Dr. Bruce Ames Presents

His Triage Theory of Aging at the American Academy of

Anti-Aging Medicine (A^4M)

By Dr. Bruce Ames

Introduction:

I'd like to welcome everyone to today's lecture in this track, Innovation and Anti-aging Medicine. It's with great honor that we introduce a rather famous professor, Dr. Bruce Ames. I first read about his work, when I worked with Nathan Pritikin nearly 30 years ago. He had developed some novel test, including the Ames test to help detect carcinogens.

Dr. Bruce Ames is a Professor Emeritus at U.C. Berkeley. He's senior scientist at Children's Hospital Oakland Research Institute. He's received numerous prizes, including the U.S. National Medal of Science, and he's among a few hundred most cited scientists in all fields with over 540 publications. His talk today is "Moderate Deficiencies of Vitamins and Minerals Accelerate the Diseases of Aging." I give you Dr. Bruce Ames. Give him a big hand.

(Applause)

Dr. Bruce Ames: Thank you. It's a pleasure to be here. And I'd like to tell you why eating a bad diet ages you more rapidly, and I think that applies to most of the world.

This is your metabolism. To run your metabolism, what do you need? You need fuel.So you eat carbohydrate and protein, and you burn it to make ATP. So, you're pulling four electrons off your fuel, adding them to oxygen to make water. That's the process that generates kilos of ATP in your body every day.

But you need something else. You need about 40 micronutrients. These are substances you need to get in your diet to be coenzymes from all these different pathways. Well, about 30 vitamins and minerals, about 15 minerals and about 15 vitamins. Then you need some essential amino acids to make your protein and a couple of essential fatty acids.

You don't get any one of these, you'd die. So the whole purpose of a balanced diet is to get all of them.What I'm going to tell you is that when you don't get enough, you're trashing some metabolic pathway. I'll explain how nature works on that.

These are the micronutrients. The ones I'll concentrate on. (Let's see. Is this on? I need a laser pointer. It's not very bright, but anyway.) The middle column is about… There

are about 15 minerals and about 15 vitamins. Those are the ones that I'm going to concentrate on. You don't get anyone, you'd die. But how much do we need? I think we don't have a clue.

The committees tell you EARs and RDAs, but it's all based on short-term. What I'm going to tell you is what happens when you're short of any vitamin or mineral. It's accelerating your aging in some particular way. (Thank you. Okay.) These are the vitamins, minerals, and then the essential amino acids, then omega-3s, and omega-6s. Okay. Are we getting enough? And by the current standards, we're not. The standards are going to be different when we start looking at long-term basis.

The two numbers most of you probably know that the official committees set – the EAR and RDA. The EAR is some distribution in the population. If you're below the EAR, that's the measure of inadequacy. So, they say that you're deficient if you're below the EAR. Then they set the RDA at two standard deviations above the EAR. Really, the EAR is the key number. It's all based on short-term. But even on that, we know menstruating women are losing a lot of iron, and 16 percent of American women are below the EAR for iron.

Magnesium, we're talking about 56 percent of the whole U.S. population. Where do you get magnesium? It's in the center of the chlorophyll molecule, so anything green has magnesium. You eat a big plate of spinach, and you're getting your magnesium. Here's to spinach. And then, nuts are very healthy food. Nuts have a fair amount of calcium and magnesium in them. But we're clearly not up to snuff.

Zinc is in 2,000 proteins with zinc-fingers, copper zinc, SOD. What happens when you don't have enough zinc? And we're talking about 12 percent of the population. This isn't a part per billion of pesticide that hypothetically might do us some damage sometimes (there's no real evidence that it does). But we're talking about some appreciable percentage of the population.

And vitamin B6, with elderly women, it's close to half of them. Folate – now they're fortifying the flour with folate, but still, we have a sizeable percentage. Vitamin E, it's practically everybody. Vitamin C, it's about a third of people. Then they haven't set EARs or they just did for vitamin D and calcium(but I haven't changed my slide yet.) We're very low in vitamin D, vitamin K, calcium, potassium (and I can't read that from this angle, but whatever it is…).

Anyway, so we're not eating a healthy diet. We're eating too much refined food with sugar. The leading source of calories in the United States is sugary soft drinks – 40 grams of sugar and no nutritive value. We're starving. Even though we're all getting fat, we're starving for vitamins and minerals. So, what happens when you're low? That's what I have been working on and I'd like to tell you.

These are some red blood cells and an occasional white cell. You all know that red blood cells don't have any DNA. But if you take a mouse and you stain it with a fluorescent reagent for DNA, one in 2,000 red blood cells lights up. It turns out that when you extrude the nucleus to make the red blood cell, if there's a chromosome break, a little piece of chromosome gets left behind. And you can stand for it. You radiate the mice. You get a nice dose-response.

A fellow named Jim MacGregor back in 1980s was looking at this micronucleus test in mice, and looking at radiating them and getting dose-response. He was looking at the effect of caffeine on DNA repair. He came to my lab on sabbatical, so I learned all about this stuff.

One day, he accidentally found that somebody had left a vitamin out of the vitamin mix, and all his controlled mice were trashed. They were all full of chromosome rays. He tracked it down. The missing vitamin was folic acid. He did a dose-response on folic acid. And it was just like radiation. So he thought, "That was pretty interesting."

He wanted to do it in people, but it doesn't work in people. The reason it doesn't work is the human spleen(which isdown here somewhere.) Its function is all the blood cells go through the spleen, and anyone that's a little stiff gets filtered out, because the body is trying to prevent a stiff red blood cell clogging up a little capillary somewhere and causing a thrombosis. But in bred mice, that have lost splenic function. They don't have any spleen that works. So, you can do this test in mice, but you can't do it in people.

Anyway, MacGregor didn't stop there. Once he found this folate thing, he went to Kaiser – a local health making organization – and found that they have hundreds of people who have lost spleens in an accident beside millions of people in their database. They gave him permission to contact these people. He found 20 people without splenic function who would give him some blood. He did that to set up the baseline of chromosome breaks in people. This experiment is really an amazing experiment.

He set up the baseline down here. But out of those 20 people, one person was over 20 times higher than everybody else. He had lots more chromosome breaks. MacGregor thought that was interesting, and he followed him for a whole year. (I have compressed the year here.) As you can see, this guy went up and down, but he was always very high. Then when MacGregor stumbled on this folic acid deficiency that breaks chromosomes in mice, he went to this guy and measured his folic acid.

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It was low, but not wildly low. It was at the level that half the poor had and the level that 10 percent of the U.S. population had. He thought that was pretty interesting, so what he did was he gave him some folic acid for a couple of weeks. And he measured folic

acid in the blood, in the red blood cells, and all of that. As you can see, the reticulocytes – which are the newly synthesized red blood cells, the one percent of the population pulls them out with magnetic beads – in a day or two, they were right down to baseline.

What's nice about this is it establishes causality where epidemiology doesn't. Then, red blood cells take time to turn over, they followed along, and they eventually came down to baseline.

Since he had only given it for a short period and this guy wasn't eating a very good diet, soon they started going up again. He thought that it would be unethical to do any more experiments on this guy. He said, "Eat a better diet or I'll give you a folic acid pill." The guy said, "Give me a folic acid pill." He gave him one and he said, "Take it forever, unless you're improving your diet." So here's… Again, they went right down to baseline.

What's beautiful about this experiment is that you know the level of folic acid that does this, because he measured that. It's only in one person, but it established the causality. Epidemiology basically sucks. It's just way too difficult. It's a treacherous field. There are some good people (and I don't want to damn epidemiologists), but it's very expensive and you're establishing association. Over half the time they get the wrong answer. But they think that they're getting the right answer mostly. It's messed up nutrition into something awful.

Anyway, this establishes causality. So when people give a vitamin pill to lots of people, follow them, and say, "Oh no. It doesn't decrease your cancer rate. It has nothing to do with cancer," that's a lousy experiment. It's not designed to detect what they're trying to detect. If you understand mechanism, then you can do things. I'll show you how you understand mechanism, and now we can tackle these problems.

Some years later, we and Fenech in Australiacompared micronutrient folate deficiency with radiation. If you go down a little bit than folate, you start breaking your chromosomes just from radiation. Broken chromosomes are the most dangerous aspect of radiation, and everybody knows that it leads to cancer and lots of other bad things. We're worried about incredibly low levels of radiation and not worried about the fact that everybody in the United States is eating such a lousy diet. They're not eating their greens and therefore, they are trashing their chromosomes.

We helped MacGregor work out the mechanism. That paper I showed… That figure from that paper I showed was in 1988, and this paper is buried in the literature. Nobody knows about it. One the reasons – I think – was that to do experiments in people, you need people without spleens. But we have figured out a way around that. I'll tell you about that. Anyway, we helped MacGregor work out the mechanism.

Folate has two main pulls: methylene tetrahydrofolate and methyl tetrahydrofolate. This one methylates the dUMP to dTMP to make your DNA, uracil in RNA, and thiamine in DNA same base pairing. We measure uracil in DNA. And you start incorporating uracil in DNA when you're folate-deficient. Then repair enzymes are always taking it out, so you may nix and break over your DNA.

Three or four types of cancers have been associated with folate deficiency.

Methyl tetrahydrofolate methylates homocysteine to methionine. Methionine goes back as a density of methionine, which is the main methylating agent in the cell that's moving one carbon unit around. It's used in all kinds of things – methylating DNA or methylating lipids. Low or high homocysteine, which accumulates under conditions where you low in folate or low in vitamin B12, is associated with cognitive decay and with heart disease. There's really starting to be quite a large literature on both heart dysfunction and cognitive dysfunction.

What about this enzyme that converts them? First of all, why methylene tetrahydrofolate reductase? There are lots of polymorphisms in this gene in Northern Europe. What that polymorphism does is increase the size of this pool at the expense of this pool. But cancer and heart disease come too late in life to really be a strong selective factor.

It's easy to imagine that the people up in Sweden didn't have a lot of folate, which you get from greens. Folium is the Latin word for leaf. But why would you have a polymorphism for these long-term diseases?We thought that it was likely to be sperm. And we published a paper showing that when you don't have enough folate, you're putting uracil in your DNA in sperm. So that nature really cares about.

Radiation gives you clusters of electrons. And when you get twonearby lesions on the opposite strands, then the repair enzymes seems start coming along.Clip this outand putit in the right base.Clip this out and put it in the right base. But if it breaks the chromosome – which it does fairly often – then you're in trouble, because it's hard to put it all back together. All the radiation biologists have worked out that these double strand breaks are the most dangerous part of radiation.

When MacGregor did all this, it got me interested in vitamins and minerals. Because if the shortage of one vitamin is affecting half the poor and 10 percent of the population and is breaking a chromosome just like radiation, that sounds important. And I wondered whether there are other cases of these things going on.

We looked at iron, next. I did this with a professor of nutrition at Berkeley, Fernando Viteri. He was interested in too much iron. Now, too little iron is the main vitamin and mineral deficiency in the world. Two billion women and children aren't getting enough iron. The women are menstruating, and the kids don't do well in school. The rat pups

don’t do well on that rat IQ test, and the mouse pups don’t do well on mice IQ test. So, it hurts your brain if you don’t have enough iron, among other things.

Then, Viteri was complaining that the World Health Organization was giving too much iron to people. Iron’s rusty nail – it’sdirt cheap. One of his graduate students and one of my post-doc did the whole range of iron in mice. Too much iron turns out to be bad for you, too.

This is all the different things that happen to your mitochondria. Too little iron trashes your mitochondria. Too much iron trashes your mitochondria. You really should be in here somewhere.*[Points at PowerPoint presentation]*Up is bad in this. One of these is mitochondrial deficiency, another one is oxidative damage to mitochondrial DNA, and another one is producing more oxidants. All these things happen to mitochondria.

Metals are very tricky. People think that if a little bit of vitamin is good, more is better. Mae West said, “Too much of a good thing is wonderful.” But she was saying that about sex, not vitamins and minerals.*[Crowd laughing]* Particularly, minerals are tricky because iron looks very much like copper and zinc. If you put in too much iron, you hurt copper and zinc. It interferes with getting in and all of that.

Calcium and magnesium look very much alike. They’re right above each other on the periodic table. If you get too much calcium, that’ll trash some magnesium enzyme and vice-versa. The body really wants about, two calcium’s to one magnesium. So all you supplement makers, don’t make magnesium pills and don’t make calcium pills. Make calcium-magnesium pills – about two to one. People do sell those pills.All similarly with sodium and potassium. This is just a little side issue, butif it’s a mineral, you really have to be careful about the amount and you have to be careful about ratios.

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Zinc – I had a post-doc, Emily Ho, who came to the lab and who have done a lot of zinc work in human cells and culture. She made human cells and culture a little zinc-deficient. They pour out oxidants. (I won’t go into the details.) They damaged DNA. This is the comet assay. Too little zinc is bad for you. It damages DNA. Too little iron damages DNA. Too little zinc damages DNA. We did three or four other vitamins and minerals in human cells and culture, and we got DNA damage all the time.

I kept on thinking, “Why is nature doing this? This seems kind of stupid to give you a cancer when your short of a vitamin or mineral.” But nature has its reasons. (This is biotin.)Magnesium deficiency crosslinks mitochondrial DNA to protein, and 56 percent of us are short of magnesium. I looked up in Google. I plugged in mutation. I plugged in all the 30 vitamins and minerals, then chromosome breaks, mutation, and cancer. All of this came out.

A lot of these in the literature; you can't take it as "gospel," because it's epidemiology again. You have to really see if it was done right and all of that. But there's a lot of material out there, which says that you damage your DNA and you can get cancer, if you're short of a vitamin or mineral. And I kept on mulling about, "Why is nature doing that?"

Then one day it hit me. It was obvious. Nature wants you to survive and reproduce. That's what the strong selection is for. If it's some gene that's helping you to live a long life, DNA repair, or something like that, nature doesn't care about it, unless when it's a matter of survival. The first priority is survival and reproduction.

So, what we postulated is that the proteins that need that vitamin and mineral for survival and reproduction hang on to it, and the ones that are helping you live a long life span, those lose it. The price that nature is charging you for being short of a vitamin or mineral is it ages you faster in some way, because all the diseases are coming with aging and all these long-term things. That's what I'm going to try and convince you throughout the rest of the lecture.

I published this paper back in 1996, and I can't say that it's attracted a lot of attention. Nutrition is stuck in its way. Most scientists are stuck in their ways. They don't like to change thinking very easily. But we're slowly changing their minds, and soon everybody will say, "It was obvious." Then, I know that I have succeeded.

One day, Joyce McCann, who's a very smart woman and who used to be a post-doc with me many years ago, came back to Berkeley looking for a job. She's super smart, so I hired her. She likes to do theoretical work. One day she came into my office and said, "Bruce, I'm a little skeptical about your triage idea…" (That's what I call this idea.) "And I think there's a better way of tackling it."

I said, "Joyce, go to it. What do you want to do?" So she said, "Let's take those vitamins and minerals that are not too complicated." If you're looking at zinc, you'd spend the rest of your life in the library visiting 2,000 different proteins. And if you're looking at magnesium, you'd spend the rest of your life in.... And vitamin D turns on 900 genes. Those are all way too complicated. Let's pick ones that just affect 10 or 20 proteins – something simple. So, I said, "Terrific. Why don't you go analyze a few of those?"

She published her first paper on vitamin K analysis, her second one on selenium analysis, and now she just finished biotin. But each of these takes almost a year of hardwork. We're looking at it from a different perspective. We're looking at what proteins are essential, what proteins are non-essential but are more long-term, and does nature favor the essential ones? Nature does.And I'll show you that it has lots of medical implications.

Vitamin K was first discovered as a coagulation factor for blood clotting. Coagulation in German – it was some German workers – begins with a “K,” so they called it vitamin K. Okay? And where do you find vitamin K? It’s used in photosynthesis in plants. So, anything green has vitamin K in it. Eat your spinach, again.

But when animals eat it, it gets into the liver,and all the coagulation proteins are done in the liver. Then it’s converted to a compound called MK or menaquinone, and that goes to all the tissues equally.And all these non-essential proteins, which are the interesting ones (we don’t really care about those). They are the ones that we’re interested about, because they’re long-term things. Those are what I call longevity proteins. Those are involved in making you live a long life.

So, I’ll just briefly run through it. Osteocalcin is in the bone. If you knock out the gene, the animals live perfectly well. But if you stress them a bit, the bones break. They make bones – they’re just not strong. Bone fracture is associated with vitamin K deficiency. There are 30 million prescriptions for Warfarin or Coumadin every year to inhibit your blood clotting, so you don’t get thrombosis. Those people are very sensitive to bone fractures. So, it kind of fits; when you look into the human gene, that fits.

Gas6 protein is involved with diabetes and heart disease (I won’t talk about that). Matrix glaprotein is really interesting. Because there, you knock out the gene, the mouse pups are born, they live, and then they suddenly drop dead. What did they drop dead of? It’s calcification of the arteries. It turns out that all of these are calcium-binding proteins, which have two carboxyl groups with this [inaudible 26:55] amino acid. You’re modifying glutamic to a “gla.” They all bind calcium. This protein’s job is to go around to all the soft tissues, and whenever it sees calcium crystalizing out of its hydroxyl appetite (which it does easily), it grabs it and takes it out of there.

In the human gene, there’s a rare genetic mutation that people die of calcification of their arteries in early age with a mutation in that gene. So, everything fits. Vitamin K deficiency is associated with the calcification of the arteries, and people getting Coumadin die at a much higher rate of calcification of the artery. But the docs who give you Warfarin don’t know about all this. This work was all done by a terrific group in Holland. We didn’t do any of the work. We’re just interpreting it in a different way.

And TGFBI turns out to be, they all die of cancer. The mechanism is that your mitosis doesn’t work as well. One understands how it all works. You get more losing of chromosomes here and there, and that leads to cancer.

The nice thing about these is a need to understanding the mechanism. Now you can do epidemiology by measuring [inaudible 28:04] versus [inaudible 28:04]. But it just illuminates everything.

There's a Japanese food called natto. Has anybody eaten natto? Yeah. Does anybody like natto? *[Laughs]* Okay. It's considered a health food in Japan. It looks sortof like a string… It'sBacillus subtilis fermented soybean. It's full of one of these MK derivers – MK7. So if you eat that, you help all these long-term proteins. They sell MK7 as a pill. It's a little yucky because of its long strings. It smells yucky and it tastes yucky, so Westerners just don't like it. But the epidemiologists all say that the Japanese who eat it have very little heart disease. They get very little prostate cancer, and it all sort of kind of fits.

Anyway, then Joyce did selenium and again, it's all these long-term things – cancer, DNA damage, heart disease, reduced resistance to infection, muscle weakness, poor cognitive function, ype2 diabetes. So again, it fits with long-term. The mechanism is really interesting. This is a special tRNA putting in selenocysteine, which is put into protein to make seleno analogue of cysteine. The way it works is that methylation of the last step in making this modified tRNA for the synthesis changes the structure, and its reaction is inhibited by selenium deficiency.

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It's a way of preferentially making the essential proteins, but not these longevity proteins. The interesting thing is that half the proteins turn out to be longevity proteins. Half in the vitamin K and half in selenium, which means that there a lot of longevity proteins.

This got me thinking that there's going to be a whole class of new vitamins and minerals nobody knows about that have to do with these longevity pathways. Nobody would have discovered them, because the vitamins and minerals are all based on short-term effects. Anyway, we think we have one, and we're looking at a few more. So, we'll see.

What have we learned? Well, we have learned that (the benefits of a triage analysis) provides a rationale for why modest micronutrient deficiencies might increase risk of disease of aging, offers a strategy for setting the RDA and the EAR based on long-term things, and it says how to do "Epi" (epidemiology) understanding the mechanism.

I think that it's going to have a big impact on the world, because I think that most of the world is eating a lousy diet. Your diet ought to be based on a balanced diet. Your mom told you that. Eat your veggies, to eat more fish, not so much meat, and all of that. And there was collective wisdom. I think moms who didn't tell their kids to eat their veggies were selected out by evolutionaries. *[Crowd laughs]* Anyway, we'll see if the nutrition community comes along. But I think that we're slowly proving all these.

Last thing on the immune system: if you look up on Google, what goes wrong with the immune system with aging, these six things pop up. We looked at adaptive immunity, and all these things pop up as being going down with age. Then I put those six things

against the 30 vitamins and minerals, and lo and behold, people have done some experiments.

One of them turned out to be a paper of ours that I have completely forgotten about. I had a French-Canadian post-doc in the lab, Chantal Courtemanche. She had found that CD4 and CD8 ratio of your T-cells change with age, and it changes when you're short with folic acid. I didn't understand what it meant, and she didn't understand what it meant, but we published it in the *Journal of Immunology*, and here it came up on the search.

One of the things you do to your immune system when you're running out of vitamin and mineral is you trash the parts that aren't immediately important. You don't remember that virus that hit you four years ago. You don't take your vaccine very well. And it's known that people in poor countries eating bad diets are much more susceptible to disease than Westerners that are eating a better diet. But this gives a reason why.

Anyway, life expectancy is going up and up and up. The top is women, and the bottom is men. Women live longer than men. Every year we increase our life expectancy, except that obesity is as bad as smoking. So, obesity is going to make a difference on this. Now just when we're giving up smoking, we're increasing obesity.

Obesity is linked to every possible disease – all the long-term stuff – because obese people aren't getting any vitamins and minerals. That's one of the reasons they're getting obese. They're eating this horrible diet. So, shape up and eat your vitamins and minerals.

These are the people in my lab, Jung Suh, who's working on this age vitamin. I call it a…This is a vitamin that only affects the long-term things. Joyce McCann did these wonderful reviews. Swapna is a post-doc from India. David does all the minerals at once. Hal is a physician who's helps work in the lab part-time. Sandy works on forensic medicine, but she's looking at mitochondrial DNA mutation by the next generation symptoms. Mark's our expert on the gut. Michelle's a physician, and so is Ash. They're helping on all our clinical trial.

Anyway, thank you.

[Applause]

[END]