Paper C1-2	***Measurement of "New" Health-Related Food Components***
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MEASUREMENT OF "NEW" HEALTH-RELATED FOOD COMPONENTS

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ABSTRACT

Results from epidemiological studies continue to validate the importance of fruits, vegetables and fiber containing foods in the diet as effective means of reducing the risk of several debilitating diseases. The elucidation of the health promotion-role of the many biologically active components in plant foods requires databases of values and analytical techniques to generate data. Many compounds in plant-based foods have biological activities which are consistent with the promotion of health. Unfortunately, robust analytical methodologies have been developed for only a few families of these compounds. Examples include carotenoids, flavonoids, isofavones, lignans, phytosterols and tocotrienols. The analyses of these components requires a chromatographic separation step, gas liquid or high performance liquid chromatography, followed by quantitative detection with systems ranging from ultraviolet detectors to mass spectrometers. While a lot of attention has focused on 'new' health-related components in foods, some of the 'old' nutrients also have received analytical attention. These include measurement of all of the forms of tocopherol, improved extraction of folates and identification of a reduced form of vitamin K in hydrogenated foods. Examples of recent advances in the measurement of nutrients and health-related food components will be discussed.

Paper transcribed from tape:

First of all, I thank the program chairs of the Nutrient Data Bank Conference for inviting me to discuss this topic. I am so happy that Chris, Dr. Christopher Beecher, made the presentation that he did this morning because he really set the stage for what I'm going to talk about this afternoon in terms of several families of components that are present in foods and that have biological activity. Let me try to set the stage for you in terms of why we are interested in a number of components of foods that are "non-nutrients." I don't like that term. I hope that sooner or later we will come up with a term that is more definitive of these components and non-nutrients. For the time being, at least, I'll talk about them in terms of food components. Other people may talk about them in terms of non-nutrients. But, nevertheless, we're talking about the same thing.

The point that I want to make in terms of setting the stage for you is that because of the wealth of epidemiological data that we now have available relative to the intakes of specific foods and the incidence of certain debilitating diseases, the general overall and overwhelming observations that we can make from these data are that fruits, vegetables, and fiber containing foods are associated with a decreased disease risk or a decreased incidence of disease. If you look, then, at the traditional nutrients, that is those nutrients for which there are either RDA's or for which there are estimated safe ranges of daily dietary intake, which I call traditional nutrients, these nutrients are important but they do not account for all of the epidemiological observations that have been made relative to the association of food intake with disease risk and disease incidence.

We also know from the plant physiology and from the studies that Chris talked about this morning, that there are many, many additional components in foods that have wide ranging biological activity. From plant physiology, we know that plant materials develop these secondary metabolites simply as a defense mechanism to protect the plant from disease, fungi, and a

number of other things. Remember now, there are very few plants that can swat a fly or swat an insect like we can or like other mammals can. So they have to develop other defense systems. It is not surprising that some of these same chemicals that the plant has developed for its defense might also be important in terms of biological systems in mammalian systems.

Biological activities that we hear about in great detail today include antioxidant activity which stimulates detoxifying enzymes. These are the Phase Two P450 enzyme series as well as other enzyme systems in the body. Some of these components have hormone-like activities or are metabolized in the GI tract into compounds that have hormone-like activities. Some of these compounds stimulate cell gap junction communication which is important in controlling the development and the expansiveness of each individual cell within the body or within an organ. And then there are a whole host of other biologic activities that I won't take time to discuss today. This sets the stage for our interest and where we are in the whole field of nutrition and human biochemistry and physiology relative to what's in the foods in the food systems that we have today.

Then there are a whole laundry list of components many of which we have heard about already today. I'm sure you've heard and seen at least some of these some place in the literature, some place in the popular press as being important relative to biological activity and also being part of specific food and the food supply in general. What I'd like to do then is rearrange this laundry list of food components and put them together chemically and to some extent with biological activity and discuss where we are and what we understand about the biological activity. This then leads us to where we are in terms of analytic methodology, and where we are in terms of having access to comprehensive data relative to the composition and to the levels of these components in foods.

I'd like to start out with the carotenoids. As you know, we have been working on carotenoids in the food composition lab for a number of years. In general, and for many years, the sole activity that was attributed to the carotenoids has been anti-oxidant activity. Remember that a handful of the carotenoids also have Vitamin A activity, that is they are converted to retinol and other Vitamin A active components by the mammalian system. That's a given for some of these carotenoids. All of the carotenoids have some anti-oxidant activity as far as we can tell at this point.

Lycopene, the carotenoid that provides the red color in tomatoes appears to be the most active in terms of anti-oxidant activity. These studies have been done by Helmut Seese and his colleagues in Dusseldorf in *in-vitro* systems. Fred Khachik in our group has observed the oxidation products of both lutein and lycopene in the plasma of human beings. This varies with the nutritional state of the human beings as well as the levels of carotenoids that are fed to those subjects. Fairly recently there has been the observation that the intakes of lutein and zeaxanthin appear to be associated with a reduction in the risk of adult macro-degeneration, AMD. This is work by Joanne Sutton out of Boston. It's interesting that from what we can tell at this point there is very little zeaxanthin provided in the food supply, but there is a considerable amount of lutein. All of the green foods have a substantial amount of lutein in them. There are also physiological studies from the University of Miami that suggest that there is a considerable amount of metabolism of lutein to zeaxanthin in the retina and also in the macula of the eye. It would appear that this is one of the first evidence that there is conversation of one carotenoid to another in the human body. I'll talk a little bit about where we stand with zeaxanthin in just a minute.

Finally, I don't think any of us have avoided the observance in the popular press that beta carotene supplements, pure supplements, appear to increase the risk of certain cancers in certain sub-populations, that is those populations that are smokers or that have recently smoked and also those people that have been subjected to environmental conditions such as high asbestos areas and those sorts of things. Beta carotene has not been the silver bullet that many people have thought that it would be. The status now with the database is: we've developed analytic methods. All of you know that we put out the database in 1993 for 5 carotenoids. Clive West and Eric Portly at Wageningen Agricultural University in the Netherlands have published a book on the beta carotene content and a few other carotenoids where the carotenoid data are available for the rest

of the world. That book was published in 1995 or 1996. I can get you the reference if you're interested in it. We are currently in the process of updating the database.

Chris Spangler in our laboratory is busy running carotenoid analysis on foods. This is a joint project that has been funded by the National Cancer Institute as well as our own group at the USDA. It's a joint study with the Nutrition Coordinating Center at the University of Minnesota and with Marilyn Buzzard while she was still at NCC; and since she has left NCC and is now at the Medical College of Virginia, we are collaborating with her there. We are updating the database with values from around the world. We are incorporating the West-Portly data into the database and also adding new data that have been published since that database was put out. We expect this database should be available late '97 or early '98, the updated version. That gives you some idea of what to expect for carotenoids.

Chris mentioned flavonoids and talked a little bit about food flavonoids. This family of compounds has certainly got a lot of press lately. Probably the work of Hertog et al. in the Netherlands through epidemiological work and also through analysis and development of a database on food flavonoids shows at least some of these compounds with a reduced risk of cardiovascular disease. This is the famous Zutphen study. The flavonoids are a very large family of compounds in the order of somewhere between 3 to 5 thousand compounds in the plant kingdom. Fortunately, we don't have to deal with quite that many in the foods that we commonly consume. Probably something on the order of 20 specific molecular entities are in the foods that we commonly consume. The work in the Netherlands was really eye-opening in terms of bringing us up to speed or at least getting us to think about the importance of these compounds.

If we look in the literature, there has been some considerable work in the Orient looking at green tea and reduced instance of cancer and reduced risk of cancer. As we look at the green teas in particular, one of the major components in the tea are the flavonoids so we're fairly certain that there is some association here between the flavonoids and the reduced incidence of cancer.

There is the whole issue of the French paradox. That is why do people that drink substantial amounts of red wine especially with meals and have fairly high saturated fat intake have a fairly low incidence of cardiovascular disease. The red wines contain a considerable level of flavonoids as well as other phenolic compounds. The important factor has been between these particular phenolic compounds as being the effectors of the reduced incidence of cardiovascular disease in this particular sub-population. One of the important biological activities that this family of compounds excels in is their antioxidant activity. Again, these are *in-vitro* systems that have been used to measure this antioxidant activity. The antioxidant activity varies from what is equal to Vitamin E antioxidant activity up to 5 times the levels of Vitamin E, so they have a tremendous amount of anti-oxidant activity. And assuming that, that antioxidant activity is one of the important biological activities for this family of compounds.

Where do we stand relative to the analytical status? Michael Hertog and his colleagues developed at the analytic methods for measuring 5 flavonoids, three of which are the most common flavonoids in the food supply. That is quercetin, myricetin, and kaempferol. They also as part of that analytical system are also able to measure lutein and anhydrolutein. Those are the flavonoids that are common in such things as onions, kale, and other commonly consumed fruits and vegetables. Bill Bronner in our lab who has been working on methodology development for flavonoids also and published a paper a couple of years ago on the development of methods for extracting and measuring the flavonoids in citrus, grapefruit and oranges. We are currently working on the methods for measuring the flavonoids in tea, that is the family of flavonoids called the catechins. We should have that methodology ready to go fairly soon. There is very little information in the literature on the quantification, the national level of flavonoids in US foods. Hertog and his colleagues have published data on the flavonoid content, that is the levels of 5 flavonoids on the food that are commonly consumed in the Netherlands. Those data have been published in the Journal of Agricultural and Food Chemistry. But there is little other analytical data that had been published using modern techniques. We're in the process right now of analyzing

foods, using the techniques that we've developed in the laboratory, and this summer we will set up the techniques that Hertog and this colleagues developed and will measure the flavonoid levels of specific foods. This is part of an NIH grant as well as with funding from our own organization we will try to develop some sort of rudimentary database on about the same schedule as updating the carotenoid database and provide it to use in your studies to assess the impact of specific flavonoids on the particular biologic endpoints that you're looking at.

Another family of compounds or groups of compounds with similar biologic activity are what I call the food estrogens. The biology of these have either directly or can be converted to compounds to that have estrogenic activity. There are 2 families of compounds that are involved here. First, the isoflavonoids which are closely related to the flavonoids and are at present in legumes but primarily in the soy-based foods that human beings eat. Then there is the other family of compounds called the lignins, which are present in high fiber foods such as foods that are made from flax and rye, to ascertain extent wheat containing foods, and then to an even more limited extent, fruits and vegetables. As I indicated, the biologic activity is estrogenic-like activity. Most of this estrogenic activity is developed in the GI tract of the human being, that is these compounds are metabolized by the microflora to produce the compounds that have estrogenic activity. In addition, these compounds do have antioxidant activity at least as measured by *in vitro* systems.

Dr. Pat Murphy, who has presented a paper at this meeting in the past, is the guru of isoflavonoid analysis in the world. She's been quietly working on it at Iowa State since the early 80's as part of plant breeding and soybean breeding to develop strains that are high in these compounds and has gotten little publicity until the human biological activity has become important.. Others working to measure these compounds are located at the Cancer Center of Hawaii and the University of Alabama at Birmingham. Relative to lignins, which are the estrogenic precursors, researchers at the University of Helsinki have just published a paper in Analytical Biochemistry describing developed methods for measuring the two lignins that are precursors to estrogenic compounds.

There are a whole family of lignins in foods we think are important biologically. Dr. Betty Li and I in our laboratory are currently recruiting a post-doc to work in this specific field. Relative to the amount of lignins from a food that have mammalian derived estrogenic activity, Lillian Thompson at the University of Toronto has developed methods for measuring those. So, relative to the database status, again we have a joint project with Pat Murphy at Iowa State to develop data to provide a database. That's really all there is at this point relative to lignins in terms of data that has been published or has been collated.

I'd like to turn next to the sulfur compounds. Again, Chris touched on this this morning with just a couple of examples. I've categorized all the sulfur compounds together because I think that their biological activity, at least from what we understand, is very similar in that their biological activity appears to reduce cancer risk, probably through the induction of detoxifying enzymes. These sulfur-containing molecules appear to turn on these enzymes. Many of these come from cruciferous vegetables. Researchers at Johns Hopkins have done quite a bit of work with sulfur compounds from broccoli and it's reasonable to expect other cruciferous vegetables contain this family of compounds. Concerning analytical status, rugged analytical methodology needs to be developed and a database as well since there is none. Again, this is down on the priority list for our laboratory. I cant assure you when we will get to it, but it certainly is coming up fairly soon on the priority list.

Chris also mentioned the saponins family of compounds. These compounds have been shown to reduce plasma cholesterol and also appear to reduce risk of cancer. The mechanism appears to be through binding of dietary cholesterol, bile acids and lipids, probably because these compounds have a structure that is very similar to cholesterol. The structure of saponins is about the same shape and dimension as cholesterol and that appears to be the reason it is active in terms of binding cholesterol. These compounds are present in legumes and some spices. We

need to develop methods and there are no databases, at least as we understand the databases that have data for this particular group of food components.

Phytosterols are compounds that are closely related to cholesterol also. We don't know whether these compounds are absorbed like cholesterol. There is a guess-timate that the daily intake of these compounds if one is eating substantial amounts of fruits and vegetables could be as much as 250 mg a day. Rudimentary analytical methods for phytosterols were developed in our laboratory back in the '80s. Katherine Phillips, and Kent Stewart while he was still at VPI, worked on the project, modifying these methods and they have now been developed to a robust level and are ready to go in terms of measuring these compounds in foods and other materials. We have no database at this point.

Let me make some comments relative to the old nutrients. I don't think that we can forget that we have extensive data on traditional nutrients for which there are RDA values. Let me just highlight some points on what is happening with some methodologies for this particular family of nutrients. For vitamin E, the tocopherols, we can now separate and measure all four forms of vitamin E. This has happened in the last couple of years. Along the same lines and using some of the same techniques, in fact some of the same analyses, we can measure all four forms of a family of compounds that are closely related to vitamin E, that is the tocotrienols. These are the compounds that are present in such things as rice bran oil and other isolated oils that may impact reduction of cholesterol and risk of cardiovascular disease. Since we can now measure these, it's simply a matter of getting to the point of developing databases that have the details for the various forms.

Folic acid continues to be a challenging nutrient. As you all know, FDA has approved fortification of flours with folic acid. That has challenged us now to be able to develop an analytical method that is rugged and routine for the measurement of this component in specific foods. I feel that all of the things are in place to be able to do that; it is simply a matter of getting around to doing it and putting it all together. There have also been advances made by the Finns in the total extraction of the indigenous folates in vegetables. This is also a very difficult area that is going to require some time before we can get a methodology put together for it.

And finally, some recent work at the USDA Human Nutrition Center at Tufts has resulted in the finding of a reduced form of vitamin K in the margarines that have been subjected to hydrogenation. We don't know what the biological activity of that particular compound is, but it's present in fairly high levels. As much as 50% of the total vitamin K in these margarines has been observed to be reduced as part of the hydrogenation process. These are some new developments for what I call "old" nutrients.

So, in summary, fruits, vegetables, and fiber-containing foods are obviously associated with health. The traditional nutrients don't account for all of the decreased disease risk that has been observed with these particular foods. There are many components in plant foods with biological activities that support health. And, yes, I think we have to remember that if we overdose on either a food or one of these components, it is quite likely that it is going to become toxic. We always need to remember that particular component of this area of biological research. And, finally, I've discussed with you the measurement systems and the database development and the state of those activities for a number of these particular components.

Thank you very much for your attention.