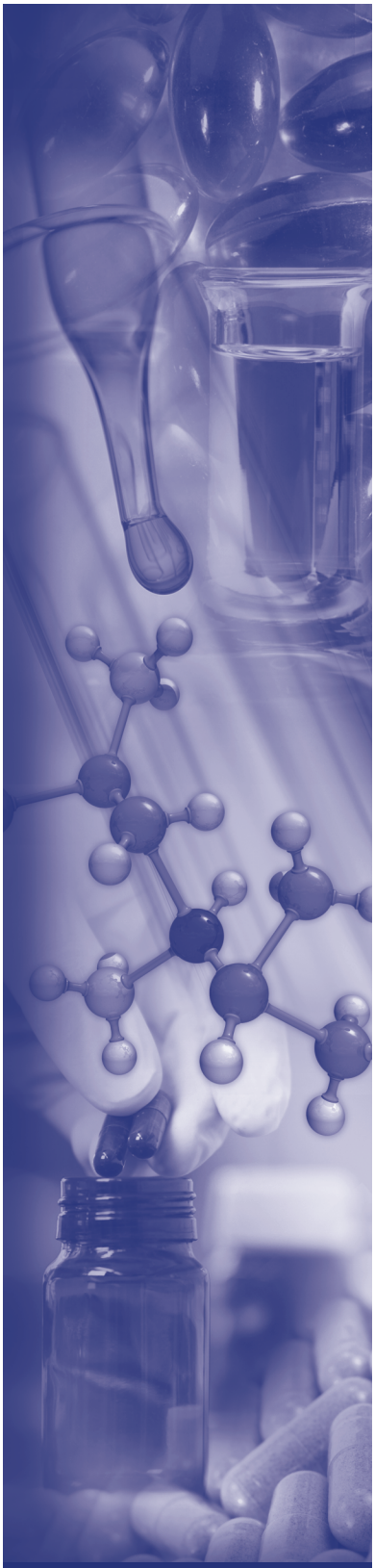

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Allergy Research Group® Newsletter April 2013

The Next Generation Vitamin E

Magnesium Stearate: A Safe and Effective Filler

The Next Generation Vitamin E
How Tocotrienols Benefit the Heart, Brain and Liver

Hidden in the stately steppes of gentle rice paddies, nestled in shiny clusters of red and purple palm fruit, lurking in tiny annatto seeds from the achiote tree… lies a quartet of potent anti-inflammatory, highly protective molecules called tocotrienols. They are cousins to the four tocopherols. Together, all eight comprise the Vitamin E family, a lipid-loving arsenal of molecules essential to health. Each has its own healing profile. According to molecular biochemist Chandan Sen, of Ohio State University, "Current studies of the biological functions of vitamin E continue to indicate that each member of the vitamin E family possesses unique biological functions often not shared by other family members."[1]

In 2008, we brought you the latest tocotrienol research, focusing then on the delta and gamma forms (*Focus* July 2008). Research has advanced at a quick pace since then, and we are devoting much of this issue to the newest findings on all of these remarkably potent molecules. Their biological impact turns out to effective in preventing and repairing stroke-related vascular damage, atherosclerosis, elevated cholesterol, and non-alcoholic fatty liver disease. Tocotrienols work not only as antioxidants, but through specialized mechanisms that directly regulate key inflammatory pathways in the body.[2]

For a long time, tocotrienols were like the Cinderella of Vitamin E science—relegated to the corner and ignored. Vitamin E research focused almost exclusively around alpha-tocopherol—discovered in 1922, isolated and analyzed in 1936, and dubbed the "fertility factor" because it is necessary for reproduction. We know that alpha-tocopherol has its own transport system (alpha-tocopherol transport protein, or TTP). This protein controls the distribution of alpha-tocopherol to cells and tissues throughout the body.

But in the last decade, a number of princes have kissed our Cinderella, and there is a growing body of impressive research on tocotrienols. In 2005, the definition of vitamin E in the *Merck Manual* had no reference to tocotrienol. Today, the manual recognizes that "Vitamin E is a group of compounds (including tocopherols and tocotrienols) that have similar biologic activities." In addition, in 2010 the FDA bestowed GRAS status on all tocotrienols. Tocotrienols turn out to offer unique protection against complex metabolic conditions, including heart disease, atherosclerosis, stroke, fatty liver disease, and metabolic syndrome.[3]

"Tocotrienols exhibit health benefits quite different from that of tocopherols, and in most cases, these activities are superior for human use," states biochemist Bharat B. Aggarwal, PhD, of The University of Texas M.D. Anderson Cancer Center in Houston, where he and his team study curcumin, resveratrol, tocotrienols and other natural compounds that might help us to defeat cancer. "We now know different isomers of tocotrienols exhibit distinct activities. While alpha-tocotrienol is highly effective in the brain for cerebral ischemia, gamma and delta tocotrienol exhibit strong anti-cancer and anti-inflammatory activities."[4,5,6,7]

Most importantly, says Aggarwal, tocotrienols work on multiple pathways: "In the last few decades, too much emphasis has been placed on designing drugs that hit a single target. I call it target practice—one gene, one target. However, most diseases are

a result of complex dysregulation of multiple genes and signaling pathways. Promising oral agents like tocotrienols are bioavailable, work on multiple pathways, and are already recognized as safe."[8]

In this issue we present:

- New studies suggesting that tocotrienols might protect the brain from stroke and repair stroke-induced damage.
- Groundbreaking research showing tocotrienols can improve and often cure fatty liver disease, improve end-stage liver disease, and protect hepatic function.
- Pathways by which tocotrienols protect neurons, prevent neuronal cell death, inhibit cholesterol and beneficially alter its fractions, and dampen inappropriate inflammation.
- Studies showing that tocotrienols reduce cholesterol, and improve atherosclerosis and arterial stiffness.

Let's Start With Stroke: Neuroprotection and Tocotrienols

If one single scientist has advanced our understanding of the brain and tocotrienols, it is molecular biochemist Chandan Sen, PhD, tenured Professor of Surgery and the Executive Director of the Ohio State University Comprehensive Wound Center. (See Interview With Chandan Sen, PhD, *From A Single Cell to the Human Brain*, pp 9). In Sen's words, "On a concentration basis, the neuroprotective effects of nanomolar tocotrienol represent the most potent biological function of all natural forms of vitamin E."[2] Uniquely, they are biologically active in the body at concentrations far lower than that of the allied tocopherols.

Working under famed Vitamin E expert Lester Packer, PhD at The University of California at Berkeley, Sen spearheaded original research into all eight vitamin E isomers in the late 1990's. One vitamin E fraction stood out. "We were astonished to find that alpha-tocotrienol was significantly more effective in preventing neurodegeneration in cells *in vitro* than any other molecule," says Sen.[1] It prevented neuronal death that might otherwise have occurred due to excess glutamate, the most abundant neurotransmitter in the brain. Later *in vitro* research found that neuronal cells could completely recover even several hours after a glutamate challenge, when treated with alpha-tocotrienol.[9] Remarkably even if glutathione remained depleted, cell death was prevented.[1]

Further research by Sen and colleagues showed that alpha-tocotrienol protected hypertensive rodents from stroke damage.[10] Stroke was induced in hypertensive rats, and their brains were removed to measure the size of the stroke-induced lesions. Rats supplemented with alpha-tocotrienol had significantly smaller lesions.[10] Then a randomized, blinded trial was conducted in dogs. Dogs were given mixed palm tocotrienols for 10 weeks, or a placebo. Stroke was induced and the volume of brain lesions measured using high resolution MRI both one and twenty-four hours after the stroke was induced.[11,12] Stroke-induced lesions in the treated dogs were significantly reduced in volume. Loss of white-matter fiber tract connectivity—the connectivity between different regions within the same hemisphere of the brain—was also reduced. Circulation to the ischemic region of the large, middle cerebral artery was measured by cerebral angiogram, and was improved in the tocotrienol-supplemented dogs.[11,12]

Stroke-induced lesions in tocotrienol treated dogs were significantly reduced in volume. Loss of white-matter fiber tract connectivity—the connectivity between different regions within the same hemisphere of the brain—was also reduced.

The researchers concluded that tocotrienols improved circulation, inhibited inflammatory

molecules, and was powerfully neuroprotective. "Tocotrienols are a natural neuroprotective agent," says Sen. "The neuroprotective property of tocotrienols hold good not only in response to glutamate challenge but also in response to other insults such as homocysteic acid, glutathione deficiency and linoleic acid-induced oxidative stress."[1,2]

Human Trials Are Next

Sen and colleagues are now setting the stage for human trials, called the Phase II Natural Tocotrienol Against Ischemic Stroke Event study. They have already concluded human trials proving that oral tocotrienols are both safe and well distributed in organs and the brain. They are currently researching optimal dosing regimens, before they design a study on tocotrienols in humans. Given the stream of positive data in every single study to date, it is likely we have in tocotrienols a potent and bioavailable, safe oral supplement to help prevent stroke and repair stroke damage.

If tocotrienols protect against stroke, they may well protect against other kinds of neurodegeneration, perhaps even the normal defects of aging. In a 2008 study, tocotrienols proved protective for a genetic neurodegenerative disorder called familial dysautonomia (FD), primarily affecting individuals of Ashkenazi Jewish descent. The mutations responsible for FD were found to occur in a gene encoding a protein (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein) termed IKAP. Over time neurons become progressively demyelinated. Autonomic "crises" include episodic vomiting, seizures, hypertensive crises, and many other symptoms. At the Laboratory for Familial Dysautonomia Research at Fordham University in New York, director Berish Y. Rubin and his colleagues screened hundreds of compounds and found that two—tocotrienols and epigallocatechin gallate (a component of green tea) were able to increase the amount of functional IKAP protein. After 3-4 months of tocotrienol supplementation, 80% of patients reported a significant decrease in the number of crises. "Individuals taking these compounds have seen a dramatic reduction in the symptoms associated with FD and have had a dramatic improvement in their quality of life," states Rubin.[13,14,15,16]

Thirty patients with non-alcoholic fatty liver disease were supplemented with mixed tocotrienols for a year, and 15 were completely cured. Another five showed significant improvement in the condition.

Tocotrienols Can Cure Non Alcoholic Fatty Liver Disease

When orally supplemented, tocotrienols reach their maximum concentration in the liver compared to other organs.[17] In fact, groundbreaking new research shows that tocotrienols can actually reverse non-alcoholic fatty liver disease, as well as improve deadly, end-stage liver disease.

Non-alcoholic fatty liver disease (NAFLD) is considered part of a spectrum of diseases called metabolic syndrome: they include obesity, diabetes, hypertension, high cholesterol, cardiovascular disease, and fatty liver. The incidence of NAFLD is surprisingly high—it afflicts 15-30% of Americans (studies in Europe, Japan and Malaysia report similar rates). Yet because it's asymptomatic, and there is no blood biomarker for the condition, it's often overlooked. The gold standard for NAFLD diagnosis currently remains ultrasound radiography.[18,19,20,21,22]

Yet NAFLD is not benign. Twenty percent or so progress to the more severe nonalcoholic steatohepatitis (NASH), and from there, some patients will go on to cirrhosis, liver cancer, or liver failure. NAFLD patients also

have a greater risk of atherosclerosis. According to pharmaceutical technologist Enrico Magosso, PhD, "The dangers of NAFLD are grossly underestimated due to two reasons: the lack of understanding of the pathogenesis; and the lack of an effective pharmacological treatment."[23]

In 2010, at the Liver Meeting of the American Association for the Study of Liver Diseases (ASSLD), Boston, Magosso and his colleagues reported a ground-breaking study that tocotrienols could improve, and even cure, NAFLD. Thirty patients were supplemented with full-spectrum palm tocotrienols for a year, and fifteen were completely cured. Another five showed significant improvement in the condition (at least one degree of amelioration of their fatty liver score, as evidence by ultrasound diagnosis).[17,23]

Tocotrienols Improve End Stage Liver Disease Scores

Tocotrienols may also improve a far more serious liver condition: end-stage liver disease (ESLD). Tocotrienols' ability to impact deadly liver disease was a serendipitous surprise to Chandan Sen and his colleagues when they began a trial to prove that tocotrienols were efficiently absorbed into skin, fat, human organs and the brain. The researchers supplemented individuals on organ transplant lists (so that when their organs were removed, testing could be done) as well as severe epileptics who needed brain surgery, and in whom at least a gram of brain tissue would be removed. Supplementation time averaged a month.

Patients awaiting liver transplant are always given a MELD (model for end stage liver disease) score.

50% of end-stage liver disease participants awaiting transplant and receiving oral mixed tocotrienol supplementation had a reduction in their end stage liver disease (MELD) score…4 of 6 participants with hepatitis C and the sole participant with hepatitis B had a reduced score following treatment.

The MELD scale ranges from 6 to 40, with the highest scores indicating poorest liver function and greatest need for a transplant. MELD score is a reliable marker for mortality, and does not improve over time. During the study, clinicians reported back that the MELD scores of tocotrienol supplemented patients were improving! Sen and colleagues amended the trial design to test the impact of tocotrienols on MELD scores. They report, "The effect… was most evident in patients with viral hepatic cirrhosis. In the current study, 50% of ESLD participants receiving oral tocotrienol supplementation had a reduction in their MELD score…4 of 6 participants with hepatitis C and the sole participant with hepatitis B had a reduced MELD score following treatment."[24]

Why are tocotrienols so effective in liver disease? Magosso speculates they impact multiple pathways. Animal studies show tocotrienols help prevent lipid peroxidation, and inhibit inflammatory mediators such as NFKappaB and TNFα, all of which are thought to be involved in NAFLD.[17] They also work on peroxisome proliferators-activated receptors, otherwise known as PPARs. Several well known anti-diabetic drugs are designed to reduce insulin resistance by working on PPARs.[17,25,26]

Tocotrienols Improve the Function of Arteries

Arterial compliance may sound like an odd phrase—are blood vessels rebellious or obedient? In fact, arterial compliance (or elasticity) is a key measurement of cardiovascular health. It refers to the action by which an artery yields to pressure or force without disruption. When arteries are stiff, their compliance decreases. Reduced arterial compliance is common in hypertension, and it can even appear in individuals with normal blood pressure, as an indicator of their potential for hypertension. Arterial stiffness is an important indicator of cardiovascular disease.

Tocotrienols increase arterial compliance. In 2008, a randomized, placebo-controlled study on 36 healthy males measured arterial compliance after two months of tocotrienol supplementation. Tocotrienols were supplied as bio-enhanced full-spectrum palm tocotrienols. The men received either a placebo, or tocotrienols (at 50, 100 or 200 mg daily). Carotid-femoral pulse wave velocity (PWV), a proven measure of arterial stiffness, was used. It measures the time it takes for the arterial pulse to move from the carotid to the femoral artery. Men taking either 100 mg or 200 mg of mixed tocotrienols daily showed significant improvement in arterial compliance.[27,28]

Men taking either 100 mg or 200 mg of mixed tocotrienols daily showed significant improvement in arterial compliance (elasticity)-a key marker for cardiovascular risk.

Bioavailable Tocotrienols Lower Cholesterol

Tocotrienols may also lower total cholesterol. They contain a side chain that increases a molecule in the cell called farnesol. That molecule signals the degradation of an enzyme responsible for the production of cholesterol (the enzyme is 3-hydroxy-3-methyl-glutaryl-CoA, or HMG-CoA).[29] We discussed this at length in our 2008 newsletter. While statins, especially in high doses, are like a metaphorical sledgehammer inhibiting this enzyme, and blocking the pathway so that co-enzyme Q10 is also inhibited, tocotrienols are far gentler, with no apparent side effects.

A 2001 study from the University of Wisconsin compared tocotrienols, statins, or a combination of the two on cholesterol markers. Researchers put 28 individuals with high cholesterol on a restricted diet for a month, and then gave them either 50 mg of mixed tocotrienols, 10 mg of the statin drug Mevacor, or a combination of the two. In the 50 mg tocotrienol group total cholesterol was lowered by 14% and LDL by 18%. In the Mevacor group total cholesterol was lowered by 13% and LDL by 15%. In the combination group total cholesterol was lowered by 20% and LDL by 25%. The researchers then tested diet and tocotrienols alone—at doses of 25 mg, 50 mg, 100 mg, and 200 mg a day. 90 people were studied, and the 100 mg a day dose worked most efficiently, lowering total cholesterol by a very impressive 20% and LDL by a clinically highly significant 25%.[30]

However, some studies on tocotrienols and cholesterol have shown equivocal results. According to researcher and pharmaceutical scientist Yuen Kah Hay, PhD, of the School of Pharmaceutical Sciences at the University of Science Malaysia, two studies showed that supplementation with 200 mg of mixed or pure tocotrienols in subjects restricted to a controlled diet led to a reduction of 8-16% of total cholesterol and LDL cholesterol within 4 weeks. Even without a controlled diet there were similar improvements. Yet other studies showed that supplementation with up to 240 mg of tocotrienols did not lower cholesterol. "The discrepancy might be due to differences in the composition of the supplements as well as differences in the bioavailability of dosage forms used," writes Yuen. "For example, a high-tocopherol content (30% and above) in the tocotrienol supplement has been found to attenuate the cholesterol lowering activity of the mixture in chickens, as well as reducing the oral bioavailability of the tocotrienols…This might explain the absence of cholesterol lowering activity observed…Moreover, the bioavailability of lipid soluble drugs was reported to be greatly influenced by the type and volume of oil administered as well as the delivery systems used. A formulation that gives both consistent and enhanced absorption of the tocotrienols will be advantageous."[31]

A 2011 study by Yuen and colleagues found that a self-emulsifying formula of tocotrienols

effectively lowered cholesterol levels. Thirty-two individuals with high cholesterol were randomly assigned to either 300 mg of mixed tocotrienols, or placebo capsules, for six months. The researchers report that "the serum total cholesterol and low density lipoprotein (LDL) cholesterol of the subjects in the tocotrienol supplementation group were decreased significantly…after 4 months of supplementation and the reduction persisted till the end of the 6-month study…moreover, there was a very impressive 22-fold increase in the total tocotrienol concentrations from baseline during supplementation compared to the placebo group, while the concentration of tocopherol recorded only a modest increase. On the other hand, the serum cholesterol, total tocotrienol and tocopherol concentrations of subjects in the placebo group remained essentially unchanged."[31]

The Cellular Pathways to Potent Protection

By what molecular magic do tocotrienols protect the heart, brain and liver? Research is now elucidating many of the pathways.

In the brain, there are at least four antioxidant-independent pathways by which tocotrienols might uniquely prevent neurodegeneration. They do so by regulating specific mediators of cell death.

Perhaps the most striking is the pathway regulated by the cSrc (short for "sarcoma") gene. Scientists J. Michael Bishop and Harold E. Varmus were awarded the 1989 Nobel Prize in Physiology or Medicine for discovering this gene. Its pathway regulates normal and abnormal cell growth. Though this pathway has been studied intensively in cancer, it turns out that cSrc is also abundant in the brain. Overexpression in this pathway can lead to glutamate-induced cell death. Too much glutamate, the most abundant neurotransmitter in the brain, is potentially deadly to the brain's glial cells, cortical neurons, and other types of neurons.[7,9,32] "cSrc is involved in neurodegeneration *in vivo*," states Sen. "Our pursuit for the neuroprotective mechanisms of tocotrienols led to the first evidence demonstrating that rapid cSrc activation plays a central role in executing neurodegeneration. Consistently, it was demonstrated in a subsequent report that cSrc deficiency or blockade of cSrc activity in mice provides cerebral protection following stroke." Alpha-toctotrienol powerfully inhibits expression of this pathway.[32]

Thirty-two individuals with high cholesterol were randomly assigned to either 300 mg of mixed tocotrienols, or placebo capsules, for six months. The serum total cholesterol and low density lipoprotein (LDL) cholesterol of the subjects in the tocotrienol supplementation group decreased significantly.

The second pathway is that of lipoxygenase, a fatty acid metabolizing enzyme, which regulates a compound known as 12-Lox. Says Sen, "Our work led to the identification of 12-Lox as a key tocotrienol-sensitive mediator of neurodegeneration. 12-Lox represents a critical checkpoint in glutamate-induced neurodegeneration." Metabolites of 12-Lox are capable of causing cell death. Alpha-tocotrienol directly interacts with this enzyme.[7]

Other pathways include that of phospholipase, another enzyme related to lipid metabolism and cell membrane viability, and the fourth involves MRP1 (also known as multi-drug resistance protein-1). All protect against neurodegeneration.[7]

For cholesterol, tocotrienols suppress the activity of HMG-CoA reductase, the hepatic enzyme responsible for cholesterol synthesis. And, according to a 2011 genomics study on tocotrienols by Dipak Das and colleagues at the Cardiovascular Research Center at the University of Connecticut Health Center, tocotrienols regulate several key, cholesterol-related proteins. "The results of

the present study demonstrate that the two isomers of tocotrienols, alpha and gamma, render the hypercholesterolemic hearts resistant to ischemic reperfusion injury by lowering several hypercholesterolemic proteins and upregulating TGF-beta." (Transforming growth factor beta is a protein that controls proliferation and cellular differentiation, as well as providing inflammation control.)[33]

Finally, tocotrienols regulate NF-kappaB, a protein complex that controls the transcription of DNA regulating genes that are critical for inflammation and immunity. NF-kappaB is particularly relevant to both chronic inflammation and cancer. This may have relevance not only to any disease involving inflammation, but also to aging, according to scientists Mary Kaileh, PhD and Ranjan Sen, PhD, of the Laboratory of Cellular and Molecular Biology at National Institute on Aging, Baltimore, Maryland. "It is widely hypothesized that NF-kappaB dysregulation accompanies human aging," they note. "It is possible that regulated consumption of tocotrienols may counter this form of chronic NF-kB dysregulation without seriously impacting acute NF-kB responses that are essential for immune responses and thereby improve the quality of life of the elderly."[34]

Tocotrienols are the Future of Vitamin E Research

Before soybean oil is refined to obtain alpha-tocopherol, gamma-tocopherol is plentiful in the raw material, but in concentrating alpha-tocopherol, gamma-tocopherol was largely discarded. Recent research shows the critical importance of balancing ingestion of alpha-tocopherol with gamma-tocopherol, and gamma-balanced tocopherol formulas are now favored.[35]

Likewise, tocotrienols obtained from fresh palm oil provide a rich balance of alpha-, beta-, gamma-, and delta-tocotrienols, along with a modest amount of tocopherols. As this newsletter shows, alpha-tocopherol does not interfere with the absorption of tocotrienols, as long as alpha-tocopherol is not artificially concentrated at excessive levels. In this particular case, nature offers a balanced combination of various vitamin E fractions that can offer therapeutic potential without interfering with each other.

We know now that meaningful research on vitamin E must define the isomer or isomers being studied. Studies that only look at alpha-tocopherol should not be titled as having researched "vitamin E". And it is time for tocotrienols to take their place in the forefront of vitamin E research. From the heart to the brain, from the liver to life itself, tocotrienols are proving to truly be the next generation Vitamin E.

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From a Single Cell to the Human Brain: A Lifelong Journey

An Interview with Chandan K Sen, PhD

A Note from Stephen Levine: When *Focus* decided to delve deeper into the unique healing potential of tocotrienols, we had the honor of interviewing a towering figure in the field: Dr. Chandan Sen, tenured Professor of Surgery and the Executive Director of the Ohio State University Comprehensive Wound Center. Dedicated to tocotrienol research for decades, Sen has unswervingly pursued his passion since his staff scientist days at the University of California at Berkeley, working with famed mentor Lester Packer PhD. Sen's research offers impressive evidence that these tiny, powerful molecules may help to minimize deadly strokes and aid in recovery for those already affected.

The pursuit has been, as Sen reflects in this interview, "a long journey of learning. Part of the challenge was learning how to progress from a model of research on the molecule to a single cell to small animals to large animals and then to humans, all the while strictly following every regulation and all the ethics required." Ethics, might I add, that went beyond the call of duty—where Sen and his colleagues found a new way to do research on canine brains, and in human brains and organs (in the living organism) that was minimally invasive and quite remarkable. –Stephen Levine, PhD

Focus: You've done remarkable work on tocotrienols, particularly alpha tocotrienol, and the possible prevention of stroke, and repair of damage in stroke. What got you interested in tocotrienols in the first place?

Sen: I was at Berkeley, and my supervisor was Lester Packer, arguably the most cited and active person in Vitamin E research at that time. I asked Lester, 'Why

do we study tocopherols, when we know Vitamin E is not limited to tocopherols?' Back then almost all the research was on tocopherols, and less than 1% on tocotrienols. Lester said, "Well, if you're interested, let's research tocotrienols." But when I looked for tocotrienols, there was nothing available in the market. No wonder nobody had studied them! Finally I found a large company in Germany that held the patent on chemical synthesis of tocotrienols, and we managed to get all eight forms of Vitamin E to study. We ordered them blinded, just coded in letter format, A, B, C, D, E, F, G, H. And we started testing them *in vitro* using an established model of neurodegeneration.

Focus: And what you found caught your full attention, and started you on your lifelong journey.

Sen: We were astonished to find that Sample C was significantly more effective in preventing neurodegeneration in cells *in vitro* than any other molecule. We published that paper in the year 2000 in the Journal of Biological Chemistry.[1] I was the first author, Lester the last author. At first, we thought perhaps Sample C was alpha-tocopherol. But no, it was alpha-tocotrienol. We then wanted to confirm our results, and when we compared the molecules again, we found the difference was not one or two fold, but a hundred fold, in terms of concentration. It was not something you could ever miss. It was a really powerful result. But, being scientists, we were still terribly skeptical at the time, so we made the observation by saying we had to report this, but we had done no supportive work *in vivo* yet. When you are publishing groundbreaking research it is not enough to say you found a molecule was protective. You must also identify the pathway or pathways through which it protects.

In 2000 we tested all eight Vitamin E fractions on neuronal cells, and found that alpha-tocotrienol protected cells from degeneration at concentrations a thousand-fold less than any other form of Vitamin E.

Focus: So finding the pathway by which alpha tocotrienol works was your next step. What pathway(s) did you discover?

Sen: We found a pathway known as cSrc (cellular-src, where src is short for sarcoma) as one powerful candidate. To put that in context, scientist Harold Varmus PhD, the previous director of the National Institutes of Health, jointly won the 1989 Nobel Prize in Physiology or Medicine with Michael Bishop for discovery of the cellular origin of retroviral oncogenes. In 1970, he identified the oncogene Src and demonstrated its significance in cancer. The discovery of Src family proteins has been instrumental to the modern understanding of cancer as a disease where normally healthy cellular signaling has gone awry. Src biology is a very hot topic in the field of cancer. However, it turns out the human brain naturally has a lot of cSrc, and nobody knew what it was doing there. We were reporting for the first time that tocotrienols, perhaps by inhibiting cSrc activity in the neural cells, could prevent neurodegeneration.

That was the beginning and we were terribly excited but we were also very worried about whether we could really 'hang our hat' on

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this finding. It takes a long time to develop a new field and call it your scientific home. So we were very interested in looking next at *in vivo* efficacy. And to do *in vivo* research on conditions like stroke requires specialist facilities. That year I moved from Berkeley to Ohio State University, where I have been ever since. So if you jump forward from 2000 to today, we are doing clinical trials today I never could have undertaken at my previous lab at Berkeley.

Focus: What was your first *in vivo* study?

Sen: I went to the director of the clinical stroke program at Ohio State, Dr. Andrew Slivka, PhD. Slivka had a laboratory where he studied stroke in rats that were spontaneously hypertensive, and I asked him to test tocotrienols in his rats. We found that just by giving tocotrienols orally, not even by injection, we could decrease stroke-induced injury to the brain in hypertensive rats. This was published in *Stroke, journal of the American Heart Association* in 2005.[2]

The scientific response to our paper in *Stroke* was huge, and many people asked me if I thought it was only stroke, or also other forms of neurodegeneration? I still get asked that question today. And I have to say, I am sorry, but I have dedicated my laboratory work to studying stroke. Other researchers are studying other forms of neurodegeneration, but in order for me to do my science well I need to take one disease all the way. And of course, people also said, there are hundreds of molecules that work in a mouse and a rat and don't work in a human. It is widely understood that the brain of a rodent is small, and its geometry is quite different than a human brain, so if you want to show this might help humans, you need to go to a larger animal first. It was time for us to test tocotrienols in a larger animal.

When we tested a model of stroke in dogs, we found that mixed tocotrienols showed a tremendous neuroprotective effect, even more so than in rodents.

Focus: So you went to study dogs. But when you saw how stroke was routinely studied in dogs, you didn't like it. You felt the dog and its brain were being traumatized. So what did you do?

Sen: I did not like the approach being used at that time to induced stroke in large animals. In one model they actually cut open the dog's skull, then went in and physically pinched a blood vessel in the brain to create stroke. To me this was tremendous head trauma, and more like a car accident than a naturally occurring stroke. And in another model, they'd take out the eyeball and go in and pinch the blood vessels in the brain. I also thought this was way too traumatic. I sat down with surgeons and neuroradiologists and said, I need a cleaner, gentler approach. It took us several years to develop that approach, and our results of the first study using this model were published in 2008 in the *Proceedings of the National Academy of Sciences*.[3] We demonstrated that we can go in with a dime size access route in the leg, and thread an endoscopy catheter all the way into the brain, into the mid-cerebral artery where most stroke occurs, and simply create a reversible occlusion there. We then wake up the animal, and it's very similar to a naturally occurring stroke. So we studied twenty dogs this way, and gave them tocotrienol-rich palm oil in a capsule (or placebo) that had already been cleared as safe for human consumption. We tested the dogs' brains by using MRI, and found a tremendous protective effect. In fact, we were able to demonstrate a far better effect in dogs than in rats.[4] And at that point, as you might imagine, there was a floodgate of enthusiasm from scientists and sponsors. Even so, I still cannot

say it would definitely work in a human until a human clinical trial is done and published.

Focus: So, on to humans. Where are you at with human studies?

Sen: The human study started the same year as dog study ended. First we proved that the dosages we were studying were safe. Then we did a study on over 80 patients who were in end stage organ failure and needed heart, lung, or liver transplants, or patients who suffered from severe epilepsy that required a surgical procedure where nearly a gram of their brain was removed. We gave full-spectrum palm tocotrienols to all these patients, and we studied the organs that were removed, and the brain tissue that was removed. To make a long story short, the tocotrienols were efficiently absorbed into all the organs including the brain. We also found something completely unexpected. We found that people with end stage liver disease and awaiting liver transplantation, began to improve. They experienced a reduction in their MELD (model for end stage liver disease) score. MELD is scored according to the severity of chronic liver disease, and determines the priority and need for liver transplant allocation. So it looks based on these experiences as if tocotrienols may have a valuable and effective hepatoprotective function. We published this in the *Journal of Nutrition* last year.[5]

We are now in a Phase II clinical trial, where we are testing different dosages and regimens to determine what is optimal. And finally we will go to a full-blown Phase III clinical human trial in stroke.

Focus: That's great. Can we address a few technical aspects of tocotrienols? For instance, one concern among researchers is that alpha-tocopherol, the most famous fraction of Vitamin E, is preferentially absorbed over tocotrienols.

We found that fifty percent of people with end-stage liver disease, awaiting liver transplantation, began to improve when given an oral mixed tocotrienol supplement.

Sen: This is only partly true. When researchers mention that, they are referring to Alpha-tocopherol Transfer Protein, TTP. But we have shown that in TTP knockout mice, tocotrienol transport is not hindered. Thus, TTP-independent mechanisms of tocotrienol transport also exist. It is true however, that alpha-tocopherol in high amounts (over 400 mg/day) taken concurrently may be detrimental to tocotrienol absorption.

Focus: What about the fact that tocotrienols are irregularly present in the diet globally? For instance, they are prevalent in palm oil, which is popular in Malaysia, but there is very little in the American diet. They are present in trace amounts in grains.

Sen: If you take an average American off the street and measure the amount of tocotrienols in his or her blood, you'd find very little. Whereas in Malaysia and Singapore they use red palm oil in cooking and so their diet has a rich source, but even so, the amounts utilized in clinical trials are higher than the amount available in red palm oil consumed as food. These amounts are not achievable clinically in the normal diet. For the effects we are claiming, tocotrienols have to be provided through oral supplementation.

Focus: What about the fact that alpha-tocopherol functions as an antioxidant? Do tocotrienols function as antioxidants?

Sen: They certainly do. All Vitamin E forms have antioxidant properties. But interestingly, what we are reporting in stroke, we believe is an antioxidant-independent mechanism. We have published four different targets or pathways by which tocotrie-

nols can actually protect neuronal cells challenged with glutamate—a substance that in excess can be damaging to cells.[6] The c-Src, which I mentioned is one. Lipoxygenase, a fatty acid metabolizing enzyme, is another. The third is phospholipase, another enzyme related to lipid metabolism, and the fourth, we finally reported in 2011, is MRP1 also known as multi-drug resistance protein-1. These are four targets or pathways that tocotrienols address, which may influence the neuroprotective properties, and not one works via an antioxidant mechanism. In fact, we showed that in cells damaged by glutamate, and in which there is a simultaneous depletion of glutathione, the cell can be prevented from dying by tocotrienols, even though glutathione levels are not replenished by the molecules. This means that even with inadequate glutathione, the cells can still be rescued if lipoxygenase is inhibited.[7,8,9]

Focus: Can you address the potency of tocotrienols? They seem effective in much lower amounts than tocopherols.

Sen: Yes. The molecular weight of something is often expressed in terms of molars, micromolars, nanomolars. Weight equal to molecular weight expressed in grams of a substance dissolved per liter is called molar strength. That is a reference concentration. When using the term millimolar, you are talking about a thousandth of a molar. Then you get to micromolar, which is a millionth of a molar. A billionth of a molar is a nanomolar. Tocopherols are often measured in the human blood in micromolars. You may find 25-50 micromolars of tocopherols in a blood sample. But tocotrienols are found at 1-5 micromolars in the blood, and in the neuronal cell a nanomolar concentration can prevent cell death. I do not know of any other vitamin that shows biological function in the nanomolar range. It's an incredibly tiny amount, and a fascinating finding. For instance, in one of our studies, we compared tocotrienols to a chemical inhibitor of lipoxygenase. Using this bonafide effective potent inhibitor of lipoxygenase requires about 2.5 micromolars. Yet the same enzyme is blocked by a hundred times lower dose of tocotrienols. That's very striking.

Focus: When you look forward, what do you see ahead?

Sen: Taking a scientific solution from the level of the molecule to the human being is a very complex, interesting and rewarding exercise. It involves learning from failures and being cautious about successes. Unless we had taken all these years to understand tocotrienols and the pathways by which they work, we would not be where we are today. I hope that eventually we will have results that will help people to void and recover from devastating neuronal and liver illness around the world. That would be the final payoff.

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Preventing and Reversing Non-Alcoholic Fatty Liver Disease

An Interview with Enrico Magosso, PhD

Focus: Before we get to your remarkable work on non-alcoholic fatty liver disease (NAFLD) and tocotrienols, tell us a bit about your research environment. You are an Italian scientist who moved to Malaysia to study fatty liver and neurodegenerative diseases. Is Malaysia a good place for this research?

Magosso: Science operates without geopolitical boundaries and Malaysia, besides being a wonderful country, has a very conducive environment for science. My own passion is for applied sciences, mostly related to metabolic diseases such as fatty liver and neurodegenerative diseases. I joined Professor Yuen's laboratory at Universiti Sains Malaysia, the top national institution here, in 2002. His laboratory already had several projects underway on tocotrienols, from pharmacokinetics to bioavailability, organ distribution and formulations. In Professor Yuen's lab, all the researchers work together and collaborate with each other closely, so knowledge is built up and shared. In 2005, our plans for large studies on tocotrienols in cardio- and neuro-protection started to come to fruition, and by 2007 we managed to secure a grant from a Malaysian Government Agency. It is fascinating to work with lesser known members of the vitamin E family, the tocotrienols, as they seem to have so much potential.

Focus: What got you interested in NAFLD?

Magosso: Family reasons, primarily. I had an uncle that had a severe manifestation, was even hospitalized, and doctors didn't have a treatment for this disease. While the main objective of our research grant was the brain and stroke prevention, we also wanted to study patients with nonalcoholic fatty liver disease (NAFLD). Alpha-tocopherol, the most studied vitamin E, showed contradictory results in NAFLD in prior research. It has been known for a long time that tocotrienols have more potent activity than alpha-tocopherol in protection against hepatic lipid microsomal peroxidation. Essentially, this type of lipid peroxidation occurs because the accumulation of lipids in the liver cell leads to increased reactive oxygen species, oxidative stress, membrane dysfunction and ultimately cell death. We also knew that tocotrienols are preferentially distributed in the liver, in fact, tocotrienols reach their maximum concentration in the liver compared to other organs, and they reach higher levels than alpha-tocopherol. So we saw great potential and we started our investigation of NAFLD.

Focus: How prevalent is NAFLD and what are the serious risks of the condition?

Magosso: In western countries and Japan, the prevalence ranges from 15-30% of the population. When we started studying NAFLD in Malaysia, we had to determine the prevalence and it was similar. That's a lot of people with NAFLD!

(*Continued on page 16*)

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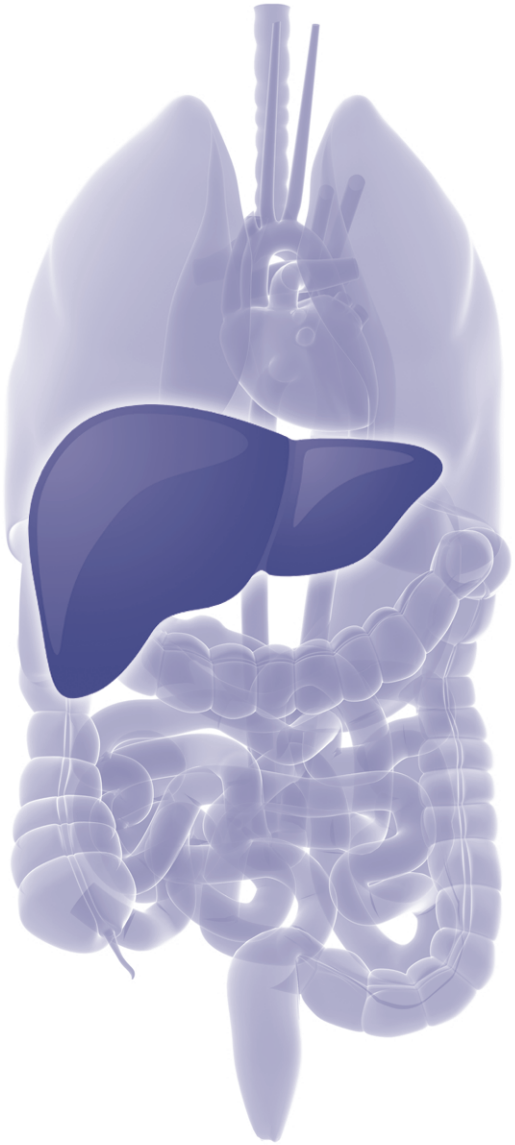
A Quick Guide: Risk Factors for Non-Alcoholic Fatty Liver Disease (NAFLD)

Genetic factors
- Gender
- Latin and black Americans
- PNPLA3
- Abetalipoproteinaemia
- Werner syndrome

Lipid metabolism and insulin resistance
- Metabolic syndrome
- Type 2 diabetes
- Mauriac syndrome
- Weber-Christian syndrome
- Sleep apnoea syndrome

Intestinal factors
- Small bowel resection
- Jejunal bypass
- Biliopancreatic diversion
- Bacterial overgrowth
- Dysbiosis
- Mitochondrial induced inflammasome activation



Nutrition
- Alcohol consumption
- High calorie diet
- Vitamin C and E deficiency
- Tocotrienol deficiency
- Acute starvation

Pharmacotherapy
- Total parenteral nutrition
- i.v. glucose
- Amiodarone
- Nifedipine
- Tamoxifen
- Glucocorticoids
- Synthetic estrogens

Hormones
- Menopause
- PCOS
- Hypogonadism
- Hypothyroidism
- Growth hormone deficiency

Intoxicants
- Organic solvents
- Volatile optrochemicals
- Dimethylformamide

Infections
- Hepatitis C

The main issue with NAFLD is that there are no specific biomarkers for it, meaning that a simple blood test will not confirm or exclude NAFLD. Even if the liver function test is normal, the person may still have NAFLD. An ultrasound examination is needed to confirm a NAFLD diagnosis, and NAFLD is highly correlated with other metabolic diseases, such as obesity, diabetes, dyslipidemia (high cholesterol or triglycerides) and hypertension. The more risk factors that are present, the higher the rate of NAFLD in that population of patients. Up to 90% of obese diabetics with high cholesterol may have NAFLD. However, even lean healthy individuals are not spared fatty liver disease.

Focus: Before we get to the success of tocotrienols in NAFLD, can you give us an overview of the disorder?

Magosso: Until the 1980s, NAFLD was not even considered a disease on its own. Because alcoholic and non-alcoholic have an analogous pathological expression, most of the patients presenting with NAFLD were considered "drinkers in denial". Fat accumulation in the liver was considered an innocuous condition. We had to wait another 20 years before the dangers of NAFLD were proven and it was shown that people with NAFLD have higher mortality, and more strokes and cancers than non-NAFLD individuals. NAFLD is actually a range of diseases of increasing severity that can progress to fibrosis, cirrhosis and in a small percentage, hepatocellular carcinoma. NAFLD is predicted to become the leading cause of liver transplants in the next 10 years.

So far, many studies have investigated drugs and supplements for NAFLD, but none resulted in a truly effective treatment. NAFLD can progress into nonalcoholic steatohepatitis (NASH). NASH includes accumulation of fat in the liver cells as well as inflammation of the liver. The inflammatory cells can destroy the liver cells (hepatocellular necrosis). Recently alpha-tocopherol was studied as a treatment for NASH at very high doses (800mg/day) for two years. But reviews of these studies were contradictory; in one study, known as PIVENS, alpha-tocopherol was superior to placebo. But in another study, known as TONIC, it was not. Both studies investigated advanced cases of NAFLD, and used liver biopsy to determine severity.[1,2]

Focus: What happened when you studied tocotrienols and NAFLD?

Magosso: Blood tests on our study population showed that all three tocotrienols we used—alpha, gamma, and delta—were significantly increased when taking the supplement. We use the blood tests to check compliance with the dosing regimen. We also measured alpha-tocopherol. In our double-blind study of 64 patients, we treated 30 with two capsules a day of 200 milligrams of a balanced, self-emulsifying formula of tocotrienols, and treated 34 with an identical looking placebo. The primary end point of the study was cure, meaning that ultrasound revealed no NAFLD. Fifteen patients on tocotrienols—50%—were completely cured, while another five had improvement—a total of 67% of all those treated with tocotrienols.

As I mentioned, a review of alpha-tocopherol and NAFLD studies is still puzzling and contradictory. Moreover, alpha-tocopherol at high doses (above 400 mg/day) has been shown to increase mortality risk.[3] The advantage of a mixed tocotrienol preparation may be that no single tocotrienol is taken at more than 260 mg/day.

The final paper is in preparation for publication, while an interim analysis was presented at the Liver Meeting 2010, in Boston.[4] We are now planning animal and cell research to elucidate the mechanisms of action. A simple antioxidant effect is not sufficient to explain the effects of tocotrienols against fatty liver. We are also planning another, large scale study in humans, hopefully with about 200 individuals. It is really

exciting to work on a disease that is so common, and presents us with so many unanswered questions. I'm a pharmacist by training, so I think that treating an early stage of the disease is better.

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Tocotrienols, Probiotics and Glycophospholipids A Perfect Prescription for the Liver?

By Michael Ash, BSc, DO, ND, F.DipION

One of my primary areas of research and expertise is the gut microbiota and its diverse impact on our health. Your liver receives nearly 70% of its blood supply from the intestine, and represents a first line of defence against gut-derived antigens. Intestinal bacteria—and the antigens they produce—play a key role in the maintenance of gut-liver axis health. Modulation of the gut microbiota to achieve and maintain symbiosis represents a new way to treat or prevent non-alcoholic fatty liver disease (NAFLD). Along with the concomitant use of tocotrienols and glycophospholipids, we may be starting to see the emergence of a truly profound intervention for a complex metabolic disease, using safe, natural compounds.

Here is how I see the three approaches merging. First, membrane consistent glycophospholipids are used to repair the bi-layer of the mitochondrial membrane. In the August 2012 issue of *Focus*, Garth Nicholson, PhD, and I wrote about the emerging role of the mitochondria—and damage to the mitochondrial lipid membrane—in integrative medicine. We explored how glycophospholipid replacement could reduce adverse spillage of reactive oxygen species (ROS), the energy-carrying enzyme ATP and oxidized DNA. The latter two are known as Damage Associated Molecular Patterns (DAMPS).

We also wrote about gastrointestinal bacterial dysbiosis, and the consequential increase in pathogen-associated microbial patterns (PAMPS) . Together, DAMPS and PAMPS drive inflammation and heighten receptor sensitivity in both tissues - with adverse outcomes in human health and physiology.

Mitochondria and gastrointestinal bacteria share a common ancestry—bacterial cells called prokaryotes. This historical genetic lineage means bacterial antigens and mitochondrial DNA are recognized by the same immune receptors on and in cells. The intracellular receptors, when activated, release two potent cytokines - IL-1β & IL-18. Research shows these cytokines are intimately linked to liver diseases including NAFLD and are instrumental in its progression to non-alcoholic steatohepatitis (NASH) .

A "two hit" mitochondrial mechanism may drive NAFLD/NASH pathogenesis. The first hit, hepatic steatosis, is closely associated with lipotoxicity-induced mitochondrial abnormalities that sensitize the liver to additional pro-inflammatory insults. The second hits include enhanced mitochondrial lipid peroxidation and increased generation of reactive oxygen species (ROS) and increased release of oxidised mitochondrial DNA.

Next, intracellular protein complexes called inflammasomes are activated. Inflammasomes trigger the maturation of the highly inflammatory IL-1β & IL-18. When inflammasomes, which are also sensors and regulators of the colonic microbiota are activated, the gut microbiota is also altered. Could this altered composition influence events in the liver? I believe so. Changes in the composition of the microbiota as a result of inflammasome activation or dysbiosis trigger the cytokine cascade with initiation and progression of inflammatory liver disease.

A cautiously optimistic idea is now taking hold that invokes using mitochondrial and bacterial induced symbiosis of the gut microbiota to track, target and treat a plethora of diseases, even ones beyond the confines of the gastrointestinal tract. Adding tocotrienols to this dual approach creates a therapeutic trinity of natural, safe agents.

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Why Can't They Just Get Along? The Truth about Alpha-Tocopherol and Its Vitamin E Cousins

Is alpha-tocopherol a kind of molecular bully, gifted with its own special passenger train (its own transport protein), by which it can reach any tissue in the body better and faster than other Vitamin E fractions? Does it actually inhibit the absorption of other forms of Vitamin E? Is it possibly dangerous?

The answers are nuanced, as biology always is. All forms of Vitamin E have their own molecular gifts, and certainly alpha-tocopherol is a potent antioxidant, a "fertility factor" necessary for reproduction, and necessary for many bodily functions and yes, it does have its own transport protein.

On the other hand, it's only one of eight. Alpha-tocopherol has been erroneously conflated with "Vitamin E" in study after study, as if it alone represented the entire family of molecules. Yet large scale investigations into alpha-tocopherol's benefits when given alone have been contradictory at best. One of the biggest studies—spanning 174 centers and over 7000 individuals, called the Heart Outcomes Prevention Evaluation (HOPE) trial, and the subsequent HOPE TOO trial—found that in patients with vascular disease or diabetes mellitus, long-term alpha-tocopherol supplementation did not prevent cancer or major cardiovascular events.[1] Even more concerning, a 2010 meta-analysis of 22 studies on alpha-tocopherol and stroke, from Harvard's Brigham and Women's Hospital found that alpha-tocopherol supplementation "increased the risk for hemorrhagic stroke by 22% and reduced the risk of ischemic stroke by 10%...Given the relatively small risk reduction of ischemic stroke and the generally more severe outcome of hemorrhagic stroke, indiscriminate widespread use of vitamin E should be cautioned against."[2,3] In addition, high doses of alpha-tocopherol (over 400 mg/day) may prevent absorption of the other Vitamin E fractions and

increase oxidative stress.[4]

Yet population studies show that individuals with diets high in Vitamin E fare better, with lower cardiovascular disease and mortality. A consensus is emerging that we should follow nature—she offers us eight different fractions of Vitamin E, and all of them are good for us.

Because alpha-tocopherol has its own transport protein, it is preferentially absorbed, and when it is given in excessively high doses, it can interfere with absorption of tocotrienols. But does alpha-tocopherol actually prevent us from absorbing tocotrienols, period? According to Chandan Sen, concerns about alpha-tocopherol are primarily based on a 1997 finding that "the transport system, TTP, responsible for carrying tocopherol to vital organs has a poorer efficiency to transport tocotrienols to tissues. The lack of relative specific affinity of TTP for tocotrienols led to the 'urban legend' that availability of dietary tocotrienol to vital organs is negligible."[5,6]

Yet biology is never that simple. It turns out that nature didn't just create one pathway by which Vitamin E fractions could do their work. New research shows there are other, as yet undiscovered pathways by which tocotrienols can be absorbed. This was stunningly obvious when a study of mice without the TTP gene (thus unable to efficiently absorb alpha-tocopherol, and theoretically, unable to reproduce), had their fertility restored. How? By supplementing with alpha-tocotrienol. The tocotrienol was successfully delivered to the relevant tissues and the mice reproduced. When scientists discover that as-yet-unknown pathway for tocotrienols, a whole new branch of research may open.

In addition, tocotrienols can impact biology at far lower doses than tocopherols—a little goes a long way. As Chandan Sen notes in his interview elsewhere in this issue (see pp 9), alpha-tocopherol can be measured in micromolars in blood, while tocotrienol can be found in the nanomolar range—a thousandth less, and yet potent and effective. "It is clear," says Sen, "that natural isomers of vitamin E do get transported to vital organs even in the absence of TTP."

Absorption and excretion of tocotrienols can be an issue, however. According to a 2012 review from the Institute of Biological Chemistry and Nutrition, the low bioavailability and rapid excretion of tocotrienols represents a major hurdle in their preventive use…bioavailability may be enhanced by ingestion with a high-fat meal, self-emulsifying delivery systems, or phytochemicals that inhibit their metabolism and excretion.[7] "In a study investigating the pharmacokinetics and bioavailability of tocotrienols under fed and fasted conditions in eight healthy volunteers," Chandan Sen reports, "the postabsorptive fate of tocotrienol isomers and their association with lipoprotein subfractions were examined." Peak plasma concentrations of tocotrienols were two to three times higher using self-emulsifying blends than the peak concentrations reported in previous studies using generic tocotrienols.[6,8,9]

The take-away? All isomers of Vitamin E have benefit. Although alpha-tocopherol has preferred absorption in the body, tocotrienols have another, as-yet-unidentified way of delivering themselves to cells. In addition, tocotrienols are potent at lower doses than tocopherols. Finally, special delivery systems protect tocotrienols further from potential competitive inhibition by alpha-tocopherol, and insure a consistant and increased absorption of tocotrienols.

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Exciting New Research
An Update from Barrie Tan, PhD

In 2008, *Focus* featured **Barrie Tan, PhD**, on tocotrienol research. Here, Dr. Tan updates us on advances since that time. Dr. Tan is the senior editor of Tocotrienols: Vitamin E Beyond Tocopherols (AOCS & CRC Press; September 2012), and organized the 2nd International Tocotrienol Symposium in conjunction with the 103rd Annual Meeting of the American Oil Chemists' Society in 2012.

Focus: What's new since we talked in 2008?

Tan: There has been a veritable explosion of tocotrienol research. We now have positive clinical studies on bone resorption, cancer, and more studies on cholesterol, to name a few.

Confirmation of tocotrienols' reduction of triglyceride levels has been repeated.[1] There is also increasing evidence that tocotrienols have a beneficial effect on non-alcoholic fatty liver disease.[2] The American Diabetes Association, a formal organization, finally recognizes that fatty liver is common in diabetic and pre-diabetic patients.

Bone resorption is another exciting area. Numerous animal studies have shown that tocotrienols prevent resorption. In animals, tocotrienols also reversed smoke-related and steroid-induced bone mineral density loss, and healed fractured bones faster.[3-7] Recently a good, placebo controlled study on post-menopausal women with osteopenia, which is bone thinning, has just been approved. It will take place at Texas Tech University, Lubbock, TX. There are also going to be cell line studies to determine just how tocotrienol promotes the longevity of the osteoblast (which forms new bone), and downregulates the osteoclast (which breaks down bone).

Another exciting new area of work is in control of adverse inflammation. Dr. Asaf Qureshi, who first differentiated tocotrienol's cholesterol-lowering function from tocopherol function in the 1980's, is now giving his attention to inflammation. He has tested dozens of compounds and found several that have substantive anti-inflammatory properties.[8] They include riboflavin, delta-tocotrienol, quercetin and resveratrol. In his 2012 paper, he tested healthy seniors, who have elevated levels of inflammation compared to children and young adults. After taking a combination supplement with the above anti-inflammatory compounds, their levels of c-reactive protein, nitric oxide, uric acid, and total cholesterol were significantly decreased.[9] Elsewhere, Qureshi has been testing cell line and animal models, while studying inflammatory mediators like Nf-KappaB. Others have examined Cox2 down-regulation.[10-13]

In addition, new data on cholesterol confirm our earlier data. Lipids will drop on tocotrienol. Our studies show that a combination of delta and gamma tocotrienol is potent.[1]

All in all, it's been an exciting five years since we last talked!

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Magnesium Stearate: A Safe and Effective Filler

Setting the Record Straight

An Interview with Dr. Dana Myatt, NMD

Note: Magnesium stearate, a common "inactive" ingredient in many nutritional supplements, has been critiqued by several nutritional supplement companies and physicians online. It has been compared to chalk, said to impair absorption of nutrients, to form a harmful "biofilm" in the intestines, and even to suppress immune function. Since magnesium stearate is an important flow agent that is widely used in the nutritional industry, we felt it important to carefully examine these claims. We found that they were all baseless. After watching a thorough, highly credible and scientifically referenced video by Dr. Dana Myatt on magnesium stearate, available at https://www.youtube.com/watch?v=Fx_ISfVVGuQ, we invited her to a question and answer session with *Focus*. – Stephen Levine, PhD

Focus: What exactly is magnesium stearate and what is its role in nutritional supplements?

Myatt: Magnesium stearate is a simple salt made of two common nutritional substances, the mineral magnesium and the saturated fat stearic acid. It is used as a "flow agent" in many nutritional supplements and pharmaceuticals. Magnesium stearate contains two molecules of stearic acid and one molecule of magnesium. The molecule is held together by ionic bonds — the definition of a salt — that break apart easily in acid, the condition found in the human stomach. Though the name may make it sound like a synthetic, space-age molecule, both magnesium and stearic acid are abundantly available in many foods in our diet. In order to really understand magnesium stearate, let's look at its two components.

Magnesium is an essential mineral, the major mineral most likely to be deficient in the American diet.[1] I don't think anyone would argue the safety of magnesium.

Stearic acid is a saturated fatty acid found in many foods including eggs, chicken, grass-fed beef, coconut oil, walnuts, cheese, chocolate, salmon and human breast milk, to name just a few.[2]

Both magnesium and stearic acid are not only safe, they are beneficial to human health.

Magnesium stearate is simply a salt that combines both of these molecules.

Focus: Magnesium stearate is used as a flow agent in countless nutritional supplements. What is a "flow agent" and why is it necessary?

Myatt: Flow agents help ensure a consistent dose of product in each capsule. Magnesium stearate does this by preventing individual ingredients from sticking to each other and from sticking to the encapsulating machines. It allows manufacturers to create a consistently homogenous mix, so the amount of active ingredients is the same from capsule to capsule or tablet to tablet. In other words, the use of magnesium stearate and other flow agents helps ensure consistency and quality control.

Focus: Some companies claim that they do not use flow agents or other "inert ingredients." Is that a good thing?

Myatt: This might sound good to consumers who do not understand the nuances of good supplement manufacturing practices. The truth, however, is that companies that don't use flow agents are more likely to have inconsistent doses of ingredients in each capsule or tablet. Almost all drugs and supplements contain inactive ingredients. These "inactives" serve multiple purposes. Flow agents, as we discussed, help ensure consistent dosing in each tablet or capsule. Some products contain fillers like cellulose which acts as a binder in tablets and helps fill out the size of tablets or capsules. Herbs can be encapsulated and additional herb used as filler, so these products may contain only "actives." So yes, it is possible to make capsules or tablets without inactive ingredients, but quality control becomes more difficult.

Focus: We have seen claims on the internet, on videos, that magnesium stearate suppresses immune T-cell function and causes the collapse of cell membrane integrity in helper T-cells. Is there any scientific support for this?

Myatt: Not unless you're a mouse.

Focus: Not unless you're a mouse?

Myatt: This claim about immune suppression is based on the gross misrepresentation of a single mouse study. Here's the "Cliff notes":

The entire claim is based on a single study — that's right, *one study* — performed in 1990, using mouse T-cells in a Petri dish. When mouse T-cells were incubated (read that as: "soaked") with stearic acid — not magnesium stearate, but stearic acid — there was indeed a collapse of the cell membrane and a loss of T-cell function.[3] This study was never repeated.

But here's the factoid that magnesium stearate naysayers conveniently "forget" to mention. Mouse t-cells are known to lack the delta-9 desaturase enzyme that converts stearic acid into the perfectly healthy fat, oleic acid. This was mentioned right in the same mouse-cell study. Mouse T-cells can apparently become toxic from high levels of stearic acid, at least in a Petri dish and at levels far above what could ordinarily be achieved from diet. That has nothing to do with humans. Human T-cells *have* the delta-9 desaturase enzyme that converts stearic acid to oleic acid, so human T-cells don't develop any toxic build-up when exposed to stearic acid.[4]

Bottom line: Mice lack an enzyme in their T-cells that humans have, so stearic acid is toxic to mouse T-cells and not to human T-cells.

Focus: What about the difference between stearic acid and stearate?

Myatt: Stearic acid is one of the most common saturated fatty acids found in nature.[5] The term "stearate" is used when stearic acid is part of a salt (as when stearic acid combines with magnesium to form magnesium stearate). The terms stearic acid and stearate can be used interchangeably.

Dr. Dana Myatt is a graduate of the National College of Naturopathic Medicine and has been in multidisciplinary, full-scope family practice for 23 years. She is the founder and CEO of Myatt Nutritionals and is author of "A Physician's Diary" (A.R.E. Press, 1994) and the upcoming "Ketone Zone Diet." She is a member of The American Association of Naturopathic Physicians, the Arizona Naturopathic Medical Association, the American Academy of Anti-Aging Medicine and the American Society for Reproductive Medicine

Focus: How much stearic acid is generally contained in supplements?

Myatt:

- The daily adult intake of stearic acid from food (US adult) averages about 7,000 mg/day.[6]

- A person taking 20 vitamin capsules weighing 500 mg each and containing 1% magnesium stearate would take in less than 96 mg of stearic acid per day. Manufacturers typically use 0.25% - 5% magnesium stearate in nutritional formulations.

- The amount of stearic acid from supplements in the above scenario is 1.3% of the total daily adult intake.

- Four ounces of human breast milk contains more than 5,000 mg of stearic acid. A 2-ounce chocolate bar will also provide well over 5,000 mg of stearic acid.

- Magnesium stearate is considered safe for human consumption at levels below 2,500 mg/kg per day. This equates to 170,000 mg per day as a safe dose for a 150-pound adult.[7] That's almost 6 ounces of pure magnesium stearate.

Focus: One claim we've seen is that the addition of magnesium stearate to supplements decreases bioavailability. Another claim is that magnesium stearate is a chalklike substance that gums up your intestines and prevents absorption of your nutrients. Fact or fiction?

Myatt: What the studies *actually* show is that absorption might be slowed somewhat but overall absorption is not decreased.[8,9] And magnesium stearate is definitely *not* a chalk. Chalks are soft, stone-like minerals, including things like gypsum (calcium sulfate) CaCO3 (calcium carbonate) and CaO (calcium oxide). Remember that magnesium stearate is a *salt*, containing approximately 96% stearic acid, which is a saturated fat. The other 4% is magnesium. Chalks are combinations of minerals, but magnesium stearate is mostly saturated fat. How could a fat be a chalk? It isn't, not by any known scientific definition of a chalk.

Even if it *were* a chalk, you shouldn't be worried about it gumming up your intestines or "caking the lining." Why? Because if you *did* eat chalk, like calcium carbonate, which is a form of calcium used in many nutritional supplements, your digestive system would break it down to its mineral components. Human digestion is truly amazing.

By the way, I used to perform quite a few endoscopies in clinic. This is where you examine the lining of the large intestine with a special scope, looking for polyps. I never once saw anyone with a "caking of the lining" of their intestinal tract. Tenacious, dry stool sometimes, yes. But "caking" with a chalk-like substance? Never. If this story about "caking the lining" is told by a doctor, it must be one who has never actually visualized the inside of the large intestine.

Focus: No T-cell collapse in humans, no "caking of the lining" of intestines. How about the claim that magnesium stearate stimulates the gut to form a biofilm? And that a biofilm is equivalent to a sludge that would act as a barrier to the absorption of nutrients?

Myatt: There is not one single scientific reference or study to support this claim. In fact, if you know what a biofilm is you'll see that this entire argument is completely preposterous. It has no basis in any known science.

To clarify for those readers who haven't seen it, there was an internet video by a well-known doctor that said that a biofilm is basically like the "sludge in your toilet tank," and that magnesium stearate *causes* this sludge and prevents nutrient absorption.

Just as there is a big difference between a chalk and a fat, a biofilm is not akin to sludge, or a "soap scum" that you might find in your bathtub (a toilet tank just holds water and won't even form a soap scum ring). A bathtub ring occurs when hard water, containing calcium or magnesium, reacts with fatty acid in soap to form so-called "soap scum". If you live in a hard-water area, you'll see this as an annoying white film on your shower curtain. Humans get significant amounts of magnesium, calcium and fatty acids — the ingredients in soap scum — from diet. But we don't form soap scums in our bodies because of our digestive enzymes and acids.

Further, soap scum is *not* biofilm — not even close.

Biofilms are layers of bacteria or yeast embedded in the gel-like substance they secrete. They tend to be highly antibiotic-resistant. As to the claim that stearic acid *causes* biofilms, this is completely without any scientific evidence. In fact, several studies have shown just the opposite — stearic acid actually helps *prevent* the formation of biofilms.[10,11]

Focus: Next claim. Magnesium stearate is made from contaminated oils from genetically engineered crops. True?

Myatt: Magnesium stearate is most commonly sourced from cottonseed oil or palm oil and it's true that cotton can be a GMO crop and is typically high in pesticides. But even if the starting cottonseed oil is contaminated, the finished product, stearic acid, is so highly purified that contamination really isn't an issue. Cottonseed-derived stearic acid is so purified and the final molecule so far-removed from the original source, it doesn't carry any pesticide residue. We might as well worry about the food-grade additive cellulose, which is also obtained either from wood waste (we call that "sawdust" out here in Arizona) or cotton waste (known as "gin trash," — the waste cotton remaining in the cotton gin).[12] Just like taking dirty water and purifying it into something clean and drinkable, purifying cottonseed oil to obtain stearic acid delivers a pure finished product. And by the way, stearic acid can also be derived from palm oil, which many manufacturers use as the source of their stearic acid.

Focus: Is magnesium stearate often contaminated during processing, as one doctor claims?

Myatt: Contamination during the manufacture of supplements or pharmaceuticals can occur anywhere along the entire manufacturing process. That is why quality supplement manufacturers test raw materials upon purchase; during manufacturing, processing, and also test the final product. Raw materials can occasionally become cross contaminated, and that's why quality manufacturers employ so many tests and inspections all along the process.

Now, is magnesium stearate one of the substances more likely to be contaminated? Absolutely not. There is *one* reported instance of a raw materials manufacturer notifying the World Health Organization that several batches of magnesium stearate had been cross contaminated with zeolite (sodium aluminum silicate), calcium hydroxide, and several other substances. The contamination was determined to be due to incomplete cleaning of air milling equipment. This was traced to a *single* raw materials manufacturer and was an isolated event. Moreover, WHO found the contaminating substances to be present in such minute amounts that they posed no health risk. And this was self-reported by the manufacturer of the raw material before it was used in product.[13]

Focus: Is magnesium stearate going to be removed from supplements by the Codex Committee on Food Additives?

Myatt: No. The Codex Committee considered removing magnesium stearate from the acceptable *food* list, not because of any danger, but because they didn't see the use for it in food. They were simply trying to trim up their list of allowed food additives. When food manufacturers pointed out that magnesium stearate is an important and safe anti-caking agent, it was reinstated. Removing magnesium stearate from Codex for use in nutritional supplements has never been considered as far as I can determine.

Magnesium stearate is currently approved by FDA regulations for use in food and supplements.[14]

Focus: You've done a brilliant job of going back to the actual peer review science and dismantling every specious claim about magnesium stearate. Any final thoughts?

Myatt: I don't always agree with my colleagues in the nutritional supplement industry. Some ingredients and doses based on the scientific literature are arguable. With most issues in medicine, there is no black and white. There is instead, "ten thousand shades of gray." In many instances, there is evidence on both sides of the question.

But the evidence is *not* "mixed" on the safety of magnesium stearate. The evidence says that magnesium stearate, a simple salt of magnesium and stearic acid, is a safe and effective flow agent that helps maintain dose consistency, and there really isn't *any* evidence to the contrary. At least I haven't found any, and I've looked long and hard at these claims because I, too, manufacture nutritional supplements and use magnesium stearate as a flow agent. So I *had* to know if any of these claims had even a shred of basis in fact. They don't.

Right now, the damning claims for magnesium stearate are completely without scientific verification or substantiation.

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How Magnesium Stearate Fell Prey to the Nocebo Effect

By Michael Ash, BSc, DO, ND, F.DipION

For over 40 years magnesium stearate, also known as octadecanoic acid and magnesium salt, has been used in the manufacture of food and pharmaceutical tablets and capsules. It is a plant based fatty acid and magnesium combination, consumed globally by billions of people each day, with no identified or scientifically recorded adverse health effects.

Halo Effect

The suggestion that magnesium stearate presents a problem for human health should be based on substantive, reproducible and testable explanations. But that is not the case. Certain opinion leaders seem to have attracted unquestioning support for their 'adverse effects' hypothesis about magnesium stearate based on the 'halo effect'; this is the phenomenon whereby we assume that because someone is so good at doing A, they will be good at doing B, C and D.[1]

Nocebo Effect

In the case of magnesium stearate, nocebo effects may have been generated by inappropriate and utterly unsubstantiated emphasis on risk by just a few

opinion leaders in the supplement industry. Nocebo effects are adverse events produced by negative expectations and represent the negative side of placebo effects. In effect people may have attributed certain symptoms, real or imagined, to their intake of magnesium stearate because they believe the unsubstantiated criticisms of its use in food supplements and medicines. As with their placebo counterpart, nocebo responses demonstrate the powerful interaction between mind and body.[2,3]

The alleged negative and undocumented "risk" of magnesium stearate has been so well distributed in the internet arena that I believe it is now genuinely responsible for a 'nocebo' effect. Nocebo effects and placebo effects that have qualifiable impact on the functionality of a patient are the direct result of the psychosocial context or therapeutic environment on a patient's mind, brain, and body. Both phenomena can be produced and amplified by multiple factors, such as written opinion, verbal suggestions and past experience. These not only have relevance in clinical management but also complicate perceptions of validity when emotionally connected to unsubstantiated hypotheses.

A fine balance must exist between communicating important nutrient information and ensuring that every attempt is made to minimize unsubstantiated claims. So, the facts about magnesium stearate:

Magnesium Stearate Facts

Magnesium stearate exists as "plate-like" crystals (or lamellae) stacked together like a deck of cards. As the blending of food ingredients or drugs proceeds, plates continuously shear off and coat adjacent particles of ingredients. The higher the concentration of magnesium stearate used or the longer this blending continues, the more complete this coating of the adjacent particles will become. For effective lubrication, you do not need and, in fact, do not want to coat everything too completely with the lubricant as it alters the speed at which the tablet and capsule contents are made bioavailable. So the approximate 1% use of magnesium stearate in supplements and medicines is not only safe but produces an effective and bioavailable food supplement with consistent dosing across all batches. The FDA states: magnesium stearate is generally considered safe for human consumption at levels below 2,500 mg/kg of body weight per day. So, for instance, 1% of a 1000 mg pill would be 10 mg. As with all professional food supplements, concentration, grade and mixing parameters must be carefully controlled and respective food supplement companies will apply their own ethos to these as well as meeting good manufacturing compliance.

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A New Perspective: Why I Believe Magnesium Stearate is Safe

Dietrich Klinghardt, MD, PhD

In 2009, I was interviewed by Joseph Mercola, MD, regarding my concerns about fillers and inactive ingredients in supplements. My patient population is often highly sensitive — I see many patients with chronic Lyme disease, chronic fatigue syndrome, and multiple chemical sensitivity. These patients often literally arrive at my office with shopping bags full of supplements.

It is a painstaking and necessary process to go through all these supplements one by one, in order to determine which ones are actually necessary and useful for the patient. An allergic or highly sensitive patient may actually get sensitized to a supplement that they need, if they are taking too much, too often. Dosage is very important. In some cases, an individual will be consuming literally hundreds of capsules a day, desperately trying to get well and feel better.

It would be nice if my patients — and in fact, all of us — could get the nutrition we need from food alone. And yet we know that most of us who live in modern society burn up a lot of micronutrients in the course of our daily lives and are dependent on good supplement program and protocol. We rely on the companies we trust to provide those supplements in a safe and effective way, and I'm very happy to say that the company I have worked longest with since I came to America in 1982 is Allergy Research Group. They work hard to try to ensure their supplements are not allergenic. When I was practicing in Germany and supplements first became available over the counter, there was not good quality control. There might be very little of an active ingredient, and too much filler. I have always felt that Allergy Research Group was careful about their inactive ingredients, using as little as necessary to assure quality control and even dose.

At the time that Dr. Joseph Mercola interviewed me, I had seen a study showing that in mice, stearic acid could build up and harm T-cell function. This was an interesting study, but it was *in vitro*, and with mice. In hindsight I gave too much credence to it. It turns out that in mice T cells, stearic acid, when applied directly to the cells may be harmful, but human cells have an enzyme, delta d-saturase, that converts stearic acid to oleic acid, a monounsaturated fat that is well tolerated and of value to health. I am not a biochemist or immunologist so did not understand or have the knowledge several years ago that humans could safely and effectively convert stearic acid. Now that I have reviewed the scientific literature more deeply, I no longer have concerns about stearic acid and T-cell function in humans.

In addition, my review of nutrition data shows that stearic acid is abundant in many of the healthy foods we eat. The backdrop of stearic acid in our diet is actually far larger than that we would ingest in our supplements daily. It really is negligible compared to the amount we naturally consume, and so magnesium stearate as a supplement lubricant and binder really is not a relevant concern for me anymore. Since stearic acid and magnesium are both part of our natural food chain, anybody sensitive to magnesium stearate will also be sensitive to the foods with these molecules.

As a physician taking care of such sensitive people I have always wanted to err on the side of caution. And frankly, when I have really looked at the stearate content of supplements available, I feel very confident that the amount utilized in Allergy Research products is reasonable. Digging deeper into the literature on stearic acid has put any concerns I raised some years ago completely to rest.

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