIL 6-8**

Diplomat Resort | Hollywood, FL

- > A4M World Congress (Apr. 7-8)
- > ABAARM/ABAAHP Written Exams (Apr. 5)
- > ABAARM Oral Exams (Apr. 6-8)
- > A4M Chronic Infections Workshop (Apr. 6-7)
- > A4M Aesthetic Module I Botox and Fillers
- > MMI Module I Endocrinology
- > MMI Module VIII -Clinical Practice Protocols (Non-CME)

#### **MAY 5-6**

San Diego Marina Marriott | San Diego, CA

> A4M Symposium

#### **MAY 17-20**

San Francisco Marriott Marquis | San Francisco, CA

- > MMI Module III Neurology
- > MMI Module V Nutrition and Exercise

#### **JUNE 2-3**

West Palm Beach Hilton | West Palm Beach, FL

- > A4M Pellet Workshop
- > A4M Advanced Symposium (June 3)



Venetian/Palazzo Resort | Las Vegas, NV

- > A4M IV Symposium (Aug. 11-12)
- > MMI Module XX A Triad

#### **SEPTEMBER 14-16**

Chicago Marriott Downtown | Chicago, IL

- > ABAARM/ABAAHP Written Exams (Sept. 13)
- > ABAARM Oral Exams (Sept. 14-16)
- > A4M BHRT Symposium
- > MMI Module IV Gastroenterology
- > MMI Module VII Autoimmune Disease & Inflammation

#### OCTOBER 6-7

Sheraton Boston Hotel | Boston, MA

> A4M Symposium

#### **OCTOBER 20-21**

West Palm Beach Hilton | West Palm Beach, FL

- > A4M Pellet Workshop
- > A4M Advanced Symposium (Oct. 21)

#### **DECEMBER 13-16**

Venetian/Palazzo Resort | Las Vegas, NV

- > A4M World Congress (Dec. 14-16)
- > ABAARM/ABAAHP Written Exams (Dec. 13)
- > ABAARM Oral Exams (Dec. 14-16)
- > A4M Symposium (Dec. 13)
- > MMI Module I Endocrinology
- > MMI Module VIII -Clinical Practice Protocols (Non-CME)



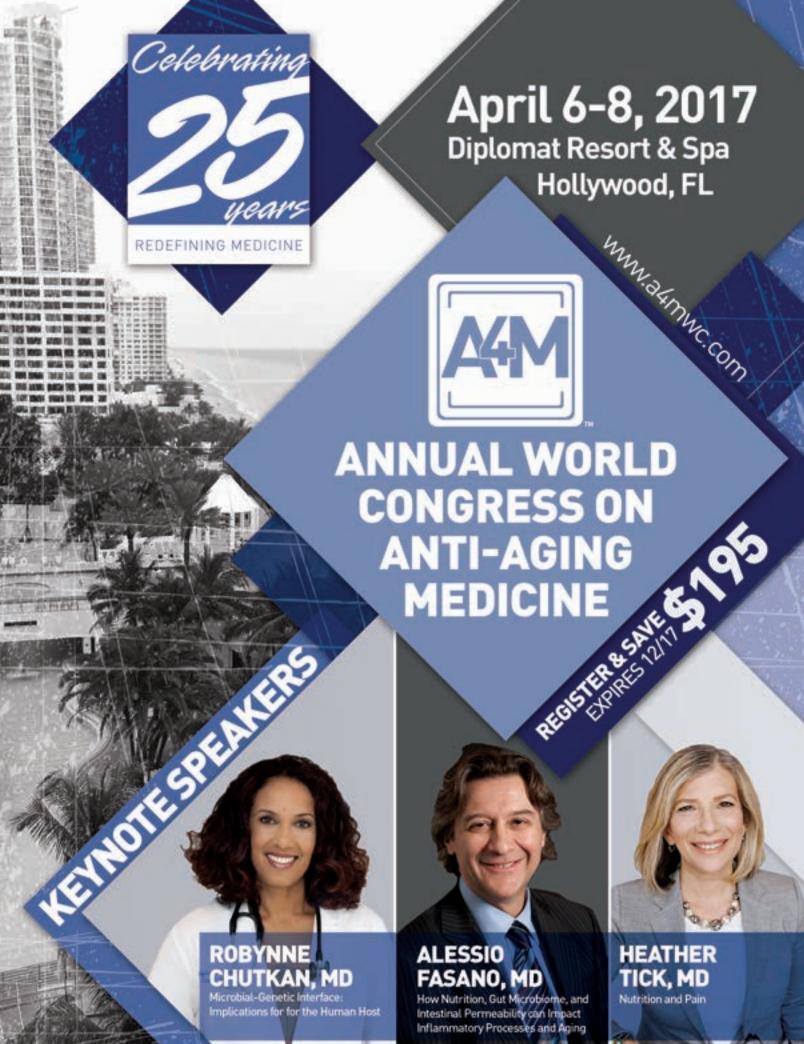




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# SAVE THE DATE

December 14-16, 2017 Venetian/Palazzo Resort Las Vegas, NV

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