January 2007 Issue | S. Jill James, PhD University of Arkansas for Medical Sciences

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Welcome to *Functional Medicine Update* for January 2007. I look forward to the first of every year, and consider how *Functional Medicine Update* will evolve. With this issue, we are starting with a high standard of identity for 2007.

I believe you will find this month to be an unprecedented example of what we consider excellence. We are going to have the longest discussion with the researcher/clinician of the month that we have ever had in our 25-year history. This discussion embodies so much of what *Functional Medicine Update* has been throughout its 25 years. It couples the best of science with clinical insight, and addresses the integration of many different findings from different fields into the understanding of a principal fundamental mechanism that underlies many diseases and cuts across multiple subspecialties of medicine. I think it is that we feel functional medicine provides an opportunity to understand: how traditional diagnostic medicine (with the *sine non quo* being the pursuit of the diagnosis) so often loses the forest for the trees.

For those of you who may be starting new with us this year, let me just quickly tell you a little bit about *Functional Medicine Update.* As I mentioned, Mr. Jay Johnson, my colleague, and I have been doing this now for 25 years-it will be our 25th anniversary as we come up to the middle of this year. As a consequence of this long-standing opportunity, we have had the pleasure, privilege, and illumination of interviewing some of the world's great agents of change in this field of health care and medicine. People who are valiant in what they are doing-warriors and pioneers and trendsetters, who have been mind changers in allowing us to understand the origin of chronic, age-related diseases in different ways that potentially lead to improved patient outcomes.

Functional Medicine Update started off many years ago as *Metabolic Update* , then it went to *Preventive Medicine Update.* Finally it evolved (a little over 10 years ago) into *Functional Medicine Update*, and we feel that the standard of identity for the product has continued to improve over this last decade. Functional medicine has been described as a different way of looking at the etiology and management of disease from a functional perspective.

The management of *Functional Medicine Update* is now through a group called Synthesis. Synthesis is my own company, and Trish Eury is our manager. Our website is www.jeffreybland.com.

The reason the administration of*Functional Medicine Update* was transitioned out of The Institute for Functional Medicine in 2006 is because to keep FMU embedded within an institution that is CME

accredited would have required that this product be edited, peer-certified, and reviewed by an outside review board. Doing this (I think) would have caused us to lose the tone of what we have been trying to achieve over the 25 years, which is for FMU to be Jeff Bland's view of what is happening within the field of health care. Our objective is to connect science with clinical work, and put it into the context of the words of the scientist and clinicians that are doing it. To be bridled by the guidelines of peer oversight through the ACCME and to try to define it as Category I credit would be different from this objective and alter how FMU provides early assessment and understanding of information that is on the front edge of evolution in clinical medicine as driven by science.

It is with this in mind that we have now taken over the responsibility for the content, editing, and format of *Functional Medicine Update*. You will see, I think, some interesting changes, starting with a feature called "Hot Breaking News" that will lead off every issue.

For Hot Breaking News this month, I want to focus on three quick topics. The first, which has been heavily in the news, is the vitamin D question. How much vitamin D do people need? We spent a lot of time talking about the vitamin D connection. I have interviewed Michael Holick, Colleen Hayes, and Robert Heaney, all of whom were helpful in helping us to understand much more about vitamin D and its connection to many different conditions.

Vitamin D Status: Evidence Suggests the Optimal Level Should Be Increased
We now recognize the best current assessment method for vitamin D status is the level in the plasma or serum of 25-hydroxyvitamin D3. These variables help us to better understand what level of vitamin D intake the patient needs to maintain proper function. It has often been said that anything above 20 nanomole per liter (nmol/L) is satisfactory. I think it is now being more and more recognized that this level (at the low end of normal reference range) is actually suboptimal, relative to physiological function for most people, and that the clinician should try to achieve a range between 40-55 nmol/L in order for the patient to have better function. This is what the evidence suggests. Some investigators suggest that clinical conditions may improve at levels up to 80 nmol/L, but I think the data from the literature would support that a target level should be in the 40 to 55 nmol/L.

For those of you who would like to read more about this, a nice review paper on this subject appeared in the *Journal of the American College ofNutrition* in 2006. [1] I would also suggest that one needs patience, as a clinician, to raise the level of 25-hydroxyvitamin D3 in a patient's serum; it may take several months on supplementation of 2000 or more IUs per day of vitamin D3 in order to raise that 25-hydroxy level to 40-55 nmol/L range.

The toxicity of vitamin D is related to the hypercalcemic effect, and to excessive levels of the hormonal form, 1,25-dihydroxy cholecalciferol. The marker for that is the plasma or serum level of the 25-hydroxy cholecalciferol. As long as serum calcium is not elevated, there is a proper calcium-phosphorus ratio in the serum and the level of 25-hydroxyvitamin D3 does not get excessive; this is why I am suggesting 40-55 nmol/L. In this situation, you should be able to continue to supplement a patient to the level of sufficiency.

Fish Oil and Fish Consumption: Does the Risk of Mercury Toxicity Outweigh the Potential Health Benefits of Omega-3 Fatty Acids?

Our next Hot Breaking News topic relates to fish oils and fish, and the question of a trade off between mercury toxicity versus the advantages of omega-3 fatty acids. There has been quite a bit of discussion over the last few years about how much benefit is achieved if we supplement patients with fish oil or we have them eat more fish because of the potential exposure mercury, which in and of itself is a health risk factor. Are we trading a benefit to an adverse risk?

This subject was revisited in an article that appeared in the *Journal of the AmericanMedical Association* in 2006. [2] It is a nice review, I think, of the relative cardiovascular incidence and mortality rate in people who eat fish, as well as looking at other co-variables of health risk associated with fish consumption and how that interrelates with total methylmercury exposure and other problems (immunological problems or oxidative problems related to mercury exposure coming from fish or even from fish oils that might contain mercury).

First of all, I should tell you that both mercury and pesticides in fish oils should have been removed by proper processing. When you use a fish oil supplement, obviously it is very important to make sure it has a standard of identity from independent laboratories that demonstrate it is free (to below the level of detectability) of standard biocides. A whole list of these are conveniently tested by toxicological laboratories, as well as, of course, heavy metals, particularly mercury. This is a standard of identity that I think clinicians should always demand of their supplier for fish oil supplements; to see an assay from an independent lab to demonstrate that it is below the level of detectability for biocides-things like dioxins, PCBs, herbicides and so forth, and also heavy metals.

With regard to fish, you can't do that. You can't go to your fish store and demand an assay of all the biologicals and the heavy metals. But what the recent *JAMA* review paper points out is that even with the imperfection of our oceans (as it relates to bioconcentration or accumulation of some of these toxins in higher carnivores in the ocean, like tuna and swordfish) the health benefits from increasing fish consumption appear to greatly outweigh the health risks. There is strong evidence for this. For women of childbearing age, however, and certainly pre-pregnancy, one might be very cautious about consumption of those fish that are the biggest accumulators of mercury (fish like swordfish and tuna).There are already some recommendations to limit consumption of these. With regard to fish oils, however, because these can be purified, one can use them safely as long as they have been demonstrated to be free of bioconcentrated toxins or heavy metals.

Neutralization of the Acidogenic Western Diet

Lastly, there has been a question about the acid or ash or alkaline components of the diet-how these influence things like detoxification, renal function, cardiovascular function, endothelial function, and insulin sensitivity. One hundred years ago, it was suggested that by increasing amounts of animal protein, refined sugars, and fats in the diet increase the acid load on the body, which has deleterious effects on function, and that eating a more complex carbohydrate, vegetable, and fruit-rich diet (which is an alkalizing diet) has a positive benefit on health outcome.

This has been re-examined in a number of papers recently, and it does appear that neutralization of the acidogenic western diet does, in fact, improve bone mass, insulin sensitivity, and endothelial function. I am quoting now from a recent paper that was published in the *Journal of the American Society ofNephrology* that shows (by using calcium citrate and potassium citrate as neutralizers of the acidogenic diet) that there was improved bone mass in postmenopausal women with osteopenia. [3] We also know that

acid-based status affects renal magnesium losses in healthy elderly people, so as you alkalize the diet, the individual retains more magnesium (plasma magnesium goes up). This is another benefit to an alkaline-ash diet; this is from the *Journal of Nutrition* in 2006. [4] Lastly, an interesting paper appeared in *Medical Hypotheses* about how acid-based balance may influence insulin sensitivity, reducing insulin resistance by modulating cortisol output. [5]

With that in mind, let's move from our Hot Breaking News section over to the extraordinary voyage/journey we are going to take with our researcher of the month.

INTERVIEW TRANSCRIPT

Clinician/Researcher of the Month
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I look forward each month with great anticipation to our opportunity to speak with a leader, clinician, or researcher. I can honestly say that I have looked forward for many months to this opportunity to talk with Dr. Sandra Jill James.

Let me quickly tell you a little bit about my first contact with the work of Dr. James, which I'm sure she'll tell us more about. I was at a meeting at the Institutes for the Achievement of Human Potential, where I have been a member of the scientific advisory board for many years. It is an institute in Philadelphia founded by Glenn Doman-who was the first winner of the Linus Pauling Functional Medicine Award-and his colleagues 50 years ago to work with brain-injured children and their families. The institution has developed extraordinary ways of improving performance and function in these children. I have personally witnessed what I would consider to be miracles there with kids who were speechless, sightless, and unable to be really self-moving and under control, who-many years later, with extraordinary efforts on the part of their parents through these programs-have been able to achieve extraordinary function, sometimes high-level function, including gymnastics and playing concert violin and doing Shakespeare. It has just been quite amazing.

A Serendipitous Meeting

As I was at one of these meetings, I met a very amazing woman, Laurette, who was the mother of a daughter who had leukemia. This was probably not your average mother in terms of her advocacy (or the lengths that she would take her advocacy) for her daughter.

Her daughter, who was receiving repetitive treatments with anti-folate chemotherapeutic drugs (I think methotrexate, and other medications like that), ended up having seizure disorders, which is not an uncommon secondary side effect from these treatments. Laurette asked the attending oncologist and physicians if this side effect could be reduced or removed. They didn't have a lot of clues about this, so she went on her own into the literature world (being a sleuth for information), and eventually hit on Dr. James' work. As you'll learn more about, this work has to do with folate, the methylation pathways, homocysteine, and all these variables, but at a different level to neurologic function.

And so Laurette came back to the people at the hospital with reams of information. They said it was very interesting intellectual stuff, but it didn't really relate to the practice of clinical medicine and her daughter. Laurette made a big case of this and her advocacy was undaunted. She said that her daughter should be treated with folic acid and other methylating nutrients and that this would reduce the seizure disorders. So the debate went on and on.

The products Laurette was requesting were not available in formulary, so the hospital staff couldn't really prescribe them, and so Laurette fought through that to get them on formulary. The long and short of it was that through her advocacy and through mobilizing the work of Dr. James and others, Laurette was able to better manage these problems with her daughter in terms of seizure disorders.

Laurette said to me, "You really have to meet this Dr. James. She is really the leader in this field. If you don't, then you aren't doing your work very well." Needless to say, Laurette is quite an advocate.

I have been looking forward to this interview because I have been following Dr. James' work. She is a Mills College graduate in biology who went on to get her PhD in nutritional biochemistry after traveling through Berkeley to UCLA, where she got her PhD. She has been in the area of research and development, and the Division of Biochemical Toxicology at the Food and Drug Administration (FDA) National Center for Toxicological Research, as well as a professor (now, of pediatrics since 2003) at the University of Arkansas School of Medicine.

We have some interesting interweavings in our histories because I also followed the Berkeley-UCLA-ultimately UCI path in my undergraduate life, and I did quite a bit of lecturing at the University of Arkansas School of Medicine on nutritional biochemistry back in the late 1970s. I guess we have kind of had this interesting web of interaction on a number of intellectual levels.

It is very extraordinary that we finally have a chance to meet together on Functional Medicine Update. Dr. James, what a privilege to have you today and thank you for spending time with us.

JJ: Thank you. It's my pleasure.

JB: In looking at your publication record, what I see (as I kind of take the broad brush) is an extraordinary example of the evolution of a scientist and the implications of their work on many fields as they recognize the spreading effect. I'd like to go back to where this all started for you, and show how it has built up to have a tremendous application across many different functional-what we might even consider disease etiology-conditions. This, of course, fits into our functional medicine model so beautifully, because we have often told our doctors that the differentiation between functional medicine and traditional diagnostic medicine is that we are less concerned about what we call it (i.e., the diagnosis as a disease) and more concerned about mechanisms (because people have processes, they don't have diseases).

The dysfunctional processes are what give rise to what we codify (to make it simpler to understand and memorize) as a disease. I think your history really is a tremendous example of the functional medicine model, because by understanding more and more about a process-in this case transsulfuration and the methylation pathways-we can see how it applies to so many different conditions that cut across a whole variety of different subspecialties of medicine. You are, in many senses, what we would consider the

premier functional medicine researcher.

With that as a kind of introduction, let me go back to where-for me-this all started. In 1971, I read a paper in the Lancet authored by Dr. Smithells in England, who was talking about B vitamins and neural tube defects and spina bifida offspring from women who apparently had functional vitamin B deficiencies. He talked about folate and B12 as important nutrients, and when he supplemented women who had previously given birth to children with NTDs or spina bifida, this really significantly reduced the frequency of another offspring having that problem. That was a very controversial area for the better part of 25 years. It was only through the later understanding of mechanisms that we started to see how spina bifida could be associated with folate problems and how that related to folate polymorphisms.

That now takes us to your work and your initial publications on methylation. Could you tell a little bit about DNA strand breaks, radiation diet, heavy metals, calorie restriction, and the folate cycle?

The Methylation-Transsulfuration Pathway

JJ: Okay. It has been quite a journey, researching this metabolic pathway. I first became involved with it in graduate school at UCLA. This is the methylation transsulfuration pathway. We started out in a rat model, using what is called a lipotrope-deficient diet (it is folate, methionine, and choline deficient). It is well known that over time, with diet change only, hepatocellular carcinoma can be induced. So that is where we started, looking at the livers of these animals that were put on this methyl-deficient (or lipotrope-deficient) diet.

Looking at DNA Strand Breaks and Uracil Misincorporation

We looked at strand breaks and how this diet affects the pathway. That was an interesting finding. The shift in the metabolites in this pathway (looking at the folate side), showed us that the dUMP, which is the precursor for thymidylate synthesis, was increased, and the dTTP (the thymidylate product) was decreased. This work was actually very early, and subsequently Bruce Ames looked at that same pathway. We had seen the strand breaks, and he followed that with the finding that there was actually uracil misincorporation. Again, this is all just diet and shift in these pathways so that this ratio of the uracil precursor (the dUMP to the thymidylate product) was increased. So what happens when the DNA polymerase doesn't differentiate dUTP from dTTP? It misincorporates the uracil. That is interesting in itself, but we think that the real problem is that there is a uracil glycosylase that will get rid of that uracil and leave a break. So that was a big insight into one of the mechanisms (again, I'm very mechanistically oriented). We thought this might be a mechanism associated with how this carcinogenesis process works and this might contribute to preneoplastic state. So that's where we started, looking at liver cancer in rats.

JB: I recall from some of your publications that you looked at different ways of inducing DNA changes, like radiation and heavy metal exposure (like nickel), and you also even did a number of studies on calorie restriction as a protective intervention in animals.

Studies on Calorie Restriction as a Protective Intervention in Animals

JJ: Yes. That was an interesting finding. Trying to understand-again, this is nutritionally related and cancer, which is where I started-why caloric restriction is protective and is associated with an increased lifespan (at least in rodents) and recently, in The New York Times, apparently in primates, as well. Did you see that?

JB: Yes, I did.

JJ: It was interesting-the fat monkey and the slim monkey and the slim monkey looked so much better and was so much more active. So, we were interested in the mechanism. If you look at rats in a cage, you can tell immediately which ones are the calorically restricted because they are zooming around the cage, they are looking for food, and they are much more active than the slobs who have all the food they want and are just getting fatter and fatter, not worrying about food so they are just gaining weight and not exercising. It occurred to me that maybe (and it was just kind of a flash moment) it could be that because there are fewer calories available, the body senses that loss (or restriction in calories) and will induce an apoptotic event in the most vulnerable cells. And so that was our hypothesis, that maybe what's happening and what is protective with caloric restriction is that the vulnerable (the preneoplastic) cells (which are well known to be most vulnerable to apoptosis), when you pull the calories, those cells go first. That paper was in Cancer Research, where we actually looked at the level of apoptotic bodies in the livers of calorie-restricted rats compared to the ad libitum fed and found a clear increase in the level of apoptosis, which did support our theory. 6

An Alternate Theory about Calorie Restriction

I have another theory about caloric restriction, which is kind of the inverse and I think is equally interesting. It may be that we are not looking at the effect of calorie restriction extending lifespan, but rather we are looking at the effect of overnutrition reducing lifespan. This depends on which side you take as your control. If you take the overfed (the ad lib fed) animals as your control, then it looks like caloric restriction is extending lifespan. However, it is equally valid, I think, to take the calorically-restricted animals as the more normal, because they are (that's what mice and rats do-look for food and they are not ad lib fed), and you take them as the control, then what you are really studying is the life-shortening effect of too many calories. So that was kind our take at that level, at that pathway.

JB: Let's talk a little bit about the assessment of alterations in the transsulfuration/transmethylation pathway. I know you have talked about glutathione and you have talked about S-adenosylhomocysteine. There are some ratios that look like they might be interesting from an assessment perspective, like the GSH to GSSG ratio, or the S-adenosylhomocysteine to S-adenosylmethionine ratio. Could you tell us a little bit about those?

JJ: Yes. Again, as I said, this pathway has been my life. It has taken me on a wonderful and fascinating journey. The transmethylation is basically the methionine cycle, which I think you are all very familiar with. What we did is take the methionine cycle through S-adenosylmethionine to S-adenosylhomocysteine to homocysteine. Then we took it down further.

Taking the Transsulfuration Pathway to Glutathione

If you take from homocysteine, now you enter the transsulfuration pathway. Most graphs (if you look at the diagrams and papers) will end at cysteine. What we did is say, "Wait a minute. We need to go all the way down to glutathione." That was really illuminating for us. What we have looked at in Down syndrome and cystic fibrosis and now, most recently, in autism is how the transmethylation pathway interfaces with the transsulfuration, taking it all the way down to glutathione.

This has been really fascinating to us because there is a lot of regulation that intersects those two pathways. For instance, methionine, which is at the top (it is the product of methionine synthase), that is

the essential amino acid. What the methionine cycle is basically doing is it is a clever way the cell has to recycle or conserve this essential amino acid. It's basically through methionine synthase and the methyl group from 5-methylfolate. It's a way to keep that methionine level high and that's critically important for the viability of the cell, for not only protein synthesis, but these essential methylation reactions because methionine then leads to S-adenosylmethionine, the major methyl donor for a multitude of methyl transferase reactions-essential DNA methylation, RNA methylation, protein methylation, phospholipid methylation, creatine, and neurotransmitters. SAM (S-adenosylmethionine) is absolutely essential to keep that up, which requires the methionine.

The methionine cycle is keeping methionine up, which then feeds to this essential methyl donor. Once it gives up its methyl group, it then becomes S-adenosylhomocysteine (SAH), which is then rapidly hydrolyzed to homocysteine. The next step is from homocysteine down through the transsulfuration to cysteine. Cysteine, recall, is the rate-limiting amino acid for glutathione synthesis.

Now if we go back up to the methionine cycle, the S-adenosylmethionine is an important regulator of CBS (cystathionine beta-synthase) which is the enzyme that pulls homocysteine down transsulfuration. When it is high, it upregulates CBS (when SAM is high you get an upregulation, which pulls homocysteine down to glutathione, which is good). There is that interaction, then, through SAM levels, of regulating the transsulfuration pathway. So keeping methionine levels high-the other important point here-is that methionine, through this pathway, down through CBS and transsulfuration, provides 50{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the cysteine for glutathione synthesis. That transmethylation-transsulfuration pathway is not only important for methylation reactions, but also as a precursor to get methionine all the way down to cysteine, and that is what leads us into glutathione. We go from methionine being high, and it is going to keep cysteine levels up. Cysteine is the rate-limiting amino acid for glutathione synthesis, and so now the whole transmethylation-transsulfuration works together, and that is actually reciprocal. When glutathione needs are high, transsulfuration is upregulated so that the SAM and homocysteine and the cysteine levels are pushed down that pathway so we can fortify the glutathione levels, which are so important for a multitude of viable options for the cell, including what we call the reducing environment inside the cell (that is what allows multiple redox-sensitive enzymes to work well). You have got to have adequate high levels of glutathione in the cell so that those enzymes are redox functional and active. It is important for the integrity of the cell membrane, for membrane signal transduction, and for gene expression.

Glutathione is an Important Detoxification Mechanism for the Cell
Glutathione is an absolutely fascinating molecule. It is important, as you know, as a free radical scavenger. It is well known to be the major antioxidant, or free radical scavenger, inside the cell. It is also important for detoxification; this is less known but equally important, and brings in an environmental aspect. Glutathione is actually a tripeptide: glycine, cysteine, and glutamate. It is the cysteine (and remember, that's the rate-limiting amino acid for glutathione synthesis) component in the glutathione and, specifically, the SH, or thiol group, that's the active part of glutathione; it donates that hydrogen from the SH, or sulfhydryl group. Well that sulfhydryl group, on glutathione, is a magnet for heavy metals. And, again, we are bringing the pathway now to an environmentally important pathway. That SH group will bind mercury, lead, arsenic, cadmium-it is a magnet for heavy metals. Once bound by glutathione, that heavy metal now is inactive (it can't damage the cell). So glutathione is an important detoxification mechanism for the cell, and that conjugant of glutathione, then, is further metabolized and is excreted from the body in the bile and also in the urine. It is not only the major detox mechanism, it is the way that

the metals are excreted, and it is actually the natural chelator of the body, if you want to think about it that way. We have sort of taken our transsulfuration down to being very interested in the glutathione and what we call redox ratio.

JB: We could spend days going through this in great detail and I know you could fill those days very eloquently with us. It sounds like there is a connection between this oxidative stress reaction that occurs when you can't detoxify oxygen and nitrogen free radicals, so your glutathione to glutathione disulfide (that's the reduced-to-oxidized-glutathione ratio) goes down as your apparent conversion of SAM, ultimately, down into incorporated cysteine for glutathione synthesis is compromised. So that would then suggest, I think, that there is a connection between oxidative stress and glutathione synthesis and interruptions in the transsulfuration pathway. Do you see, then, increased levels of 8OHdG (8-hydroxydeoxy-guanosine) in animals or humans when there is a diminution of the reduced glutathione levels?

JJ: We haven't done that. However, we have just gotten a 5-year NIH grant and that will be part of what we'll do. Our grant is more focused on autism, but it is the same thing. We see a low GSH-GSSG ratio and whether that is associated with 8-deoxy-guanosine is a fascinating question. My bet is that it should be.

JB: That, then, also opens our thoughts because we have heard so much about homocysteine as a marker for interruptions in this pathway (at least a surrogate marker). And then there is literature that you have described in some of your publications suggesting that S-adenosylhomocysteine might be a more sensitive marker. Could you tell us a little about the homocysteine connection, as a marker to defects in this pathway?

The Relationship between Homocysteine and S-adenosylhomocysteine (SAH)
JJ: Yes. That is a very interesting area. We were interested in the SAH reaction. What we showed (I think in a Journal of Biological Chemisty paper, I think in 2000) is that when homocysteine is elevated,that comes from S-adenosylhomocysteine to homocysteine and adenosine. 7 That is the sole source of homocysteine in the body (that one reaction). It turns out that that reaction is actually reversible. That is getting to be better known, but it was not really fully appreciated early on.

The only reason that that methionine cycle proceeds in the clockwise direction, if you will, from homocysteine then back through to methionine synthase (the reason the SAH hydrolase reaction proceeds in that hydrolytic direction) is because the products (homocysteine and adenosine) are rapidly removed. The metabolism is set up to keep those levels low. The homocysteine gets sucked into methionine synthase or sucked down into the transsulfuration pathway. Adenosine goes on to adenosine kinase or adenosine deaminase. So the products of the SAH hydrolase reaction, under normal physiologic conditions, are kept very low. That keeps that SAH hydrolase reaction going in the hydrolytic direction. However, under pathologic conditions, where homocysteine is elevated, or adenosine is elevated, that SAH hydrolase reaction will reverse, and, in fact, the thermodynamics of that reaction actually favor the reverse direction (from homocysteine back up to S-adenosylhomocysteine, or SAH).

We showed that in our paper-actually a very nice relationship-that as homocysteine is elevated, so is SAH. But the real punch line is that SAH is a potent product inhibitor of all of the methyltransferases, and it is involved in, for instance, DNA methyltransferase, which was our particular interest at the time. When

SAH with homocysteine levels go up, it backs up and SAH levels go up and then the hypothesis is-and it has been shown in multiple in vitro studies-that the SAH will inhibit the methyltransferase and cause a decrease in the methylation potential or capacity. And so in that paper, we showed also that the DNA methylation level decreased as SAH went up. I think that is where it has actually been reproduced, and now there is a lot of talk and theory (which I think is still at the theory level, but certainly worth pursuing) that maybe homocysteine is just a marker and it is really the SAH (which inevitably has to go up when homocysteine goes up because of the thermodynamics of that reaction) and the SAH well known effect on phospholipid methyltransferase, DNA methyltransferase, and protein methyltransferase.

We are getting into a whole new area that could be related to an increase in homocysteine, but is actually an indirect downstream product of an increase in homocysteine. I think that's still in its infancy, but there is an awful lot of active work going on now looking at methylation changes (whether it be protein, DNA, etc.) associated with elevated homocysteine.

JB: For the clinician, who is probably the major listener of this, is it still valid or valuable for homocysteine assessment to be done as a surrogate marker?

JJ: Absolutely. Mechanism is where we are delving now. Without question, homocysteine is much, much easier to measure than S-adenosylhomocysteine. I'm not even sure that it is commercially available. This is done in the research lab. We have HPLC with electrochemical detection, which actually was a breakthrough because SAH levels in the plasma (readily available to clinicians) is very, very low and very difficult to pick up with fluorescence. But it absolutely holds that if you see an elevation in homocysteine, you can assume that the SAH is up. You can also assume that if you lower homocysteine with intervention such as folate, B12, B6, or betaine and get homocysteine down, SAH came down as well. So you really don't have to measure SAH. That whole area of trying to understand the mechanism behind atherogenesis or carcinogenesis, for instance, may or may not be important to the clinician directly. If you can get the homocysteine down you are going to get the SAH down, and that is what is important, clinically. The mechanism-whether a hypomethylation is involved with atherogenesis or carcinogenesis-that's kind of a research area.

JB: I hope that our listeners are hearing that you are delivering on my introduction. You have cut this discussion across a number of potential clinical conditions, including cardiology. We have talked about neurology. We are going to talk about developmental biology. We are obviously talking about areas of endocrinology. It is a pretty fascinating that we have even talked about oncology (carcinogenesis).

Mechanisms are probably where the action is in medicine. The outcomes that we call disease are really the secondary effects from altered mechanisms. You are making this point very, very clear.

Let's go back now. The clinicians who are listening have survived through some very arduous biochemistry, so now the payoff is to talk a little bit about the applications. Earlier in your career, you started applying this concept to Down syndrome, looking at some of these polymorphisms of the folate cycle, like methylenetetrahydrofolate reductase and catechol-O- methyltransferase. Can you tell us that chapter in your history?

Down Syndrome and Polymorphisms of the Folate Cycle

JJ: Yes. That was, again, serendipitous. A colleague called who was involved in Down syndrome

research, and pointed out that the cystathionine beta-synthase gene is on chromosome 21, and it overexpressed, obviously, because there are 3 copies of CBS in children with Down syndrome. This perked my interest because, of course, that's part of our pathway (and, again, that's the beginning of the transsulfuration pathway). So we were interested in looking at children with Down syndrome (to examine whether this pathway was altered). We did find alterations in the pathway.

We also looked at cells (the blastoid cell lines) from children with Down syndrome. What we see with Down's kids is that homocysteine is low because it is being pulled down the transsulfuration pathway with the overexpression of CBS. But, interestingly, we also found the glutathione levels were low, which you might not expect with overexpression of CBS. It turns out that superoxide dismutase (which leads to hydrogen peroxide) is also on 21 and that is overexpressed, and so we did see some oxidative stress in the children (the GSH-GSSG ratio being decreased), but (again) for a very different reason (secondary to another gene on chromosome 21).

We also were interested in the parents, and looked at the frequency of the MTHFR polymorphism (the 677T). This was actually a surprise. We just thought it might be interesting and it was a huge payoff. It was actually very controversial when our paper first came out.

Most geneticists involved with Down syndrome thought there really wasn't a genetic cause, but that it was somatic. What we found was a significant increase in the 677PT. The hypothesis (and I always have to have a mechanistic hypothesis to get interested in a project) was that basically Down syndrome is a nondisjunction event 95{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the time in the mother (two chromosomes don't separate properly). Thinking about that and the folate cycle was the rationale behind even thinking about looking at MTHFR. It turns out that where methyl groups are most concentrated is in the pericentromeric repeats (CG repeats) around the centromer. I hypothesized that if the mothers had a MTHFR polymorphism, that should affect the methylation of the DNA, and where most methyl groups are is in this pericentromeric region. 8 With fewer methyl groups in the centromeric region, what happens is that the DNA is in a more open configuration and more likely to tangle and may, somehow, be involved (mechanistically) with why those chromosomes aren't segregating properly. That was the rationale.

We came out with this paper and it was very controversial. It usually takes 5 to 10 years before a hypothesis is replicated or not. The good news is that it is being replicated. And actually even more interesting, other genes that are involved in this pathway are now being found to be elevated (in MTRR and MTHR1298-other polymorphisms affecting the same pathway that would affect methylation). Data are coming out of different countries now that really seems to support that we were on the right track. Subsequent to that paper (our paper with this kind of far out hypothesis), came several papers showing that, in fact, the MTHFR677TT is clearly associated with DNA hypomethylation, which would fit with our hypothesis that maybe it is the methylation around the centromer that is involved with the nondisjunction event.

JB: I think this is an enormous bit of science and discovery. Over time, this will probably weave its way down in the same way Smithells' early observational association between B vitamins and neural tube defects has changed our thinking about epigenetic effects of nutrition on gene expression and developmental biology. Your work is just right at the cornerstone of that and I really want to compliment you and your colleagues.

Before we jump to autism (I know a lot of our listeners are very excited about hearing about your recent work in that area), I want to quickly cover a couple of sound bytes that I know are part of your publication record. One is the postmenopausal woman. Looking at lymphocyte DNA methylation patterns, do we have any relationship, do you think, of functional folate, B12, and B6 insufficiency in the postmenopausal woman who is eating the standard American diet?

JJ: Are you talking about an estrogen effect?

JB: I'm talking about the paper that you published in 1998 in the Journalof Nutrition where you looked at lymphocyte methylation patterns in postmenopausal women. I thought it was quite interesting.

Elevated Homocysteine in Postmenopausal Women

JJ: That was the SAH paper, I believe. We were showing that elevated homocysteine could be associated with the postmenopausal state. This actually goes back to the difference between men and women and their homocysteine levels. It is known that men have higher homocysteine levels than women, all the way up to menopause. It is fascinating to me what that could mean.

Estrogen upregulates the methionine cycle, believe it or not. When the MAT is increased, methionine synthase is increased (and this is in rats as well as humans). The methionine cycle is much more efficient in women, and that may be the reason why we see lower homocysteine levels in women at all ages up to menopause, and then the difference is less. Somehow estrogen-and it could be, again, by upregulating the methionine cycle-keeps homocysteine low. I think that was the paper where we were showing when homocysteine went up, SAH levels went up and the DNA methylation was affected. 9 It is, again, back to a relationship between the homocysteine level being elevated and backing up to increase the SAH, which affects methylation.

JB: What really struck me about your paper was multiparametered input into postmenopausal women and their risk to cardiovascular disease and cancer and neurological deficits. The cause may be more than just the lack of estrogen, in and of itself. There may be a number of other secondary variables like alterations in methylation pathways, which may be modifiable factors if we can just look at these from a different perspective. I think your work is very helpful in that regard.

This is such an extraordinary and important topic I think we would be remiss if we didn't also address the work you did on the p53 tumor suppressor gene and how that relates to methylation and the folate cycle. This question about whether folate supplementation in women is increasing or decreasing the risk to breast cancer is a very interesting topic right now. Could you tell us a little about your work on p53 tumor suppressor gene and methylation?

Methylation and the p53 Tumor Suppressor Gene

JJ: Yes. Again, this was in our rats on the choline, methionine, folate-deficient diet-looking in their livers for methylation changes. We picked the p53 gene because it is a well known tumor suppressor gene that should be expressed highly as a tumor suppressor gene (when it is working). We were interested in whether the methylation pattern would change with this diet (again, the concept that just alone could affect changes in DNA methylation and gene expression).

As background, I guess you need to know that where DNA methylation works, generally, is in the

promoter region of genes. When we talk about DNA methylation we are talking about cytosine methylation. It is a methyltransferase that is taking a methyl group from SAM and putting it on the number five position of cytosine (at CG dinucleotide-that's the target). This is really how tissue-specific gene expression is produced and maintained.

These patterns of methylation are set down very early in development (during embryonic development). If a gene is methylated, it is turned off. That is the way it should be in a tissue-specific manner. We want the albumin gene to be turned on in the liver, but not in the kidney. So what you see is a hypomethylated promoter for the albumin gene in the liver; that means it is going to be turned on. Whereas in an inappropriate tissue (like the kidney), that same gene (because all cells have all the same genes) is hypermethylated and turned off. DNA methylation is really a way that tissue-specific gene expression occurs.

What we found with our diet (which we showed previously) is that it will cause global (what we call "global") DNA hypomethylation. That means there is a loss of methyl groups in the DNA, globally (when you look at the whole DNA). It is fascinating and complicated, unfortunately, that when there is a global loss of methyl groups, it is accompanied by (and this is paradoxical) a local or regional hypermethylation and turning off of inappropriate genes.

JB: I apologize.I had to interrupt you in the middle of your sentence for technical reasons. Could you continue your thought about this methylation pattern?

JJ: Right. You were asking about the p53 gene. It was another step after the strand breaks: looking (in a rat model) at totally and nutritionally-induced carcinogenesis (really a nutritional-deficiency-induced carcinogenesis). We looked at a common tumor suppressor gene (p53). I was explaining that what we found is that when you have a global loss of methylation, it can often be associated with a promoter region hypermethylation. Although complicated, it is basically a dysregulation of methylation.

We looked at the promoter region of the p53 gene and saw an increase in methyl groups, and that (when the promoter is methylated) is turned off. This finding fit into a mechanistic approach to this diet-induced carcinogenesis: maybe the methyl groups were dysregulated and turned off in a very important tumor suppressor gene-the p53 gene. This was an important tumor suppressor gene that was turned off by diet.

JB: I want to highlight one last clinical tidbit from your publication record. It has to do with the methylation patterns of phosphotidylethanolamine to phosphotidylcholine and how that relates to construction of cellular membranes that you had described earlier, including phospholipids that are associated with myelin. I know you have had at least one paper where you looked at undermethylation and its relationship (in an animal model, maybe) to MS, which we consider a dymyelinating condition. Is there any emerging thought that methylation may play a role in some of these neurologic autoimmune disorders?

Phospholipid Methylation: The Role of Methylation in Neurologic Autoimmune Disorders
JJ: Yes. Actually we looked at DNA methylation because that was kind of our emphasis in this rat model. Frankly, I think phospholipid methylation and protein methylation are going to be affected more quickly than DNA methylation. You are not going to get a change in DNA methylation unless you've got cell turnover, which is fairly slow. Phospholipid turnover is very rapid, and protein turnover is also rapid

(or more rapid than DNA). I think it is phospholipids methylation where you'd see changes in the availability either of the SAM (the methyl donor) or the product inhibitor inhibiting the methyltransferase. I would think that phospholipid methyltransferase would be the most sensitive.

We did a study-it was actually on cystic fibrosis, not MS-looking at that ratio in children with cystic fibrosis. 10 Once again, it was kind of a serendipitous observation that homocysteine levels were elevated in these children with cystic fibrosis. I felt this must mean that SAH levels were also elevated. When we looked, sure enough they were, because the two go hand in hand. If you see elevated homocysteine, you can assume SAH is also up.

My collaborator, Sheila Innis, at the University of British Columbia, is a phospholipids expert. She measured the ratio of phosphoethanolamine to phosphocholine, the precursor of phosphotidylethanolamine methyltransferase (the PEMT), and found that indeed the phosphoethanolamine, the precursor, was elevated, and the product, phosphotidylcholine was decreased. So that was evidence, then, that the phosphotidylethanolamine methyltransferase (or the methyltransferase involved in membrane lipid turnover) was indeed affected by changes in homocysteine.

JB: Thank you. That's very interesting. Now let's turn to what people have been waiting on the edge of their seats for. I will read a quote from one of your recent papers. "The current study was promoted by the serendipitous observation in a previous study that the metabolic profiles of dizygotic twins, one with Down syndrome and one with autism, were virtually identical with respect to methionine cycle and transsulfuation metabolites." 11 Here we go. Now we go in to ASD (Autistic Spectrum Disorder).

JJ: Let's go back to Laurette, where we started, because Laurette has just been instrumental in my life. This is a mother. Her child has Down syndrome, and developed leukemia. I got a telephone call out of the blue from this mother, who was obviously well read. She really had no biochemistry background, and it was fun because she couldn't really say the words right, but she knew what she was talking about. We formed a wonderful relationship, helping her child get through chemo, because children with Down syndrome are very sensitive to methotrexate. We formed this wonderful relationship, which has held today.

So through that Down's project, and through Laurette, we were doing a study (as I mentioned previously) looking at this pathway in children with Down syndrome. As researchers, one of the problems we have in doing human studies (in children, especially) is the availability of normal control children.

I had a great idea. The mothers of these Down's children were so helpful and so grateful. I asked them if they could bring in a normal sib. Usually this wouldn't be a good control, but for Trisomy 21, it is a fine control. And, of course, the moms were more than happy to bring in the group of kids, so we got a lot of controls.

A Unique Set of Twins Leads to Autism Research

One mom had twins, and one twin had Down's and the other twin had autism (no Down's, but just autism). That is what triggered my interest in Down syndrome. It was truly an N of 1, but it was so unusual. I couldn't tell which one was the control; I had to call to ask. It was usually very obvious which child was the control child. Based on that N of 1, and with Laurette's help, we decided to follow up. Was this was a fluke (something had been wrong) or was it real?

So it was truly serendipitous that I got to autism through Down's and through Laurette. She arranged a physician in Buffalo, and I said I needed 10 plasma samples from autistic children. Laurette, bless her heart (her daughter was somewhat autistic, as well as Down's, as well as getting through leukemia, so she's been through the gamut), had relations with the autism community there and arranged and got 10 plasma samples.

We repeated our profile and it was so consistent I really didn't believe it. I called the physician back (kind of embarrassed) and said, "You know, Paul, I'm not sure I believe this data because it is too consistent, and you don't see that in humans, generally." I asked him for another 10 plasma samples, and again (with Laurette's help), they sent me a second 10 samples. The results were the same (a little more variation, but basically the same pattern). That launched me, full speed, into autism and I haven't quit yet and don't intend to. That is how we got started.

JB: We are very happy to hear that you have just gotten started because the work you are doing is absolutely pioneering. The first paper that I saw out of your group, which is a collaborative paper with Dr. James Neubrander (and it was very kind of you to have Laurette as a coauthor) was the one in the American Journal of Clinical Nutrition in 2004 (vol. 80, pg 1611) titled, "Metabolic Biomarkers of Increased Oxidative Stress and Impaired Methylation Capacity in Children with Autism." And then more recently, a 2006 paper that you might want to tell us about. I think it is an absolutely fantastic contribution to our understanding of this subject. This paper is titled, "Metabolic Endophenotype and Related Genotypes are Associated with Oxidative Stress in Children with Autism," and I think it takes this discussion to the next level. 12 Maybe you could tell us a little bit about that?

JJ: Okay. In the first paper (in the Journal of Nutrition) we discussed a small intervention trial that was fascinating. I had been looking at using trimethylglycine and folinic acid in the Down's kids. We decided to try that in the autistic kids as well, because what we want to do is bring up their methionine, cysteine, and glutathione levels. So we initiated the folinic acid TMG in a small subset (and this was part of the publication). And then Dr. Neubrander came out with the methyl B12 information, so we added the methyl B12. So in that first paper, there is a small subset of 8 kids that we took through this intervention with folinic trimethylglycine and methyl B12.

With 3 months of intervention, we were able to bring up methionine, SAM, and glutathione levels in these children. But, again, that was a very small study, and we were just looking at the biochemistry; we did not have any behavioral outcome as part of it. We are following up now at Arkansas Children's Hospital Research Institute (where I work), repeating that intervention with the methyl B12 and folinic, and we are doing a behavioral evaluation (before and after treatment). That will be very interesting because (just anecdotally), I know you know (through Dr. Neubrander), the methyl B12 does seem to have a dramatic effect in many children, behaviorally. That's the next step with that project.

The more recent paper in the American Journal of Medical Genetics was a follow-up to our research on 20 kids-the first 10 (that I didn't believe) and then the second 10 (that I did believe); that was an N of 20. In this more recent paper we have an N of 80 children, and this is through many of the physicians that are involved in the Defeat Autism Now movement (and they are coauthors as well), who helped get the plasma and sent it to us for analysis. So now it seems this is a much more powerful statement; we have 80 autistic children compared to 73 controls. Now we can see what the real variability is when we look at a much larger population. Basically, it was the same story (much more variation, but, again, it was the same

story in most of the children).

Recent Study Reveals Low Methionine Levels in Autistic Children
What we see-and this gives us so much insight into this imbalance in metabolism and what it might mean for etiology and for treatment targets-is a decrease in methionine levels, and that was highly statistically significant. In the paper, I break it into subgroups because the mean difference was significant (statistically), but within that mean (if you look at a subset), what we found is that half of those kids had extremely low methionine levels. That was kind of our approach. The mean differences were exactly as we had seen in the previously smaller set, but now that we have a larger group I can get more definitive because, as you know, autism is highly heterogeneous. We were really interested in what subset of kids was severely affected, and could we isolate that subset and then look at their behavior and try to make some sense of biomarkers versus behavior. What we found, again, was similar: decrease in methionine (very reproducibly), decrease in its product (S-adenosylmethionine) in about 50{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}, and then an increase in S-adenosylhomocysteine (the product/methylation inhibitor) in about 20{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}. Even though the mean difference was statistically significant, it was really a subset that I would consider functionally affected by the elevated SAH (which would affect their methylation). So then, of course, the ratio was decreased. We look at this ratio as the best indicator of methylation capacity because you have a low methyl donor (SAM) and (in a subset of the kids) a high SAH; that is a set-up for methylation problems. That was a new finding that we thought was very interesting.

If we look at the transsulfuration pathway, low cysteine is highly consistent. As I said, it is the precursor for glutathione synthesis. You would anticipate (with low cysteine) that you would have low glutathione, and we did see that as well. I think the most interesting (and the strongest) indicator that these children are under oxidative stress is the increase in plasma GSSG (that is the disulfide oxidized form of glutathione-it is the spent form that has given up its hydrogens and it is not being converted back to the active GSH as rapidly as it should be). The only reason that GSSG would be increased in the plasma is if there is a problem intracellularly. What the cell will do as a last-ditch effort when it can't keep up and that absolutely essential redox ratio begins to creep into a dangerous level is to get rid of that GSSG-export it, get it out of there, reduce the denominator-and keep that essential redox ratio in a good range. When we see an elevated GSSG in the plasma that is proof positive that there was a problem inside the cell, which is where we are really interested, mechanistically. That, I think, was our strongest indication that many of these children appear to be under chronic oxidative stress.

JB: I think we are all applauding as we are listening to you talk. This is, again, a history of scientific evolution. It is really extraordinary to listen to this journey through your story.

As we close, I would like to talk just briefly about how (in functional medicine) we might interpret some of your discoveries. As I said in the introduction, we are less concerned with the diagnosis and more concerned about the imbalances, defects, or alterations in basic physiological mechanisms. Our assessment is more focused on antecedents, triggers, and mediators that give rise to signs and symptoms. You have been talking at length throughout this whole discussion (and through your many, many studies) about antecedents-genetic variabilities that may point a direction towards an increasing risk or prevalence to a condition. Then we have to talk about a trigger that would take that antecedent and convert it into a different expression pattern. These markers you are describing (like elevated S-adenosylhomocysteine, or

lowered levels of glutathione in the reduced state, or elevated glutathione disulfide in the plasma) are the so-called mediators or markers that give rise ultimately to a complex metabolic event that leads to the signs and symptoms of certain conditions. Knowing that we have these antecedents that are emerging, does this, then, allow us (you being at the principal hub of this wheel) to understand something about this triggers? I'm thinking about thimerasol and the connection (or the Andy Wakefield work) with lymphoid nodular hyperplasia and GI-immune relationships, or certain other kinds of environmental triggers that might encourage the expression of these patterns that give rise to these unique oxidative injuries in the nervous systems of certain children.

Considering Autism as an Environmentally Sensitive Metabolic Imblance

JJ: Basically what we have is a phenotype. When you look at the metabolic profile that gives you (in my mind) the sum total of the genes and the environment for that individual. It gives you clues about genetic susceptibilities. We have found (it was part of our most recent paper in the American Journal of Medical Genetics), several polymorphisms that are increased in autistic children that might be responsible for this abnormal profile. The profile, itself, gives us clues possibly to etiology, as well. The problem with autism, intellectually, I think, for many physicians, is that it is a behavioral diagnosis and so you are thinking neuro; you are thinking brain. We are introducing a metabolic component, and that means it is going to affect systemically; it is going to affect beyond the brain because that pathway is in every single cell of the body. It brings out the possibility that if this is a genetic predisposition to this metabolic imbalance that is very environmentally sensitive, that maybe we are affecting more than the brain. In fact, maybe the brain is downstream.

We know there is a significant GI component through Wakefield's work. We know there is a significant immune component to autism and there have been multiple studies looking at immune imbalance in autistic children, most recently related to thimerasol. Issac Pessah, at the M.I.N.D Institute, has shown that thimerasol in very low levels affects antigen-presenting cells (the dendritic cells). Because glutathione is the major detox for heavy metals, if it is low in these kids, you would expect they would have a reduced ability to detox environmental exposures and that is where we get in to the environmental components and the environmental sensitivity of this pathway. What this would mean is given an exposure, with their lowered glutathione levels, these children would be less able to detoxify. 13 They are also going to have a reduced homeostatic reserve. You can think of it that way. Basically, these children have a fragile, environmentally sensitive, metabolic imbalance, and when exposed to environmental toxicants, they are going to be the most vulnerable. That is really scary because we are all exposed. Autism is often referred to as the canary in the coal mine. If, genetically (and possibly through this pathway), they are more sensitive because they are less able to detox, and if we don't do something about the environment, the next level is going to be affected, and the next level.

Bringing Attention to the Need for Environmental Testing

I think you may have seen that recent release in the Lancet about Dr. Grandjean (from Harvard) who is coming out with a paper making the point that just so desperately needs to be made that there are a lot of chemicals in our environment that have never been tested in our children for developmental problems. 14 This is a huge advance that this is coming out. Why do we have so many neurodevelopmental problems in children today? Could it be the environment? This new paper in the Lancet really makes that point and hopefully will open some eyes in the government in terms of research. We need to understand the impact, developmentally, on the nervous system of all these chemicals that are in our environment now.

JB: I can't tell you how much we have appreciated this. This is the first of our 2007 editions and this couldn't be more noteworthy precedent to start the year with. Your work is just pioneering and I know it reflects many, many other investigators that have collaborated with you. It is a community of evolution in thought.

In the course of this discussion we have touched on Down syndrome, neural tube defects, spina bifida, autistic spectrum disorders, leukemia, coronary heart disease, cancer, and MD. All of these are connected to these mechanisms you are describing. I think it is fascinating to watch the transformation in medicine. I really believe that the age of the primacy of diagnosis is fading and that we are seeing the emergence of understanding fundamental mechanisms and the dysfunctions in those mechanisms as being what will create both the prevention and treatment of these conditions in a more successful way. Your work is certainly helping to guide us in that direction. Thank you for giving us so much time. It has just been extraordinary. We are going to follow this journey and your evolution as it moves forward.

Understanding Autism as a Medical Condition
JJ: Thank you. I did want to make one last point regarding autism to the clinicians, and that is that I think all of this-our work and the work of others-is beginning to change the view of autism-that it is much more than a neuropsychiatric condition, that there is systemic involvement. There are real medical problems-gastroenterology and immunology-in these kids, and if we can treat them, they are going to get better. That is a whole other area that I think is so important in the transition to understanding to autism more as a medical condition rather than a neuropsychiatric disorder, and treating those medical problems with the children and some of them can get better. It's wonderful.

JB: We thank you. That's a very wonderful, optimistic view where we are heading. And we thank all of your collaborators-these are all pioneers that have really often fought against the standard of fear and the belief systems of the age which are sometimes difficult to overcome, and have done so at some personal peril. I think this is what will make great revolutions in our future. A lot of children who have been adversely affected hopefully in the future will be protected.

JJ: I hope so too.

JB: Thank you so very, very much.

JJ: You're quite welcome. It is my pleasure.

Bibliography

1 Viljakainen HT, Palssa A, Karkkainen M, Jakobsen J, Lamberg-Allardt C. How much vitamin D3 do the elderly need? *J Am Coll Nutr*. 2006 Oct;25(5):429-435.

2 Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health. *JAMA*. 2006 Oct 18;296(15):1885-1999.

3 Jehle S, Zanetti A, Muser J, Hulter H, Krapf R. Partial neutralization of the acidogenic western diet with potassium citrate increases bone mass in postmenopausal women with osteopenia. *J Am Soc Nephrol*. 2006 Nov;17(11):3213-3222.

4 Rylander R, Remer T, Berkemeyer S, Vormann J. Acid-base status affects renal magnesium losses in healthy, elderly persons

5 McCarty MF. Acid-base balance may influence risk for insulin resistance syndrome by modulating cortisol output. *Med Hypotheses*. 2005;64(2):380-384.

6 James SJ, Muskhelishvili L. Rates of apoptosis and proliferation vary with calorie intake and may influence incidence of spontaneous hepatoma in C57BL/6 x C3H F1 mice. *Cancer Res*. 1994 Nov 1;54(21):5508-5510.

7 Yi P, Melnyk S, Pogribna M, Pogribny IP, Hine RJ, James SJ. Increase in plasma homocysteine associated with parallel increases in plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation. *J Biol Chem*. 2000 Sep 22;275(38):29318-29323.

8 James SJ. Maternal metabolic phenotype and risk of Down syndrome: beyond genetics. *Am J Med Genet A*. 2004 May 15;127(1):1-4.

9 Jacob RA, Gretz DM, Taylor PC, James SJ, Pogribny IP, et al. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. J Nutr. 1998 Jul;128(7):1204-1212.

10 Chen AH, Innis SM, Davidson AG, James SJ. Phosphatidylcholine and lysophosphatidylcholine excretion is increased in children with cystic fibrosis and is associated with plasma homocysteine, S-adenosylhomocysteine, and S-adenosylmethionine. *Am J Clin Nutr*. 2005 Mar;81(3):686-691.

11 James SJ, Cutler P, Melnyk S, Jernigan S, Janak L, et al.

Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *Am J Clin Nutr*. 2004;80:1611-1617.

12 James SJ, Melnyk S, Jernigan S, Cleves M, Halsted CH, et al. Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *Am J Med Genet B Neuropsychiatr Genet*. 2006 Dec 5;141(8):947-956.

13 James SJ, Slikker W 3rd, Melnyk S, New E, Pogribna, et al. Thimerosal neurotoxicity is associated with glutathione depletion: protection with glutathione precursors. *Neurotoxicology*. 2005 Jan;26(1):1-8.

14 Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. *Lancet*. 2006 Dec 16;368(9553):2167-2178.p>