IN THE U.S. DEPARTMENT OF AGRICULTURE

IN THE MATTER OF:)
)
SYMPOSIUM:)
)
DIET AND GENE INTERACTIONS:)
EQUAL PARTNERS IN HEALTH?)
)
USDA Center for Nutrition)
Policy and Promotion)
)
The Center for Genetics,)
Nutrition, and Health)

The Jefferson Auditorium
USDA - South Building
14th St. and Independence Ave.
Washington, D.C.

Wednesday,
December 6, 2000

The parties met, pursuant to the notice, at 9:02 a.m.

APPEARANCES:

RAJAN ANAND, Ph.D., Cochair
ARTEMIS P. SIMOPOULOS, M.D., Cochair
DAN GLICKMAN, Secretary of Agriculture
SHIRLEY WATKINS, Undersecretary of Agriculture

Speakers:

ARTEMIS P. SIMOPOULOS, M.D.
RONALD M. KRAUSS, M.D.
STEVEN C. HUNT, Ph.D.
ANNE M. MOLLOY, Ph.D.
ROBERT F. MURRAY, JR., M.D.
DONALD B. JUMP, Ph.D.
DENNIS A. SAVAIANO, Ph.D.
BRUCE A. WATKINS, Ph.D.
FRANK W. BOOTH, Ph.D.

P R O C E E D I N G S

(9:02 a.m.)

DR. ANAND: We're delighted to welcome you to our sixth all-day symposium. I see many faces that have come to all of our symposia. We appreciate your help. We also appreciate your e-mails complimenting these efforts. I also want to acknowledge the people who have watched us on the web site because these have been webcast.

I may not get this chance again. So I want to pay tribute to two most decent human beings that I had a great privilege to work with. I don't remember anyone in recent history, any sector of agriculture, who has taken more interest in nutrition and nutrition programs as Secretary Glickman has. He is the inspiration, and he is the force behind these symposia.

(Applause)

DR. ANAND: When we announced the symposium on Great Nutrition Debate, believe me, we got lots of criticism why we are doing it. But it was Secretary Glickman who said if we don't do it, who will do it? It was a great success, and we got lots of compliments after that. It has been a really great privilege to work under his leadership to promote good nutrition for all Americans.

The second person is my boss. She has never ceased to amaze me with her energy, enthusiasm, and commitment to her work and to promote good nutrition for all Americans. She has made a personal commitment of feeding families and fighting hunger. She is the one whose idea it was to have these symposia open to all people, and she has steadfastly supported these efforts all the time. I really appreciate her work very much.

(Applause)

DR. ANAND: Thank you. I want to bring her and present to you the undersecretary of Agriculture for Food, Nutrition and Consumer Services, Shirley Watkins.

(Applause)

MS. WATKINS: Thank you so much, Dr. Anand. And I want to thank all of you for getting here this morning in the cold morning that we have in Washington. It feels like winter time. We didn't have much of a summer, but we sure are getting ready to have a winter. So thank you for being here, and thank goodness you didn't have to stand outside in the cold to get inside. So I hope you didn't anyway.

I want to welcome all of you to our symposium on diet and gene interactions. And I want to especially thank Dr. Anand and his staff for all of the hard work. And I deeply appreciate them in putting together the

symposia that have provided a great deal of insight into some issues that are perhaps uncommon to be discussed at the Department of Agriculture.

The great American poet Ralph Waldo Emerson once said it is superstition to insist on a special diet; all is made at the last of the same chemical atoms. Science has told us that we are all made out of pretty much the same basic material, but what we eat combined with who our parents are or our grandparents were can contribute, or who our grandparents are can contribute to enduring questions about the real nature of our composition.

We have long believed that diet and genes are competing forces with the genes having an effect on a person's life quite independent of an individual diet. Traditionally, it has been a debate on ‘nature versus nurture,’ and which of these two seemingly contradictory or competing elements has a large role in determining the aspects of life.

Today, our understanding is much more complex, and we know that in some cases genes trump diets, and in other cases diet trumps genes. I guess you think I play poker or bridge or something, and perhaps that might be right. We are in fact products of both our genes and our environment.

For example, we are aware that some racial

groups are lactose intolerant, particularly people of color, African Americans, Asians, Native Americans. Yet in Central Africa, the people of the Masai tribe herd cattle and drink milk with no ill effects. Why aren't they lactose intolerant? Were they ever lactose intolerant? Well, if so, how many generations did it take for them to develop a gene which allows them to drink milk without any discomfort?

Well, to me that is pretty fascinating, and it is one of the questions that we probably will hear raised today. We'll probably hear a lot of things discussed, and we'll probably get a lot of answers, and we may leave here today still with no answers. We still may have lots and lots of questions. And I think that is the purpose of these symposia and why we think it is so critical that we open the dialogue, start the questions and ask a lot of questions. And whether we get the answers or not, at least it brings all of us together to start talking together to see if we can find some answers.

I want to especially thank Dr. Simopoulos, who has been very helpful in helping us to pull this symposium together. She is the president and founder of The Center for Genetics, Nutrition and Health. And it is the first time we have had someone to cosponsor these symposia. And we're delighted that she agreed to work

with us in cosponsoring this, and we want to thank her for joining us and producing this insightful, thought-provoking symposium. And I'm sure we are going to call it some more adjectives before this day is over. As a matter of fact, the last symposium that was sponsored -- I guess this is the last one, Raj, that will be sponsored by the Clinton-Gore administration. And I couldn't close my remarks without paying tribute to the people who have made these groundbreaking events possible over the past three years. Dr. Anand and his staff at the Center for Nutrition Policy and Promotion (CNPP) have just done an outstanding job. And as he indicated, sometimes we didn't always meet eye to eye on some of the symposia that we held, the first one being that one on childhood obesity prevention. And people asked, of all places, the Department of Agriculture would talk about child obesity and childhood obesity prevention.

Well, we broke that ground and decided, well, why not, and just do a bunch more. And that is exactly what we have done. So they advanced the scientific research, and it has raised the public consciousness in some very, very significant ways. We had the dietary behavior, the breakfast and learning, the nutrition and aging, and all of them have been covered by CNPP, and each one has been enthusiastically received. And as Raj

said, some of the faces I see out here today are faces that I have seen at each one of the symposia, and we're delighted that you have found these to be helpful.

The other one that the secretary wanted us to do and challenged us to get done, and that was the Great Nutrition Debate, and that was one of the most popular events ever held in this auditorium and at USDA, with both national and international press covering the session. And the secretary said, 'I have never seen so much press in all my life as we had at that one.' And many of you were here sitting on the front row and standing outside waiting to get in when the department opened, and let us know that that is something that we need to talk about. And we still have more discussions that will continue in the future on that issue.

These could not have been possible had it not been for the commitment of the staff. And I want to thank them again and again and again. In my office, we are all accustomed to hearing Raj to say to me and to all of us that he has no money to do any of these. But invariably, he has been able to produce a first class, well-organized, and a timely event.

I remember the first one. He came and said, Shirley, we are going to need a lot of people to do this, and we are going to need a lot of money, and it is going

to cost lots and lots of money. And I said, what is lots and lots of money? And he said $100-, 150,000. I said, Raj, get out of my office. You know that I don't have $150,000, and neither do you. We ended up doing it for about $25,000, and then the next one got cheaper and cheaper and cheaper. And now you see people come without any money because they are so delighted to be here. And to our speakers, I want to thank you because you understand we don't have any money. You are just delighted to be here.

(Laughter)

MS. WATKINS: We certainly appreciate all of you. You may know that Raj came to the United States from India in the '60s to do his graduate studies. And 100 years earlier, India was known as the crown jewel of the British Empire. And today, Raj and the center, for us at FNCS, are the crown jewels of the Food, Nutrition and Consumer Service. Thank you for a job well done.

(Applause)

MS. WATKINS: Well, today you are going to hear national and international speakers that are extraordinarily organized and recognized for their great work. And they are going to discuss all of these issues in greater depth on diet and gene interactions. But as Raj said earlier, none of this could have been possible

for us to attain had we not had strong support and leadership from this administration. And we're extremely fortunate to have a secretary of Agriculture who could not only talk about roads and cotton and soybeans and organic food and food safety and nutrition, but he is also able to articulate what it means to talk about production agriculture and nutrition in the same voice.

He has been extremely supportive of all of the nutrition issues. It is not uncommon for us to go to subcabinet and have a message on nutrition passed out by the secretary of Agriculture, the latest research on blueberries, or the latest research on diet and cancer. And I think his being able to connect all of the dots at the Department of Agriculture has been more than just helpful for us in looking at health risk, nutrition, hunger, food safety issues. He has had a very balanced approach on all of the issues in this department. And it is my honor to introduce our beloved secretary of Agriculture and my friend, Dan Glickman.

(Applause)

SEC. GLICKMAN: Thank you. That's the first and last time I am going to be called ‘beloved,’ probably, but from Shirley it is an honor, you know. When you are about ready to leave, they call you a lot of good things.

(Laughter)

SEC. GLICKMAN: Anyway, this is a delight for me to be here, and I want to thank Shirley and Raj both for doing outstanding work. And Raj, of course, Shirley talked about in terms of his leadership on nutrition and diet issues. Shirley, of course, has a rather large portfolio. A majority of spending of the Department of Agriculture is in her mission area. Most people think of us as the cotton or soybeans or wheat or corn department, and we do a lot of that.

But a majority of the money that we spend are in federal nutrition programs. The Food Stamp program -- we buy about a third to 40 percent of all the food that is served in over 100,000 schools every day in the United States of America. We run the Women, Infants, and Children program. We run a breakfast program. We buy a tremendous amount of food for the nation's food banks. And we are really the hunger agency as well as the nutrition agency. So this is a very large portfolio, and it is a very important portfolio. And I think that Shirley and her team have elevated this portfolio.

For years and years, this department dealt almost exclusively with traditional production agriculture and related conservation issues. And then in the early and mid-1960s, people began to realize that the consuming side of the picture was critical, not only in

terms of how Americans were fed, but also to strengthen the political base for the farm side of the picture, where only about 1-1/2 percent to 2 percent of Americans live on farms any more, and you had to have some political base to do that.

So a couple of very famous gentlemen, none other than George McGovern and Hubert Humphrey and Bob Dole, and others, were responsible to kind of bringing together a coalition of members of Congress together that worked to deal with both issues, nutrition issues, hunger issues, and farm issues. And that coalition is largely still intact today. And it was perhaps one of the great bipartisan coalitions of the modern era to allow us to keep our eyes focused on both the production agriculture issues, but at the same time deal with the serious issues of hunger in America as well.

So anyway, I thank Shirley for the great work that she has done. I thank all of our speakers. I see that all of them are doing this for nothing, right out of the goodness of their heart. I have to think about this because after January the 20th, I am looking for things to do, and it worries me that you are all doing this for nothing. But I guess I could do that as well.

Dr. Simopoulos is my neighbor. She reminded me she has seen me walk my dog in the neighborhood. I hope

I'm a good neighbor is all I can say.

In any event, my daughter works for a company that manufacturers and sells jeans. And I said I'm doing this conference on diet and genes, and she says, Dad, you can't fit into yours or anything else any more. And I couldn't help but -- I mean, that is obviously something somebody was going to say today, I'm sure.

But the fact is that these subjects are very important. And I just want to repeat, what we have done here in the department is try to get a focus on some of the diet and nutrition issues. You know, when Benjamin Franklin said you are what you eat, it is probably the greatest input into what makes up a human being and how he or she lives, how healthfully he or she lives, and how long he or she lives, is what they eat. It is the one thing we can really control in our lives. So we have taken that seriously here. And we have tried to do several things.

We have had a conference on childhood obesity, which I think helped focus the attention of the scientific community on perhaps the greatest public health issue of our time, the issue of obesity and how that relates to diseases like diabetes and cancer and heart disease, and how -- well, there are other public health issues of very great significance in terms of

smoking and AIDS and other issues. Clearly, obesity was not an issue that was elevated in the public debate. And I think we have done a great job of getting that up into folk's attention.

We held a breakfast and learning symposium, which led to a school breakfast pilot program that is up and running in six school districts around the country, and there is recent data to indicate that there is a profound impact on kids' learning ability, absenteeism, and disciplinary activities when they have breakfast. When you consider that a majority of kids of all income classes go between dinner and lunch the next day without any food, you begin to see how significant that is in terms of people's patterns.

As Shirley mentioned, we are in the middle of an important follow-up on last February's Great Nutrition Debate, which featured Dr. Atkins, Dr. Ornish, and other doctors who many of you know because they sell a lot of books. And they are also very much involved in promoting a certain kind of diet which they think is healthy for Americans.

Today I am announcing that on January 11th, USDA's research, education, and economics arm will host a public meeting on health and nutrition effects of popular diets. So this is another follow-up issue. And the

reason for this is because a great deal of Americans, millions and millions of Americans, are desperate for information on diets and what to eat. Now most people are looking for the quick fix. I understand that. That's human nature. Not a lot of hard work. People are thinking that this is an easy road to go down.

But still, I think we need to get input on how we should approach our research in these popular diets. At that time, we will also release a white paper summarizing the existing popular diet research. Diet books represent a multibillion industry, so I think they should also be subjected to the highest levels of scientific scrutiny as well. So these symposia, in which Raj has taken the leadership on, are important because they demonstrate the breadth of USDA's mandate and constituency, and they also reinforce the role of food content, quality, safety issues, and research that make up a big part of health issues and lifestyle issues in this country.

As I have said before, too often we are pigeonholed as the department of farms. That is a critical part of our constituency. We have an abundant food supply. We produce about two times as much food in this country as we consume domestically. We sell a lot and give a lot of it overseas. And we have, in my

judgment, the safest food in the world. But it is more accurate to say that we are the department of food, and that includes food production as well as food consumption. We are also involved in the whole issue of food safety.

In addition, what we have tried to do with our programs over the years, particularly our school meals programs, is to try to ensure that they meet some basic nutrition standards without the government becoming a national nanny and telling everybody exactly what to eat, because any of you who have kids know that you tell a kid to eat A, that kid will eat B. And you have to use reverse psychology and really tell the kid to eat B when you want that kid to eat A in order to get that kid to eat A. And that is sometimes a very tricky proposition.

But what we have tried to do in our school meals program is to launch a program called Team Nutrition that helps educators teach children the basics of healthy eating. And in many cases, they will bring that educational experience home with them. We have also designed a new food guide pyramid tailored specifically to the needs of young children. Last spring, in conjunction with the Department of Health and Human Services, we released the 2000 version of the dietary guidelines for Americans. We have a new interactive

healthy eating index, which you can find both on the web as well as information directly from us, which is an online tool that helps Americans assess the quality of our diets.

We have also begun a new behavioral nutrition initiative, which will use USDA's research capacity to explore the reasons behind the food choices that we make. And one of the reasons certainly food choices have to do with genetic composition. Thanks in large part to the Human

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(Laughter)

SEC. GLICKMAN: -- were words like "the naked truth" and "no gene beans." And it struck me after that -- and then, plus, we have been through a lot of other things, that the whole issue of genetics and food is a very tricky issue, for a lot of different reasons. The science is new, and it is interesting, and sometimes difficult to understand or accept. There are

preconceived views of the world, cultural views, historic views, geographical views that are sometimes inconsistent with what we might think the best science is.

The regulatory schemes are different around the world. Ours is, I think, the best in the world, but ours is developing. It is not perfect. We are changing it as time goes forward. In other parts of the world, their views of food are different than our views of food. Some places, for example, in central and western Europe, there is almost a spiritual or a religious view of food, of the meal, of the diet, which often we in America I don't think share, particularly as our eating patterns have changed. And so here we have a great opportunity to deal with the issue of genetics in food, to understand, of course, what makes our bodies tick.

But then the next step is what do we do about it. And if it is just changing our behavior, that is one thing. But if it is changing the nature of the food itself, which I think is a very likely proposition and one that we should clearly explore, particularly if we can make this food more nutritious, better for you, use less pesticides, less herbicides, keep our environment cleaner, it is something clearly that is a road we need to go down.

So the issue of food and genes and diet relate

both to the understanding of the human body, and then the decision is what do we do about it once we understand the human body, and what should our role be in modifying those foods in order to make us thinner, happier, live longer, healthier, or all of the other things that are there. And then what is the role of ethics and public policy, and just pure politics in all of this kind of effort?

I say this because if you looked at the morning paper this morning, the Europeans are going to destroy about 1.1 billion animals, cows mostly, beef cattle because of fear of Mad Cow Disease. I'm not going to prejudge whether that is a legitimate thing they are doing or not a legitimate thing they are doing. There is such a disease. There is such a problem. It hasn't affected us in the United States, and it is not an extensive problem. But they have made that decision for a lot of reasons, whether it is fear or politics or science that is governing that decision.

Well, coming back to what we're talking about today is that we have an opportunity to really explore the issues of science as they relate to the genetic makeup of people, what their predisposition is towards disease, how food interrelates to that, how it affects our diets, and then we can make some changes and

recommendations there. And then on the other hand, we can also look is there something we can do to the foods themselves to make them better.

That is more controversial. That road is going to be a rockier road to go down. But it is one that we should not shirk from at least looking at. As human beings, we should welcome new science in looking at these kinds of issues as well.

Now Shirley talked about I'm a little bit of -- I would say I have great respect for science, but I'm also one of these people that reads a lot. Some of what I read is nontraditional. So I did come in one day, and I gave people a lecture on blueberries. I said, do you know that these are some of the foods highest in antioxidants that you can find, and we ought to eat them three days a week? Then I came in one day with some recommendations on another food group. And one of our research scientists said with all due respect for Mr. Secretary, stick with soybeans, you know. You're not an expert in this; stay out of this kind of stuff.

But the fact is that people are vitally interested in food and what they eat. This is an issue that 250 million Americans are experts on. And we can't just say that some people are stupid just because they have a different perspective on a lot of this stuff. So

this is also, in addition to the science battle, this is also an issue of understanding human nature and dealing with the public in terms of how they perceive food.

I remember growing up, food was the most important thing in the life of my mother. I mean, our life revolved around food. I mean, to her it was a glorious time when she would make food. It was love. It was family. It was everything else. And as I look back, I'm not sure that the content of that food was exactly what perhaps Dr. Simopoulos and others are recommending that we eat today. And so the other thing I think we have learned is people's attitudes do change and should change as we learn more information.

For example, we have recommended daily allowances. Do those mean anything? Are they relevant to everybody? We do an average there. Are more people outside the norm than they are inside the norm? And then you ask the questions like, you know, there are certain kind of foods that are less good for you than others, and should we use genetics in a way to either change the food to make taste better, find out what people really like, and then play around with it, and then come up with something, that brussel sprouts is the best thing since sliced bread. You know, is that a right thing to do or not? Should we be doing that kind of thing?

So, you know, just getting a better understanding in the relationship between diet and genetics, I think, will allow us to move forward to help control chronic health conditions like coronary heart disease, like hypertension, like diabetes, obesity, and cancer with specific food products. But I will have to tell you out there, the public is crying for information out there, good solid information. People care about this subject very much.

When we had this debate on diet and nutrition debate, it was a little bit of theater between Dr. Atkins and Dr. Ornish and a few of the other doctors there. But -- and they both have very strongly held positions on the issues -- we had more cameras here where these three cameras are than ever in the history of the Department of Agriculture, and we have been around -- I was going to say since 1862. Back then they didn't have too many cameras. But we had more public interest on that than anything else we have ever done.

Just think about that. Think about how people care about these things in their life. So what you all do -- I know that there are a lot of folks here from various parts of the scientific community and the medical community that this is an extremely important subject. And let me just close with one final point.

Over the years, I think the medical community was extremely slow in jumping onboard and understanding the relationship between diet and health. That is changing, but it is not changing fast enough. And I think there is not enough of perhaps an information flow into the traditional providers of medicine as to what we're doing here, or maybe it is not exotic enough for them to think that it often has a major impact on people's lives.

So those of you involved in the medical community, and those of you who are in collateral professions, I think we need to do much more in terms of the training of health care providers, from doctors, nurses, physician's assistants, you name it, everybody throughout the system, to let them know the value of the work that we are doing.

So anyway, I have talked too long. But I do want to thank you for being here. This is an extremely important conference. And I know that the next administration will continue the work that Shirley and Raj started here. Thank you all very much.

(Applause)

DR. ANAND: Thank you, Secretary Glickman. We are going to start the program now. And before I introduce Dr. Simopoulos, I want to acknowledge once

again her cooperation and the cooperation of our center staff. Without their help we could not have put together this symposium. So it is my pleasure to introduce now -- we are not going to have very long introductions because all of the background is given in your program.

Dr. Simopoulos received her M.D. from Boston University School of Medicine. She is a physician and endocrinologist whose research was originally on the genetic aspect of endocrine disorders in children. Dr. Simopoulos actually has written lots of papers and lots of books, and she is really an expert on this area of genetics and diet and gene-nutrient interactions. So please join me in welcoming Dr. Artemis Simopoulos.

(Applause)

DR. SIMOPOULOS: Thank you, Dr. Anand. Good morning, ladies and gentlemen. It is indeed a pleasure for me to be here to speak on genetic variation and nutrition and try to give an overview of this very important subject, which you might be surprised to know it is not really new. It is just using the methods of molecular biology we have been able, in essence, to verify what was postulated in the fifth century B.C. by Hippocrates under the concept of positive health.

At that time, the physicians were vitally interested in disease prevention. But because prevention

has negative connotations in Greek, they called it positive health. They emphasized that:

> ‘Positive health depends on man's constitution, what indeed today we call genetics, and of the powers of various foods, those that are natural, and those that are actually made by people (today’s processed foods). But eating alone is not enough for health. There must also be exercise, of which the effects must likewise be known. The combination of these two things makes regimen. When proper attention is given to the season of the year, the changes of the winds, the age of the individual, and the situation of his home. If there is deficiency in food or exercise, the body will fall sick.’

In essence, what determines our health is the interaction of genotype and the environment on the phenotype throughout development. In other words, what we become, and the way we look, is the result of the interaction between the genetic and environmental factors. It is not a question of nature or nurture. It is the interaction of the two that determines overall health.

Now we are all very much aware that major changes have taken place in the food supply over 3 to 4 million years of evolution, and definitely in terms of

physical activity. Today we lead a rather sedentary life. So these changes in the environment in essence create a situation where the genes need either to adapt or they interfere with health.

So if we were to review the main steps in human cultural evolution, we note that the very first attempt on the part of humans was to develop stone tools about 2 to 3 million years ago. And then a million and a half years later, they were able to make fire. And then speech developed just 100,000 years ago, followed by clothing and art. But food production as we know it today did not begin until about 10,000 years ago, which in terms of evolution is a very short period of time. About 99.9 percent of our time on earth was in an environment where the people did not cultivate the food but they ate what was available in their environment, namely, lean meat and fish, wild berries, honey, fruit, and wild green leafy vegetables. That diet is, in essence, quite different from the diet that we have today. Yet the genes that we have are Stone Age genes, because the spontaneous mutation rate for nuclear DNA is estimated at 0.5 percent per million years. So for the past 10,000 years, since the agricultural revolution, there has been time for very little change in our genes, perhaps 0.005 percent. And this is a very important

concept to keep in mind.

So if we were to look at the three phases throughout evolution, the period of hunter-gatherers, which started about 4 million years ago (Phase I), and then look at the agricultural revolution (Phase II) that led to the domestication of animals and cultivation of plants about 10,000 years ago. During that very long period there were very slight changes in the food intake, but major changes have taken place the last 150 years or so (Phase III). For example, both vitamin E and vitamin C were much higher in the food supply prior to the industrial revolution. And there was a balance between the omega-6 and omega-3 essential fatty acid intake. Trans fatty acid intake was minimal because in nature less than 2 percent of energy comes from trans fatty acids. But then over the last 150 years, major changes have taken place that are characterized by a decrease in vitamin E and vitamin C, an increase in trans fatty acids and omega-6 fatty acids, and a decrease in omega-3s, with an overall increase in total fat and saturated fat.

Considering then that the genes we have are Stone Age genes, they now need to adapt in an environment for which for them is in essence foreign, and some people have the ability to adapt better than others. And in many situations, because of the interaction between genes

and various environmental factors, we may have the development of a disease. For example, people who are sensitive to gluten, and they eat wheat and rye, they develop enteropathy, because genetically speaking they have not been able to adapt to total digestion of gluten.

During the Paleolithic period, when we were actually eating wild plants, we had a much higher amount of all the vitamins, and a much higher intake of the antioxidant vitamins, than is in the current U.S. food supply, because cultivated plants contain less vitamin E, vitamin C, and beta-carotene than wild plants.

So let me focus a bit on genetics, having shown that there are major changes from the dietary standpoint that have taken place. Genetics -- most biologists today I think would agree that the definition of a gene is a DNA sequence that determines primary structure of a protein, that then provides for a particular structural or catalytic function in development.

When we began to look for genetic variation, it became obvious that humans, as well as other animals, are storehouses of genetic variability. We have a large number of gene variants. A gene variant, or an allele, or polymorphism is what makes all of us different. And it is these polymorphisms or variant genes, that actually have maintained genetic diversity. So it is perfectly

fine to have alleles and to have gene variants. The definition of an allele or a gene variant is the presence of one or more alleles at a frequency of about 1 percent or more in the population. And when studies are carried out, it has become evident that 30 percent of our genes contain variants, that is, are polymorphic. In other words, at the same locus in the chromosome, there is more than one form of a gene.

So what does this mean? It means not that the rest of the 70 percent of the genes actually do not have any variance, but 30 percent of the genes are allelic and are present in more than one form, and account for genetic diversity, and account for the fact that the human species has remained robust, and we have not all disappeared because of being terribly homogeneous.

Furthermore, each one of us is a heterozygote for 10% of the genes. Again, it is very important to understand that this genetic variation in 30 percent of the genes being allelic forms, and 10% heterozygotes, is something that is very good, and has normal physiologic functions. It is just that when some of these alleles find themselves in environments that are not consistent with their normal metabolism, then we begin to manifest disease, or we begin to show differences in how we absorb a certain nutrient or how we metabolize a certain

nutrient, or how we handle a certain nutrient.

The fact that there are differences in the genes, or genetic variability in the population has been known for many years. For example, 30-40 years ago, when we would test a population for a certain enzyme, we knew that there was enormous variation in the quantity of the enzymes. There was enormous variation for some of the proteins. Blood groups are the best example of genetic variation. Some people are type A, others type B, others AB, and others type O. And then when we look at the HLA system, the human lymphocyte antigen, we know that there is genetic variation. And certain types of the HLA are associated with a higher incidence, for example, of type 1 diabetes or arthritis.

It is easy to measure genetic variability, and it can be shown by doing (1) genetic linkage analysis. And that was one of the very early methods that was used. Or one could do other family studies. (2) And then later on, as methods became available, somatic cell genetic hybridization studies were used to demonstrate genetic variability. (3) And of course today we have molecular genetic studies, so that if you look at the level of the protein or enzymes, you see variation. (4) If you look at the level of DNA and single nucleotide polymorphisms, SNPs, you see a much greater degree of

variation.

Genetic variability has been shown to exist for many, many years. And it is now a well-established concept both in genetics and in medicine. So because of genetic variability, not all of us are susceptible to disease to the same degree or have the same biochemical levels of a nutrient.

For example, the serum cholesterol level is genetically determined to a certain extent. Studies show that 50 percent of the level of serum cholesterol is genetically determined. Blood pressure, anywhere from 30 to 60 percent is genetically determined. Fibrinogen, which is again a risk factor for heart disease shows genetic variation. In some populations, 15 percent is genetically determined, and in other populations it is 50 percent. Bone density is mainly genetically determined, about 75 percent. All of these very important factors have a strong genetic variance. And this needs to be taken into consideration whenever we plan any formal dietary studies or give dietary advice.

The importance of genetic variation and nutrition and the interaction of nature and nurture becomes very important in many of the chronic diseases, such as coronary heart disease, diabetes mellitus, epilepsy, hypertension, manic-depressive psychosis,

because all of them, we know that they run in families, and we also know that they carry a much higher risk in first degree relatives. This was known long before we had done any studies on the human genome.

And if we were to review what are some of the causes of coronary heart disease, we know that it is not really limited to a high serum cholesterol level, but it involves the coagulation system, the cellular elements of the arterial wall, inflammatory components, thrombosis, and injury. All of these are genetically determined to a great extent. There are a number of studies that show genetic variation, for example, in terms of factor VII in coagulation or genetic variation in terms of interleukin-1 that leads to inflammation, as well as having genetic variation in terms of the control of serum cholesterol level.

There are many genetic and environmental factors that are associated with coronary heart disease. The major risk factor for coronary heart disease is family history at an early age. And then the LDL cholesterol level, and HDL cholesterol and other lipoproteins that are associated with cholesterol, lipoprotein(a) [Lp(a)], LDL receptor activity, thrombosis coagulation parameters, triglycerides, RFLPs, other DNA markers, blood pressure, diabetes, obesity, insulin level, insulin resistance, and

homocysteine.

It is evident that there are many genetic factors that contribute to heart disease and that we need to be able to understand and dissect them in order to make proper diagnoses and plan effective treatment. The most important environmental risk factors for coronary heart disease are smoking and sedentary life style. About 100 years ago, 30 percent of physical activity and energy expenditure came from muscular work, whereas today less than 1 percent comes from muscular work because of the mechanical devices, automobiles, and machines that do the work. So a sedentary lifestyle and smoking, both of which are very new in terms of our evolution, are detrimental factors to our health. And, of course, diet, excess energy intake being the most serious one, followed by high saturated fat, high omega-6 fatty acid intake, high trans fatty acid intake, low omega-3 fatty acids, and psychosocial factors, type A personality and social class.

Next, I'm going to briefly give you an example of a genetic variation and serum cholesterol levels. The genes that have been associated with lipid metabolism are the genes that have been studied most extensively. And ApoE, the ApoE gene, is the one that has been studied more, both in the U.S. and other parts of the world. The

ApoE gene is polymorphic and has three alleles. One allele is called ε2, the other ε3, and the other one ε4. So these three alleles produce one, two, three, four, five, six genotypes, three of which are homozygotes (2/2, 3/3, 4/4) and three of which are heterozygotes (4/2, 3/2, 4/3). Individuals that have the ε2 allele with the 2/2 genotype have the lowest plasma cholesterol level, whereas individuals that have the 4/4 genotype have the highest cholesterol level. In the normal population, you are going to have individuals that because of the genetic variation, because of the polymorphism, because of the alleles of the ApoE gene, they will have enormous differences of serum cholesterol level, up to 16% higher level if they have the ε4 allele.

There is enormous difference in plasma cholesterol level between the mean and the individual that has the 2/2 genotype versus the one that has the 4/4. It is very important to keep in mind because the frequency of the alleles varies in different populations. The frequency of ε3 is the most common one (60 percent), whereas the ε4 accounts for about 9 percent of the population, and the ε2 accounts for about 4 percent of the population in the U.S. But in some populations, the ε4 allele has a very high frequency, almost 23 percent in

the Finns. The Finnish population also has the highest frequency of heart disease.

Very extensive studies have been carried out trying to see why individuals with the ε4 allele have higher cholesterol levels, much higher than those with the ε2 allele. In a study consisting of two groups of patients, the 2/2, 3/2, and the 4/4, 4/3 under normal dietary conditions, that is, at their regular dietary intake, the 4/4, 4/3 group had much higher total cholesterol and LDL cholesterol than the 2/2, 3/2 group. And when they were put on a low fat, low cholesterol diet, both groups lowered their total cholesterol and LDL cholesterol. But the drop was much higher in those with the 4/4, 4/3 genotype. And when cholesterol was added back into the diets, so we continue the low fat but we add high cholesterol, the only people who raised their cholesterol were those with the 4/4, 4/3 genotype.

So this is a very good example why you should not give the same advice to everybody in the population, particularly when you are dealing with populations where the 4/4 frequency is very high. The polymorphism of the ApoE gene locus would have important consequences for the plasma lipid profile since it will account for up to 7 percent of the genetic variance and 16 percent of total serum cholesterol in the normal population.

The ε2 and ε4 alleles have cholesterol lowering and cholesterol raising effects respectively compared to ε3. For this reason, the distribution of the ε4 allele in the general population has been studied in several countries. And what we see is that the distribution of ε4 varies enormously. It is the highest in people in New Guinea (35 percent of the population) followed by the population of Nigeria (30 percent). Whites in general, have a frequency of about 15 percent (Europe, average). But when you compare northern Europe to southern Europe, Finland has a much higher frequency than Italy, 23 percent and 9 percent respectively. And Sweden (20 percent) has a much higher frequency than Italy. And when you look at Europe, coronary heart disease is much higher in the north than in the south. ε4 allele, of course, is only one of the genes that will account for it.

If you are going to compare the coronary heart disease mortality in three populations, Japan, Minnesota, and Finland, you'll notice that they have a much lower incidence of coronary heart disease in Japan, and there is the highest incidence of coronary heart disease in Finland, with a mortality rate being the lowest in Japan, the highest in Finland, and in Minnesota somewhere in

between.

Now if you were to do a survey and measure the mean plasma cholesterol of these three populations, you will notice that again the cholesterol is lowest in the Japanese and highest in the Finns. If you were to measure the percent of saturated fat in their diet, the higher the saturated fat, the higher the plasma cholesterol. And when you compare the percent of saturated fat in Japan it is only 2.9 percent whereas in Finland it is 23.7 percent.

If you were to stop here and didn't do any further studies, and you didn't look at the genetic variation, you would draw the conclusion that the people in Finland have a much higher incidence of heart disease, have a much higher death rate of heart disease, have a much higher serum plasma cholesterol level, and have a higher saturated fat intake. And you would draw that conclusion that it is the saturated fat that caused the coronary heart disease. But, if you were to continue the study and do the genetics of the population, you would see that in Japan the frequency of the $\varepsilon 4$ allele is 11 percent, where in Finland it is 23 percent.

So this is a very good example where the presence of genes in an environment that is high in saturated fat is associated with a much higher risk for

heart disease. And therefore, the advice that you will recommend, it will be tailored more towards the individuals that have the ε4 and much less towards the individuals with the ε2. This way, you don't have to have people suffer to change the diet when it is not really necessary.

Again, continuing with the information about the ApoE gene, we know that women have higher HDL cholesterol than men. So coming up with a recommendation to increase the P:S (polyunsaturates:saturates) ratio for the whole population, in women it is not the appropriate recommendation because by increasing the P:S ratio you are going to lower the HDL, which is exactly what they need more of. And in the studies that have been done, the women with the 3/2 genotype, benefited the least from a high P:S ratio. But the men who had the 4/3 genotype, benefited the most, because these are the people that would absorb more cholesterol because of the ε4 allele, whereas the women would have lost their protective factor, which was the HDL, would benefit the least.

And then you know there have been many studies with oat bran, some studies showing that there is definitely a benefit from oat bran and other studies showing that there is no benefit from oat bran. When you look and see who are the people who benefited the most

from oat bran, these are the ones who have the ApoE3/3 genotype (which is the normal Apolipoprotein E gene) were those who benefited from oat bran (oat bran decreases the cholesterol in the individuals with ApoE3/3 genotype). Where those who had the ApoE4/4, the oat bran had no effect, because the effect of ApoE4/4 in absorbing cholesterol is so strong that having oat bran and fiber did not make such a difference.

In closing, I want to emphasize that in determining nutritional health, we need to take into consideration not only the food supply (the environmental factors), but we ought to understand a lot more the genetics in terms of proteins, receptors, carriers, enzymes, and hormones that influence genetically the digestion or food absorption, distribution, transformation, storage, and excretion. And in looking at individuals that have many of the so-called diseases of civilization, we ought to understand that it is the interaction of the genotype and the environment, such as diet, calories, lipids, physical activity, stress, and drugs that determine the phenotype.

In many of these conditions, physiologically speaking, we have abnormalities in insulin action so that here we have many genes that are involved in these chronic diseases, such as obesity, hypertension,

diabetes, that implicate insulin secretion, glucose intolerance, body weight, and all of them are influenced by the environmental factors for the manifestation of disease.

Until now, medicine has been in a situation where the individual who is sick goes to the doctor, and the doctor makes a diagnosis and prescribes treatment. And what we're going to be seeing in the 21st century is that we're changing the paradigm. We are going to have a DNA based diagnostic test followed by metabolic monitoring, and then will prescribe treatment to prevent an illness. We are at the beginning of the molecular era, and I think we are very fortunate to be in a country where a lot of the research in the human genome has been given top priority so that between now and 2010, we believe that we are going to have predictive tests for at least 10 to 12 conditions.

Thank you very much.

(Applause)

DR. SIMOPOULOS: It is now my pleasure to introduce to you the next speaker, Dr. Ronald Krauss, who is senior scientist and head of the department of molecular medicine at the Lawrence Berkeley National Laboratory, and adjunct professor in the department of nutritional sciences, University of California at

Berkeley, who is going to speak on genetic variation and dietary response, its implications for cardiovascular disease. Ron.

DR. KRAUSS: Thank you very much, Dr. Simopoulos. And I would like to particularly thank you and Dr. Anand for organizing this important meeting. A number of us have been involved in this area and have been convinced of the importance of diet-gene interactions for a number of years. And it is really very gratifying to see the attendance here today, which testifies to the growing interest in this topic.

Now as Dr. Simopoulos has indicated already, cardiovascular disease and prevention of cardiovascular disease involves a number of very important interactions between genes and diet that can affect heart disease risk and have a profound effect on risk. However, despite advances in our ability to identify risk factors for heart disease and understand some of the factors that control risk factors, we have not succeeded in controlling the heart disease epidemic, although we have made significant improvements. Coronary heart disease remains the leading cause of death in this country.

Now the paradigm that we have been talking about and will be talking about today is illustrated in another version of the diagram that Dr. Simopoulos showed a

moment ago. And that is that genes and diet interact to influence disease risk through effects on traits. And in the case of coronary heart disease, we have called these traits risk factors. We understand a great deal about risk factors. We know many of them Dr. Simopoulos reviewed. Among these, cholesterol ranks as perhaps the most important.

However, again, we are understanding the mechanisms by which genes influence cholesterol, and how diet influences cholesterol. But we are failing to appreciate the fact that genes have a very important effect on dietary response. And also, as we'll be hearing, from some of the work I'll be describing as well as from other speakers, diet can have a very important effect on the expression of genes.

So there is really a bidirectional connection where genes and diet interact very closely in affecting disease risks. And today, in the next few minutes, I will give you a case study of how this paradigm can be used to improve our understanding of heart disease risk, identifying those individuals most likely to benefit from specific dietary interventions. And one of the main messages is that in addition to our exploding knowledge about genetics and different gene types that we are hearing about, it is extremely important to understand

what it is that we are measuring, the phenotype or the trait.

And in the case of cholesterol, as well as many other biological variables, we are learning that it is extremely important to define in as precise a way as possible, in molecular terms preferably, the trait itself because genes operate through molecules. Molecules affect a disease. The closer we come to understanding the effect of genes on these molecules, the more likely we'll be to decipher some of these important interactions.

Now in the case of cholesterol, as we have already heard, it is not cholesterol that causes heart disease. It is lipoproteins. Cholesterol is transported in the blood in a series of lipoprotein particles, of which LDL, is the principal one that winds up in the artery wall. However, LDL is formed through a metabolic pathway that originates in the liver with the production of VLDL that actually carry triglycerides as well as cholesterol. And triglycerides are gradually broken down and used by the body for energy. And in the process, these VLDL get converted to remnant particles, partially degraded VLDL, ultimately to a particle called IDL, and then, only then, do we get the LDL.

LDLs are taken up by receptors, largely in the

liver, and that is how we control our blood cholesterol level. However, if there is too much LDL in the blood, it cannot be taken up by the liver, it can wind up in the artery wall, and it can oxidize and initiate inflammatory and thrombotic events that eventually lead to a heart attack. Now acting to antagonize this mechanism to a large extent is another lipoprotein, the HDL, high density lipoprotein, which as a protein, Apo-1, that can block some of the damaging effects of LDL in the artery wall.

So we have already, moving cholesterol to this system, an understanding of an interplay of factors that regulate not just cholesterol but a profile of various lipoproteins that overall dictate disease risk. Now how does diet impact on this system? I'm going to focus on the LDL because that is the principal target of our current dietary guidelines for heart disease risk reduction. In those guidelines, we emphasize restriction of saturated fat in particular, oftentimes in the context of low fat, high carbohydrate diets. However, oftentimes in the context of low fat, high carbohydrate diets, there is a second effect which paradoxically operates to antagonize this benefit, and that is increased production of VLDL and triglyceride by low fat, high carbohydrate diets that can actually feed more LDL into the system.

So we see that in addition to the heterogeneity, the different players we have in the lipoprotein pathway, we also have heterogeneous effects of diet. And this makes this area extremely challenging to decipher these various contributions to the net effect that we're looking for in terms of heart disease risk reduction.

There is a subset of the candidate genes that Dr. Simopoulos reviewed that are important in regulating this pathway. And you'll see ApoE is here, as was mentioned. There are many others of which there are many variants in the population. And you can see that there are rather extraordinary opportunities for variance in these genes to influence the efficacy of this pathway.

However, there is even another dimension to this story. And again, this is going to be the case study I will describe for you in the next few minutes because there is also heterogeneity in the types of lipoproteins that are involved in this pathway, and in particular LDL consists of a series of particles that are rather various in their distribution. I am going to describe those in a moment. And the net result is that when you put people on a low fat, high carbohydrate diet, you see a tremendous variation in the LDL cholesterol response.

The total LDL cholesterol in a diet such as shown here, in which we studied 105 individuals feeding

rather low versus a high fat diet for six weeks each in a randomized crossover design, we got results that are very typical for other studies in the literature, namely, an average cholesterol reduction, LDL cholesterol reduction, of about 15 percent. But you see that there is a tremendous range of LDL cholesterol response around this mean. And we really tend to think when we read articles and read reports that a diet has a given effect on LDL. This is an average. And we often fail to consider the variation around that average in this variable as well as many others. This variation is enormous. And there are people whose LDL actually goes up on such supposedly therapeutic diets.

Now as I mentioned, there are several factors contributing to this heterogeneity of response: genes, nutritional factors. But also, heterogeneity within the LDL itself. And here is shown a simplified diagram of a scheme that can separate LDL into several components based on their size, from larger particles which overlap with the IDL to a large LDL and a small LDL. Now this heterogeneity is not yet something that has been appreciated in the clinical arena to any great extent. However, as we understand the molecular basis of this heterogeneity, we're beginning to see that this has tremendous potential clinical importance, which I'll be

describing shortly.

One of the things that we have learned is that if we measure the size of the LDL, larger and smaller LDL particles, we can learn some other important metabolic facts that can influence heart disease risk. LDL particle size, which we can measure in the research laboratory very carefully and precisely in angstroms in ranging from 230 to 280 angstroms, varies very significantly as a function of the blood triglyceride level, so that as triglyceride levels go up, you can see that the LDL particle size gets smaller.

This is a group of 317 individuals, in which this relationship is quite strong. But you also see that there is a clustering, that there is a subgroup of individuals who have lower triglycerides and larger LDL particles, and another subgroup that has higher triglyceride and smaller LDL. And if we look at the distribution of LDL diameters in this and other populations, we can see that this clustering represents a bimodal distribution of LDL size, such that the majority of healthy individuals have a predominance of larger LDL particles. We call this the A group. But a significant subset of the population, anywhere from 20 to 30 percent, have a predominance of the smaller LDL. And again, this bimodal distribution speaks very strongly to an important

genetic determinant of this, which we do have evidence for, as I'll show you in a minute.

From the standpoint of coronary disease risk, the relationship of LDL size to triglyceride has opened up another dimension to this understanding of metabolism, and that is that small LDL, the pattern B LDL, is associated with higher triglyceride, but also lower LDL, higher levels of remnants in IDL, which are highly atherogenic. And also, there has been a great deal of attention given to the relationship of this lipid trait, particularly high triglyceride and low HDL, to insulin resistance, which is a metabolic disturbance that can be a precursor of type-2 diabetes, adult onset diabetes.

So we have moved from cholesterol to understanding the molecular basis of LDL to a subtype of cholesterol that is associated with an extremely important metabolic syndrome in the population that is becoming an increasing concern not just for heart disease but for diabetes and obesity and other related diseases. And we have now a handle on this, a discreet marker that we can identify in individuals that tells us about this constellation of factors which are indeed coronary risk factors and for which we have evidence from mechanistic studies that the small LDL is indeed a more potent actor in the artery wall and has more pathologic effects on

atherosclerosis than the normal form of LDL.

And I won't have time to go into all of the studies that have supported this. But we now know that there is roughly a threefold higher risk of heart disease in patients with small LDL than with large LDL. We have called this now the atherogenic lipoprotein phenotype. The small LDL can get into the artery. It actually tends to hang around in the blood longer because it interacts less well with the LDL receptor. So it has more time to find its way to the artery. And once in the artery, it binds more tightly to the artery wall and it gets more oxidized than normal LDL. And it can lead to more clinical events, heart attacks, as a result.

So we have refined our understanding of cholesterol metabolism in this direction to identify a common trait in the population associated with a threefold higher risk of heart disease, which is not revealed by the cholesterol measurement. These individuals, many of them have normal blood cholesterol levels. But it is the type of cholesterol rather than the amount that can cause the problem.

Now I mentioned that there is evidence for genetic susceptibility. The bimodal distribution of the LDL size profile is an indication of that. But we now have more formal evidence from family studies that about

40 to 50 percent of the variation in LDL particle size is genetically influenced. We have evidence both using what are called segregation models, where we look for the transmission of the trait in families, so-called Mendelian model. But we also have evidence from more formal genetic linkage studies in which we have identified five candidate genes in the pathway affecting LDL metabolism in which variance of those genes have been statistically linked to variation in the small LDL profile.

One of those genes, which I'll come back to in a moment, is in fact the LDL receptor gene. A variation around the LDL receptor gene has been linked for variation in the small LDL profile. So this is putting some real statistics and some real molecular definition behind a genetic trait affecting heart disease risk.

But here we are talking about gene/diet interactions. And this system, like many of those that we'll be talking about today, is one where genetics is only part of the story. As I say, only 40 to 50 percent of the variability is genetic. The remainder is influenced by other factors. And among these are gender, age -- this is not commonly expressed in childhood -- and menopause. It is also influenced by adiposity, particularly around the middle. And here, for the

purposes of today's discussion, we need to ask, well, how does diet affect this trait. Is diet an important determinant of this syndrome, and do individuals who have differing LDL profiles respond differently to the kinds of diets that we prescribe?

So we embarked a few years ago on a series of experimental diet studies in humans. In healthy subjects, when we stratified based on their LDL profiles and fed a series of diets in which there was variation in both total fat intake and in particular saturated and polyunsaturated fat, where we reduced both of these in parallel, substituting carbohydrate, trying to minimize variation in the other components, the carbohydrate was a mixture between simple and complex carbohydrates. And I should emphasize this is not necessarily a therapeutic diet in any way because we are really trying to keep many of the variables in the diet constant.

Ordinarily, in recommending a more healthful diet, we would try to put more fiber and perhaps more polyunsaturated fat. But this was an attempt to isolate a response to fat and carbohydrate manipulation in the diet and determine what effect this may have on these LDL subtypes.

Now when you look at the standard lipid measurements, LDL cholesterol, HDL cholesterol, and

triglyceride, we were quite pleasantly surprised to find that those with the high risk small LDL trait, pattern B individuals, 18 of these 105 men showed a significantly greater LDL cholesterol reduction than unaffected individuals. The majority of men with a pattern A trait had less than half of the LDL lowering.

We were initially concerned that the genetic basis for the pattern B trait might limit the dietary response. These people might be more resistant to diet. But as we heard from Dr. Simopoulos, sometimes it is the higher risk gene that shows the great response, as was the case with the ApoE4, which we also demonstrated in these subjects. Very gratifyingly, those individuals at highest risk are the ones that showed the most beneficial response.

Now both groups showed some reduction in HDL cholesterol, pattern A perhaps less than the pattern B. And this is a fairly common response to low fat, high carbohydrate diet, as is the increase in triglyceride, which was somewhat spiked here by the sugar content of these diets. But overall, we did not see a significant difference between the pattern A and pattern B. There is a great deal of variation in the triglyceride response, which we think has other genetic factors underlying that.

But the LDL was the one that really we need to

focus on for heart disease risk. And we can break the LDL down, as I mentioned, into large and small LDL. When we do that, we see something rather surprising. And that is that when you look at the pattern A subjects, most of their LDL is large, and the diet that we feed reduces that large LDL. That is to be expected. But what is surprising, that if we measure the small LDL in these subjects, it actually goes up. So these individuals have a reduction in large LDL but an increase in small LDL. And we can actually measure the particle number, the number of LDL particles in these subjects, and there is really no change. What we are seeing is a shift from larger to smaller LDL, whereas in the pattern B subjects, the higher risk individuals, we see a reduction in their main LDL form, the small LDL, and a somewhat lesser reduction in large LDL. Overall, a much more beneficial response, particularly in the small LDL. So there is a significant difference between pattern A and pattern B in their response to diet when you look at small LDL particles.

So what this leads to is an appreciation that there are two fundamentally different mechanisms operating to lower cholesterol in the population. In the higher risk pattern B subjects, we see the expected reduction in the number of atherogenic small particles

that these individuals have, whereas in the pattern A subjects, there is a small reduction in cholesterol. But it represents primarily a shift from the larger cholesterol rich LDL to the smaller cholesterol poor LDL. So there is actually a reduction in cholesterol, but not a reduction in the number of LDL particles. And in fact the LDL that are formed on lower fat diets resembles quite closely the LDL that we find in pattern B individuals on their usual diets.

Now this shift from larger to smaller LDL and pattern A subjects is also demonstrated when one looks at the distribution of LDL size on the high and low fat diets. This is the particle size distribution. Here we see the pattern A and pattern B modes, mostly pattern A on the high fat diet. But the shift to smaller LDL results in a qualitative quantum shift to pattern B in 36 of the pattern A men. So we can actually induce the expression of pattern B in a subset of healthy individuals on these low fat, high carbohydrate diets.

And now we have three groups of individuals. We have those that remain pattern A, those who remain pattern B on the low fat diet, and a group that switch from pattern A to pattern B. And we'll see that the only significant benefit in the ratio of LDL to HDL, which is a standard marker for heart disease risk, was found in

the pattern B individuals. So these individuals have a beneficial response in terms of reducing their more atherogenic small LDL and their LDL to HDL ratio. And we don't see the same benefit in the other groups. In fact, we even have to wonder about potentially adverse effects on risk, particularly in those individuals who induce pattern B.

So carrying out these and other studies on a larger number of individuals using various combinations of fat and carbohydrate, we have observed a rather striking relationship between reduction in fat, substituting carbohydrate, and the prevalence of pattern B. In nearly 600 men that we studied, as you go to lower and lower fat and higher carbohydrate intakes, you get a higher percentage of the population expressing pattern B.

We have hypothesized based on the genetic evidence for the effects on LDL particular size for certain genes that perhaps individuals as we lower their fat intake and increase carbohydrate are beginning to turn on one or another gene that they may be harboring in response to this diet that produces the pattern B phenotype. So this would be a gene induction and an example potentially of diet on gene expression.

So in our recent studies, we have carried out studies to test this hypothesis again using families to

show whether or not there is a genetic influence on this dietary response. We have carried out several studies in families. One just published earlier this year was carried out in children in whom the pattern B or phenotype B is incompletely expressed. It only shows up in adulthood in genetically predisposed individuals. But we hypothesize that if we looked at the parents of these children, those children with a pattern B or phenotype B parent would be more likely to carry a predisposing gene to pattern B, that offspring of B by B parents would be much more likely to have the gene than offspring of A by A parents. And therefore, genetic influences underlying these phenotypes might be detected in these children as a function of their parental lipoprotein patterns.

And so we put a group of children, 50 children, on an extreme low fat diet as a test diet, and looked at these children as a function of their parents. Nineteen of the children had two pattern A parents. Thirty-one children had either one or two pattern B parents. And we looked at the percentage of people converting from pattern A to pattern B. We found that six out of these 50 children did indeed show conversion to pattern B. And all of these children were offspring of B by B parents, indicating very strongly a genetic influence on this response.

In addition, offspring of B by B matings, had a significant reduction in their LDL size on the low fat, high carbohydrate diet, whereas there was no significant change in offspring of A by A parents. So this supports rather strongly a genetic influence on the induction of this phenotype in individuals as a function of their genetic predisposition.

And the final step that we take in this process is to actually determine which genes might be involved in mediating this dietary response so we can counsel individuals by genetic testing as to whether or not this diet, a low fat, high carbohydrate diet would or would not be beneficial. And for these studies, we involved larger numbers of families using what is called a subpair approach, where we take families with at least two brothers. We are now doing studies in women as well, of which most of them were two, but several three, four, and five siblings, healthy middle-aged men that we then feed a diet series high and then a low fat diet, looking at the lipid response, but measuring genetic variance in both the sibs and their parents to determine whether or not specific genes were associated with a diet response.

In these group of sibs, we showed, as we have seen previously, that a significant percentage convert from pattern A to pattern B. In this case, about 25

percent of men in the low fat diet shifted from the A to the B pattern. When we looked at genetic markers to see if we could identify whether one or another gene predicted this response, we focused on the LDL receptor gene locus that was one of our candidate genes for determining small LDL profiles. And in fact, P values showed the linkage with the LDL receptor locus and various LDL traits that we can measure indicating size or density of the particles.

The one I want you to focus on is the conversion from pattern A to pattern B, the A to B conversion. The change on reduce-type low fat diet was significantly linked to the LDL receptor locus, indicating that indeed at least one of the genes responsible for a predisposition to this atherogenic form of LDL can be induced or the expression of this can be induced by a low fat diet.

So to summarize, I have given you a case study of a particular system, I think an important one, related to cardiovascular disease risk in which gene/diet interactions operate such that we can determine which individuals are most likely to benefit from diets that we commonly recommend to reduce heart disease risk, and showing that heritable factors contribute to differences in LDL subclass response to low fat diets. Individuals

with a high risk trait associated with small LDL seem to derive the greatest cardiovascular risk benefit from diets that we commonly recommend for heart disease risk. But this is a minority of the population.

Again, it is a similar observation to that involving ApoE4. A relatively minor variant accounts for a significant percentage of the benefit of such diets. And so therefore, we could focus some of our energy on these individuals. It is hard to get people to change their diets. These would be the individuals most likely to benefit from these more extreme diets. And what solidifies that approach perhaps even stronger is the subgroups of healthy individuals may be genetically predisposed to adverse blood protein changes with such diets. And this has been shown for the first time to be related to genetic predisposition.

So we must consider both a positive and potentially negative effects of more extreme dietary manipulations as we direct our attention to reducing heart disease risk by diet in the population. And, of course, the gene/diet interactions involving cholesterol and lipoproteins are just a model for many other systems that you will be hearing about a little bit more today, both with regard to heart disease and other disease conditions. And just keep in mind we're still at the

very early stages of this. We're just beginning to uncover the specific genes involved and how they work. But there really are many, many opportunities for putting this information together in a way that can help promote positive health.

And as again you'll be hearing more about today, the new tools that are emerging in genomics and genomic technology will enable panels of genetic markers to identify individuals most likely to respond either favorably or unfavorably to given dietary manipulations.

Finally, I just want to mention and acknowledge the contribution of my colleagues in the laboratory at Berkeley, where we have been fortunate enough to be supported both by the National Institutes of Health and also by the National Dairy Council for a number of years carrying out these studies, and to make a plea for supporting research in this area because studies involving genetic influences on heart disease and other forms of diet responsive phenotypes in humans is very laborious, requires a great deal of effort, and we hope that more research support will be forthcoming in this next century.

Thank you very much.

(Applause)

DR. ANAND: Thank you, Dr. Krauss. We're going

to take a break, so stretch yourselves, use biological break, or have some coffee in the cafeteria. So be back here at 10:45.

(Recess)

DR. ANAND: Our next speaker is Dr. Steven C. Hunt, who is a professor in the cardiology division of the University of Utah in Salt Lake City. He received his PhD from the department of medical biophysics and computing, specializing in genetic epidemiology and biostatistics, University of Utah, in 1980. He has authored over 115 peer reviewed manuscripts, 22 invited interviews, and 15 book chapters. My gosh. So Dr. Steve Hunt. Dr. Hunt.

(Applause)

DR. HUNT: I am going to spend a few minutes reviewing some of the genes that have been involved in hypertension and how they may interact with certain dietary factors. Obviously, one of the problems with hypertension, before you can identify some of these dietary factors that interact, you have to find the genes that are involved in high blood pressure. And this has proven to be a much more difficult task than originally thought five years ago, mainly because the effects we're trying to detect are much smaller than we thought they would be. But the effect of these genes are really at

the limit of detection for some of our statistical methods that we are looking at.

One of the reasons for this is that once you get up to the endpoint of hypertension here, you have a lot of different genes that are involved that form intermediate phenotypes. And every step along the way, there is potential for confounding and interactions that can remove or mask the signals so that even if the gene tends to have a fairly moderate effect on any one of its proteins that it is making, it can be easily masked by the time it gets out to hypertension. And so one of the strategies has been to try to study the intermediate phenotypes.

But, even if you do that, suppose there is a gene that increases some level of some protein or decreases it? Well, they are all part of a system. They are not isolated events. And as soon as there is some perturbation of that system, some other factor is going to come along and try and compensate for that. And it is these compensating factors that can actually bring the abnormality almost back to normal. And it is only going to be if there is multiple -- either multiple factors or a long time period where eventually the body fails to be able to compensate any more that you are going to develop some disease due to these mild genes. And I think, that

in a classic case of high blood pressure, we see that it takes 40 to 60 years to develop this high blood pressure trait because the body can compensate for many years before finally the control is lost.

There is continuous distribution of blood pressure and there may be specific genes, such as angiotensinogen (AGT) and kallikrein (KAL). And there are many other possible genes that have been proposed to be responsible for the shift of blood pressure in the distribution.

Now, unfortunately, if you take an example of the angiotensinogen gene, you would expect that that gene should have a very sizable effect upon the angiotensinogen level, which you can measure in plasma, when in actual fact, if you do that -- I mean, here are the significant differences. You can see between the three genotypes -- here is one polymorphism in this gene. And you can see that there is a significant effect in the angiotensinogen level among the three genotypes. It was significant in three studies. It wasn't significant in another study, although this study did find a linkage of this trait to high blood pressure.

Nevertheless, this particular polymorphism explains at most 20 percent of the angiotensinogen levels. And in some studies, it has been 10 percent, so

that a gene that makes a specific protein is only explaining a small proportion of that protein's level. And so there are obviously other things that are confounding that. And obviously one of those is a negative feedback loop in the renin angiotensin system that once angiotensinogen is elevated and angiotensin-2 is formed, the negative feedback loop is activated and lowers the renin level and tries to normalize angiotensinogen.

This is another interesting interaction showing that there are a lot of important interactions going on there. This is actually with the interaction of hypertension and lipids. This was a study published by Selby back in '91. And it shows that in twins after 16 year follow-up for CHD death, those that have both dyslipidemia and hypertension, so that lipids and blood pressure are interacting, leading to increased mortality.

And so gene/gene and gene/environment interactions are really the rule. I would say they are not the exception. In a multigenic condition they cannot be otherwise because physiologic system integrate. They don't separate components as specific systems. And even if two genes are additive, it is likely that a compensating mechanism will be invoked that in turn will turn this additivity into some type of interaction,

making it statistically very difficult to identify.

And I think some of the varying results that you see in the literature for two genes commonly studied, alpha adducin and angiotensinogen, are due to this very fact that you get different populations with different frequencies of these interacting factors. And sometimes these factors mark the effect and sometimes they don't. And so you need physiological studies to go and confirm these associations and linkage studies that are being published.

So let me review three of the most important genes or the best studied genes that seem to be involved with sodium sensitivity, and also with diet, and with the development of blood pressure. And the first one I am going to talk about is kallikrein, which is a vasodilator. This is tissue kallikrein. It is decreased in hypertension. There are decreased levels in those with a positive family history of hypertension. It has been shown to inversely correlate with blood pressure. If you give a person extra sodium in their diet, you can decrease kallikrein. If you give them extra potassium in their diet, kallikrein will increase and thus supposedly protect you, lower your blood pressure. And there are familial correlations of this trait at all ages, showing that it is expressed early in childhood and that these

correlations remain fairly constant over different age groups.

Well, if you look at a pedigree study, where you again fit a segregation model, which is a statistical method to try to separate genotypes, this is the model that results if you look at the effect of urinary potassium on this axis and urinary kallikrein on this axis. So this is representative of the dietary potassium that they're taking in. And here are the three genotypes that this model predicts. It predicts there are those who are homozygotes for high kallikrein and a homozygous group for low kallikrein. The urinary kallikrein level was independent of urinary potassium. So these two slopes are fairly flat.

But if you look at this middle group, the heterozygotes, which is 50 percent of the population in this study, they can be anywhere along this curve here. And so that if they have a low potassium diet, this model would infer that they look very much like the low homozygotes, at risk for hypertension. Whereas if they have high potassium diets, they look very much like the high genotype. And so there is a strong interaction here of a dietary variable with this supposed major gene.

Now we are trying very hard to find the polymorphisms that are explaining this major gene in

kallikrein. We think we have a couple now. And we're beginning to test these particular polymorphisms to see if this model may fit. But in the meantime, the philosophy would be that those that have the high -- you can't read that very well. It says high homozygotes. So this is the low risk for hypertension, and these are the people at high risk for hypertension. And then these people would be the susceptible people that depend upon their diet.

Now let me go back to the angiotensinogen gene for a minute. Here is the M235T polymorphism, which is tight disequilibrium with what appears to be a more functional polymorphism in the promoter. I said we had to go back to physiology. So this is a study looking at the renal effects, the renal artery effects when you infuse angiotensin-II into a person.

So if you put them on a high salt diet, which makes the reactivity of the renal blood flow very much higher -- it activates this, so you can tell very easily what the person is like -- and you divide it by genotype, you see that these subjects that are the TTs that are at risk for hypertension -- this is the allele at risk for hypertension -- have much lower response to angiotensin-II in the renal plasma flow. They can't react as well. We call them nonmodulators. They have a blunted

response. It is abnormal, and they can't respond to the salt load. And so -- and these other two genotypes respond normally.

Well, let me just pause for a minute. This TT genotype seems to be the at risk allele, and yet in many populations it is the most common allele. Back to the other two talks you have already heard, this appears to be the ancestral allele if you look at any of the primates or the mouse or the rat or a few other species. This is the allele that is always there. Assuming that back in the ancient times when they needed to preserve salt, this allele helped them to preserve it. And in our current environment, where we have too much salt, this allele now becomes deleterious and we develop high blood pressure.

These are the correlates of that renal vascular response. So if you look at the baseline renal blood flow or plasma flow, you'll see that is highly significant. BMI has a very significant negative correlation so that the more overweight you are, the less response you have. HDL is correlated, insulin, triglycerides, and gender. So you have a lot of things that can confound the supposedly genetic trait that we're looking for, whereas if you were to look at another trait, instead of putting them on high salt, if you put

them on a low salt diet, it activates the aldosterone system.

And you can look at the aldosterone response on a low salt diet to angiotensin-II infusion. And if you look at the correlates of that, you no longer see obesity or lipids or insulin correlating in there. Now you get a gender difference and a mild age difference, but the others are not correlated. So when you are looking for genes, this is very important because you would rather study the aldosterone response and get away from the confounding of obesity and lipids, although there are merits for studying the other also.

Now if you were to try and predict people who have this blunted response to angiotensin-II, you can look at those that are concordant. So we have the two criteria. We have the renal plasma flow on high salt and the aldosterone response on low salt. And this is very low salt, 20 millimils per day. They get all of their food fed to them from a clinical research center, and they come into a hospital overnight. And you classify them using both criteria, whether they are nonmodulators.

It turns out that almost 70 percent of those that are TT genotype turn out to be nonmodulators, whereas only about 15 percent of the MM genotype are nonmodulators if they are concordant for that

abnormality. And it turns out that those that are discordant between the two classification criteria tend to be those that are obese so that they look like the nonmodulators because they are obese, and that makes them discordant when they shouldn't be.

So this type of classification based on salt, salt levels in response to A-II, seems to be fairly predictive for these people. But what about the genes that can represent or that can predict salt sensitivity or responsiveness to A-II? Well, if you look at the ACE polymorphism, you'll see there is a relative odds (this is from a logistic regression) of only 1.3, which is not significant. If you look at the aldosterone synthase polymorphism, again not significant. And if you look at the additivity or interaction of these two genes, again it is not significant.

If you look at the angiotensinogen gene alone, you now have a significant effect. I already showed you that there is a significant effect of this polymorphism on the response, on the adrenal response to A-II infusion, a relative odds of two. If you then add in the aldosterone synthase gene, which is responsible for the synthesis of aldosterone, you now get up to relative odds of 2.4. If you add in the ACE gene with AGT, the odds ratio jumps up to 3.7.

There is a highly significant interaction between ACE and AGT. And that can make sense because now you have two mechanisms, ACE which converts angiotensin-I to angiotensin-II -- if you have increased levels of that, you can get increased conversion -- and the increased production of angiotensinogen itself. So both of these substances together seem to be increasing the risk. And if you add a third gene in, aldosterone synthase, again the relative risk increases. But now the sample size is so small that you hardly believe that number, and we're in the process of trying to double our sample size here to see if we can actually replicate this. But you can see one of the difficulties, as you start studying gene/gene interactions, you need huge sample sizes.

Now there is evidence that there are dietary interventions or manipulations that will also affect angiotensinogen production. And this is a study in rats where they took control rats, measured the level of angiotensinogen, and then fasted them, and the angiotensinogen decreased. They refed them and there was a rebound so that it went up to 187 percent of the control value. And this is now becoming known that the adipocytes, the fat tissue, produces angiotensinogen. It is about the third most prevalent tissue that expresses

this, so that the more weight you gain, the more angiotensinogen you can produce. And this again can confound that genetic effect to any gene, but it also all by itself can mimic that genetic effect and produce hypertension. We know that obesity is one of the strongest risk factors for hypertension.

Now another interesting interaction that has been published, that goes along with the twins study, is a lipid interaction. Dyslipidemia is known to highly correlate with blood pressure. This is a study showing a systolic blood pressure change, again when you infuse angiotensin-II. And what the authors did is they took a control group of people, showing the blood pressure response, 20 millimeters of mercury increase at 20 nanograms per kilogram per minute infusion. And then they took a group of subjects who had high cholesterol. And they had a much greater response to the A-II infusion, a blood pressure response, when they had high cholesterol. The investigators took these same people and gave them a statin. And that statin reduced their cholesterol a little bit more than half. And you can see that it brought them about half way back down to the normal blood pressure response.

So by upregulating the LDL receptor with statins, they actually were able to show a reduction in

blood pressure. So lipids interact with these blood pressure genes.

We tried to replicate that in our own data. We found the exact same thing, that if you divided by tertiles of LDL, both systolic and diastolic pressures had significant increases. If you were in the top tertile of either one for diastolic or systolic, you had the greater response to A-II infusion.

So lipids also interact highly significantly with these genes and with blood pressure. And another clinical trial, a double blind crossover clinical trial, also showed that if you take people with high blood pressure and just give them a statin, pravastatin, that decreased systolic blood pressure, diastolic blood pressure, the pulse pressure, and the reactivity to a cold pressor test, so that by just intervening on lipids, you have reduced their blood pressure.

This is a large clinical trial, the trial of hypertension prevention had four arms: (1) a usual care group; (2) sodium and weight reduction combined; (3) weight loss; and (4) sodium reduction (a decrease of 40 millimol of sodium chloride per day). They followed them for three to four years. The people were borderline hypertensives and they were fairly young. The overall effect of this trial was to decrease blood pressure

significantly by a few millimeters.

We then went and genotyped these people for the angiotensinogen genotype. This is a different polymorphism, but it is in complete disequilibrium with the other one I described earlier, so that the AA is the TT genotype in the earlier study. The people with the AA genotype, after three years, had a greater incidence of hypertension than the GG group.

Even though these persons had a higher incidence of hypertension, they had the greatest decrease after sodium reduction, so that the relative odds of developing hypertension was only .57 compared to the usual care. So it is just like the other two studies you just heard, that the ones that were at greatest risk had the greatest reduction in risk when you remove one of the risk factors. And you see these same results for weight loss. The at-risk group had relative odds of only .48 after intervention. So in both of these groups, weight loss or sodium reduction, intervention reduced the risk of that high at-risk group.

This is a second clinical trial, a Dutch Saga salt trial, where they again randomized persons to sodium chloride or to Saga salt. Now Saga salt has a lot of potassium in it and a lot of magnesium, only 41 percent sodium chloride. They also replaced the salt in their

breads, their cheeses, and a lot of other prepared foods and had them eat this for six months. And then these groups -- these people were untreated hypertensives, and they were older. So they were about 15, 20 years older.

And here are the results of this clinical trial. Again, if you divide it by AGT genotype, overall -- before dividing it, overall there was a bigger effect of this study. And the effect was about 7 millimeters of mercury in this study. And then when you divide by genotype, you see that most of the effect is in these two genotypes, again the TT or the MT for both systolic and a little bit less for diastolic.

So in two clinical trials now it is shown that there is a subgroup of people defined by the angiotensinogen genotype that appear to be more salt sensitive than the other genotype.

And finally, here is another very interesting clinical trial, the DASH study, which I'm sure most of you are familiar with, where they held sodium constant, but they gave them a high fruit and vegetable diet, and then they gave another group high fruit and vegetable and lower fat diet with the dairy foods. And overall, they found a very significant reduction, with those diets, in blood pressure. And at the last hypertension meeting, they presented these results, where they subdivided this

DASH study by AGT genotype again, and they found the same thing, that those with this AA at-risk allele had the greatest reduction in systolic change with the fruit and vegetable diet, with the full DASH diet, and with diastolic blood pressure, and also the change with full DASH. There is no change in the GG genotype.

So it doesn't have to be sodium. It can be weight loss. It can be high potassium fruit and vegetables and a lower fat diet, something that will lower cholesterol, the LDL level from the previous studies. So it looks like you have this conglomerate of risk factors. And if you were to remove any one of those risk factors to a significant degree, it can counteract that genetic effect that you may be predisposed for.

And the last gene I want to talk about is the adducin gene, which is another salt sensitivity gene, and with both an acute test of salt reduction and a longer term chronic test. They subdivided it by the two genotypes. There are very few Trp-Trp alleles for this particular polymorphism. But they found about a 7 or 8 millimeter difference between the Gly-Trp and Gly-Gly genotypes and salt sensitivity. And this particular gene affects the sodium potassium ATP-ase pump. So it is on the opposite side of the cell from the epithelial sodium channel.

So here is another sodium affecting gene that seems to have a strong interaction with dietary sodium. And it is strongest in certain populations. The odds ratio on the whole population is about 1.7, 1.4, depending on the study. I won't go into this slide, but this is a way of subdividing groups statistically. And when you do that, you can predefine what group has the highest odds ratio. And the group that this particular gene has the greatest effect in is those with high BMI, older age, moderate triglyceride levels. And in that subgroup, their odds ratio is now 4.2. And if you calculate attributable risk for that, population versus the subset, the attributable risk for hypertension in the population is 17 percent.

And in the subgroup, it is 47 percent. If we find out in clinics that patients fit these criteria, it becomes even more important to genotype them to see what polymorphism they may have for this adducin gene to see if they would be responsive, possibly a 7 or 8 millimeter mercury blood pressure reduction to this salt reduction.

And so let me just conclude and say what is our goal eventually? We would like to predict CVD or treatment response in individuals. Is that practical now? Well, probably not now because the size of the genetic effects that seem to be present are very small.

And there is a huge overlap in the distribution. So on an individual level, it becomes very difficult to predict response. But we should also point out that that is also true for other things, like BMI, blood pressure, lipids, glucose, smoking. There are huge overlaps in those distributions. And yet they are still very useful in predicting group treatments.

So our goal for present may be to identify those most likely to develop CVD or to respond to treatment. And if so, then we can go on to genotype them and use genetic studies and other strategies that we'll have to really be successful in preventing or treating these individuals. Thank you.

(Applause)

DR. SIMOPOULOS: Thank you, Dr. Hunt. There is going to be a change in the program. The next speaker is going to be Dr. Robert Murray, who is chief of the division of medical genetics of the department of pediatrics and child health and professor of pediatrics, medicine and genetics at Howard University here in Washington. He is also chairman of the graduate department of genetics and human genetics in the graduate school of arts and sciences at Howard University. Dr. Murray will speak on methodology, state of the art present and future. Dr. Murray.

DR. MURRAY: Thank you, Dr. Simopoulos, Dr. Anand, colleagues, and members of the Department of Agriculture, visiting guests. I'm pleased to be here. And I must say that I am here because Dr. Simopoulos is a very persuasive person. Any of you who have dealt with her know that. We have worked together for many years going back to 1971, when she persuaded me to chair a committee that I was glad I did finally, but didn't want to at the time. In any event, this is a busy time of the semester at Howard, and I'm going to have to leave right after my talk to go teach.

For many years, researchers in the field of nutrition and fitness paid little attention to the importance of genetics and genetic variation and nutrition in their investigations. But recently, scholars, again influenced by Dr. Simopoulos and others, have recognized the necessity of including genetic considerations in any serious research in nutrition and exercise. Even human geneticists scarcely recognize just how critical genetic factors were in nutritional studies until recently, even though there has been a large body of knowledge about metabolic errors that are treated by dietary modification.

I'm going to give you a brief overview of the current state of knowledge of human genetics and

nutrition and its development, and we'll consider the current status of the progress of the genome and structural genomics, some potential areas in which this new structural genomics knowledge can be used and will be used as the basis for the next major advances in genetics, something which is now called functional genomics, and how this might relate to a proposed plan of action of research for the 21st century that will hopefully lead to reduced human disease and suffering for all inhabitants of planet Earth.

The genetic advances do not only include mapping and sequencing a variety of human and other genomes, but include developing techniques of automated sequencing and DNA based testing. The latter techniques involve the use of the so-called DNA chip technology, specially designed wafers of silicon about the size of a postage stamp which are designed to make it possible to test for tens, hundreds, but even thousands of genes simultaneously.

These advances have been considered a continuation, an expansion, of the new genetics of 1979. Some of you may remember those years in which we were studying recombinant DNA, a molecular genetic technology which related to what was called genetic engineering. And now we might deal with what can be called a new, new genetics that some have more recently called the genomics

era.

Now subareas of genetics have been renamed, pharmacogenomics, microbial genomics, or environmental genomics, because the genomes and their composition can be investigated directly. The goals, status, and expected time for completing the complete sequence of the genome has undergone considerable modification over the years. The original rough draft of the total sequence was expected to be finished in 2005, was later predicted to be completed by the end of 2003, and now the official completion of the rough draft of the genome was announced on June 26th of this year, 2000.

Things moved very quickly, especially when money was injected. It is a great motivator. This is considered by most scientists to be the most remarkable achievement of modern biological science. At the moment, all of the chromosome number 22 and almost all of chromosome 21 have already had their sequences completed by December of '99. And there are 21 specific disease genes that have been connected with chromosome number 21.

When this achievement was announced, some people suggested it was the completion of the first chapter of the book of life. And, of course, there would be 23 chapters because there are 23 different chromosomes. Now draft sequences of a number of other chromosomes were

completed in March, and we still have to call this a draft because the sequencing technology which is used is not perfect. And there are regions of the DNA which are either improperly sequenced or not sequenced at all so that we cannot say that we have a complete sequence of the so-called human genome.

In addition to the sequence of human genome, we are studying the sequences of microbes, and 11 of those have been completed. Sequencing the genomes of several nonhuman species is also underway.

This just gives you an idea of the percentage of completion of the 3 billion base pairs which supposedly constitute the DNA of human beings. And 97.7 percent -- some people will say 99 percent -- of this is completed. But when you realize that the 1 percent that may not be completed constitutes as many as 30 million base pairs, that still is a lot of information that is missing. Chromosomes 5, 16, and 19, those were worked on because they are thought to have important disease genes encoded in them and the microbes. And now the mouse genome is being given a special attention because of its homology to the human genome and because there are many mouse diseases that have been created to mimic or simulate human disease.

Some of you who are familiar know that knockout

genes are mouse models where genetic defects are artificially produced in mice to supposedly simulate human disease, and with that to be able to study the corresponding mouse genes and how they function. Realize that having the gene in hand today does not tell you necessarily what the gene does. We have now identified many mutant genes, cloned them, understand their sequence and structure, but we still don't know what function they perform, even if we may know what organ system or even what cells they function in. And that is where the idea of functional genomics comes in, and what business we need to focus on now.

Now the final representational genome that we will have at some point, maybe not until as late as 2002 or 2003, we hope will be at least 99.99 percent complete and accurate. Remember, when you are counting 3 billion things, it is easy to make a mistake, and mistakes certainly will be made. But it will not be made up of any single individual because on the planet there are billions of genomes, human genomes, and each, except for those in identical twins -- and I predict that we will find that so-called identical twins even have different genomes at a molecular level -- are different.

So we can't say that any one genome represents everybody. And then, of course, there are subgroups of

the human species, homo sapiens, which are different people in different populations of the world. And a truly representational genome would have to include DNA from the most populous ethnic groups on planet Earth. And most of the genomic work has been done in Europeans so far. And that work for the rest of the planet remains to be completed.

We are still adding new genetic material to the database. And as many as 20 million new base pairs are added to the bank, the gene bank files, central gene bank files, each night from different laboratories. And so it now holds something like 8 billion base pairs in its database. Supercomputers have been developed which make comparisons between genes of known function and new genes to find out what the similarities may be and perhaps the possibility of similar function.

So the characteristics of the final human genome will be accuracy; all lengths will fit into the original genomic DNA. It will be affordable, that is to say we will be able to sequence a particular genome at relatively low cost, and will be readily accessible. We want all DNAs to be available for scientific study, although the issue of privacy now becomes a huge one. If DNA -- people's DNA are stored in central databases, how do we keep that private from those prying eyes who might

misuse the information.

In keeping with these changes in terminology, renaming subareas of genetic study, ecogenomics for the study of total

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’Ü@ÂÜò@ÆÂæÊX@èÐÂè@Òæ@Â@èÊäÚ@îÐÒÆÐ@’@èÐÒÜÖ@æÐÞêØÈ@ÄÊ@ê È@ÒÜ@èÐÊ@ÌÒÊØÈ@ÞÌ@Üêèä0èÒÞÜ@ÄÊÆÂêæÊ@ÄÂæÊÈ@êàÞÜ@îÞäÖ@èÐÂè@ ÐÂæ@ÂØäÊÂÈò@ÄÊÊÜ@ÈÞÜÊX@îÐÒÆÐ@òÞê@ÐÂìÊ@ÐÊÂäÈ@ÂÄÞêè@èÐÒæ@ÚÞ äÜÒÜÎX@ÂÜÈ@îÞäÖ@èÞ@ÄÊ@ÈÞÜÊX@îÊ@îÒØØ@ÜÞî@ÄÊÎÒÜ@èÞ focus on nutritional research based on genomic information. And the specific definition would be the study of the genomic basis for individuality or individual variability in the response to specific nutrients or nutrient exposure.

Ultimately, the biggest payoff in completing the sequence and thusly completing the so-called book of life would be the knowledge of the function and control of expression of the working units of the structural genome.

Now one of the things that the genome work has emphasized and will emphasize even more so is that there is more variation than we anticipated, and more I'm sure will be found. So genetic variations within populations is much greater than that between populations. I'm sure many of you have heard that before. But people have tried to explain differences that we observe with respect to disease frequency and so forth based upon the population differences between populations. And that may be true. But for each human gene, there is estimated to be at least 1,000 common variants.

We focused on the uncommon ones that cause disease. We know about those. But there is lots of

variation that is common that does not produce disease but that may influence reactions to diet.

So how are we to proceed with research programs to search for functional genes and gene complexes that influence or significantly affect human nutrition and related functions? And these are four strategies that can be used. I'm sure there are many others that we'll develop as time goes on to study genes that cause nutritional disease or serious malfunction. Look at genetic variance found in animal models showing atypical or deficient function, studying animal models that show superior or more adaptive. We have tended to emphasize in our studies disease and maladaptation. But it is clear that there will be examples where animals more efficiently use or humans more efficiently use food. And that can become important, particularly in developing nations or if we have some of the catastrophes that are predicted, and it becomes important to know how to more effectively use food. And then finally, by studying teratogenic effects of selected nutrients in addition to the chemical nutrients -- chemicals that we have been examining.

Now this is a quick summary of some of the variation that we see within mammals, some human and the mammalian DNA sequences. Among monozygous twins or

identical twins, we think there is no genetic difference. Between brothers and sisters in a single family, the ratio of variance is 1 per every 4,000 base pairs, so one difference based on genetic factors or approximately 750,000 base pair differences between a brother and a sister or brothers, what have you.

Between unrelated individuals, one per every 1,000 base pairs or 3 million differences between any two people sitting in this audience who are genetically unrelated. There will be 3 million base pair differences. We are only 1 percent different in our genetic makeup from the chimpanzee, our closest relative, and there are 30 million differences, base pair differences. And the mouse, which we are using as our model, has a difference of 1 in every 30 base pairs or 100 million base pair differences. And, of course, where those differences exist and what factors they effect will make a difference in how those differences are expressed.

One of some of my colleagues that suggested we don't need to study people for different ethnic groups because we are so much all alike. But the fact is that although we are very much alike, we are also different, and we need to understand those differences.

There are already well known categories of nutrition disease dysfunction caused by single mendelian

genes available for study, inborn errors, for example, cystic fibrosis of the pancreas, hereditary hemochromatosis, and a variety of other conditions I could mention. But more important and revealing would be the study of conditions that are the result of quantitative rather than qualitative effects, those you have been hearing about with respect to cardiovascular disease and hypertension, where multiple genes interacting with the environment produce a broad range of expression, much broader than the variable expressivity seen in Mendelian traits or single gene traits, and are far better able to provide for the organism's adaptability.

Attempts to assign a particular nutritional recommendation in these conditions fails to provide uniform preventive response, probably because of multiple genetic differences, both qualitative and quantitative.

Now, of course, we are all familiar with the attempt to establish dietary standards. And those have focused primarily on disease initially, minimal dietary standards or minimal daily requirements to prevent disease or function. But they have focused on what might be considered optimal standards, that is, what is the best diet for the optimal physical and mental health according to population, the population average, or age.

And so what we would do is look at the optimal diet in relationship to the specific genotype of the individual. And you might say, well, how can we do that? Well, the improvement in the technology will some day enable us to sequence the genome from a single individual and identify the variability within a single individual. And there is a film called Gattaca some of you may have seen in which that supposedly technology had been developed in the future so everybody could be identified in a matter of minutes using such technology.

But be that as it may, if we begin there, we can then focus on some of the specifics that you have heard about already and that some of those that we will learn about as we continue our studies on functional genomics. And what we can use is a model and develop in pharmacogenetics, well advanced beyond where we are in nutrition, I believe, where multiple polymorphic variants have been related to the response of different individuals to therapy with particular jobs. And a study of these are called single nucleotide polymorphisms, single base pairs, which are called SNPs as the acronym for that single nucleotide polymorphisms. And people are now working on trying to tailor drugs to the specific genotype, say, within the enzyme function or the metabolic pathway through which a drug may be metabolized

or excreted or detoxified.

And we know that there is individual difference because many people -- there are always outliers who have a problem taking innocent -- what we might consider innocent drugs such as aspirin, for example. And I think this is a kind of model we might want to adapt in nutrigenomics. So now we would develop designer diets rather than designer drugs. And so we would have people come in for their profile. And we wouldn't just take what their cultural background is, which we consider nowadays, or what they like to eat or their various kind of nutritional history that dieticians traditionally take nowadays. But as a part of that background, we would get their genetic or genomic or nutrigenomic profile. And we would now factor that in in developing a diet for a particular person, and wouldn't necessarily have to wait until they had diabetes or hypertension. But we would take into account the predictive character of this information, namely, who are at risk and modifying their diet so they could avoid the complications.

So we would try to use this detailed knowledge of genotypic variation in relationship to genetic determinant or influence health consequences to develop these diets. So no more one size fits all diets.

Now what are some of the means we have used to study assessing various factors in a genome? Biomarkers have been particularly useful. The study of so-called DNA adducts, which are chemicals or toxins that bind to DNA, or protein adducts, which can inactivate or harm them, are associated with -- or that may be associated with an increased frequency of chromosomal breaks or anomalies. Some harmful effects may include DNA strand breakage, activation of oncogenes from proto-oncogenes, which lead to the production of organ system dysfunction and/or cancer or malignancy.

Identifying disease susceptibility and its relationship to DNA polymorphisms may also reveal useful markers. A widely use biomarker involves the assessment of oxidative DNA damage or protection by mycotoxins, dietary polycyclic hydrocarbons, et cetera, et cetera, et cetera. And none of these studies, however, that have been reported in the literature -- and there are numerous ones of these -- refer to the genotypes of the subjects. And now it is very clear that any study of these factors, these responses, must include some genotypic analysis.

A number of studies have been made on the influence of diet on the expression of the P53 gene and its mutations, which mutations are related to the

causation of cancer in a number of organ systems. These assessments also include the effectiveness of DNA repair mechanisms. Additional studies designed to evaluate the effectiveness of influencing this biomarker need to be performed.

Now there are, as I say, a number of oxidative studies that have been used adding such factors as tomatoes, vitamin C, red wine, and so forth, all of which are thought to reduce the risk of a variety of common disorders. But I would like to call your attention to the comments of a colleague, geneticist Dr. Bruce Ames, who is a well-known geneticist and toxicologist. And he suggested that we should focus on some things which we could still influence without knowing a lot about our specific genotypes, suggesting that there are many micronutrients that we don't pay much attention to but he thinks are important dietary parameters that may set us up for susceptibility to malignancy or other chronic diseases.

He thinks that these mimic radiation effects that we see in animals. And this is a preventable source of DNA damage. He also points out something which some of us would like not to hear about, namely, that there are a variety of naturally occurring carcinogens, and that this exposure to them is much greater, in his view,

than the artificial or chemical ones.

One of this is aflatoxin, found in peanuts. And those of us like myself who like peanut butter, and who love peanuts, don't like to think that every time you throw a handful in your mouth, you are taking in some potentially carcinogenic agent. Nevertheless, Dr. Ames' work has not been proven in humans, but he has got animal studies to support his contention.

So one might add to Dr. Ames' observation the requirement that of equal or greater importance is a specific genotype of the individual who is exposed to those agents. And none of his work takes that into account. And that would definitely affect the genetically controlled protections and the genomic repair mechanisms.

And finally, in the genomic age that is upon us, we can look forward to the development of systems of nutritional treatment as well as prevention that will be developed as a consequence of the hoped for advances that can be expected in the new and exciting field of nutrigenomics. Thank you.

(Applause)

DR. ANAND: We're going to spend the next 25 minutes to give you the opportunity to comment or ask questions. There are two microphones on both sides.

Please keep your comments as brief as possible so the speakers can answer.

Dr. Krauss, would you come up here? Dr. Hunt and Dr. Murray. And in the meantime, I would like to really acknowledge the tremendous work done by the Center for Nutrition Policy and Promotion. I would like to acknowledge some of the staff members who are here, Dr. John Webster -- John, could you raise your hand?

(Applause)

DR. ANAND: Andy Fitzgerald, Nancy Gaston. Would you stand? Kim Thigpen. Would you all stand, all the staff who is from Center of Nutrition Policy and Promotion? Would you stand? Come on, stand, Peter. Peter, Carole, Carole Davis, Peter Basiotis.

(Applause)

DR. ANAND: Thank you very much. They have done tremendous work and I'm really proud to have a staff so good as this staff. So now come on, please. Could you have the lights on, please? Thank you. So please come forward and ask any question or comment on the presentation that you heard this morning, please. Introduce yourself.

MS. TALLMADGE: Okay. I'm Katherine Tallmadge, a consultant nutritionist in Washington. I have a question about the types of fats and how they may affect

various genomes. I understand that cutting saturated fat across the board for most genomes is important for lowering LDL. But it also may -- cutting total fat may have a harmful effect. Could you talk about specifically whether you get a beneficial effect from mono- or polyunsaturated fats or omega-3s? What are the most beneficial fats, and who needs to have those types of fats in their diet in terms of genotypes?

DR. SIMOPOULOS: Well, that is a very good question. And let's start first with total fat. From the evolutionary standpoint, we need to moderate the amount of total fat in the diet. However, many studies, particularly the seven country study, showed very clearly that the people in Crete who had the lowest rate of heart disease and lived the longest, they had a diet where the total fat intake was about 37 to 38 percent of energy. That study and other studies have shown that what appears to matter is the saturated content of the diet. And this becomes important for people who overeat, who are obese, and who are inactive.

There are minimal data for people who are not obese and who are active that there is no need for them to lower the saturated fat intake because the physical activity takes care of the metabolic changes that are beneficial, such as lowering LDL and increasing HDL.

When it comes to the essential fatty acids, the omega-6 and omega-3, it is important to try to keep them in balance. By that we mean that the ratio of omega-6 to omega-3 shouldn't be more than four to one. And the reason I say that four to one is because this is what was shown both in terms of the diet of Crete as well as the Lyon heart study, where two groups were studied. One group was put on American Heart Association diet, step one, and the other group was put on a modified diet of Crete with a ratio of four to one of the omega-6 to omega-3. And the death rate from heart disease and total mortality decreased by 70 percent in those on the diet of Crete.

So the ratio of the essential fatty acids is very important. The omega-3 fatty acids should be included in everybody's diet because they have antithrombotic, anti-inflammatory properties, and they also lower triglycerides without affecting HDL so that if I were to summarize everything I have said, the ratio of the essential acids becomes very important, four to one or less, eating fish two or three times a week, or take fish oils if you do not like fish. If you are a vegetarian, using oils that are high in omega-3 fatty acids such as canola or using flax seed or flax seed oil to improve the omega-3 content of the diet is another

factor.

Who should be on this type of diet? I would say this is the type of fatty acid content that is consistent with evolution. So in essence, I would say this is the type and amount of fat that everybody should include in their diet. And those who are predisposed to disease conditions, they are to make appropriate modifications depending on their condition or disease.

DR. ANAND: Dr. Krauss?

DR. KRAUSS: Yes, thank you. Considering that this is a session on diet-gene interactions, I think it is important to point out and anticipate I think what Dr. Jump will be talking about a little bit later this afternoon, and that is that the unsaturated fatty acids, the omega-3 and omega-6, are potent regulators of gene expression. And this could have enormous consequences for individual variability in both beneficial and perhaps adverse effects of high consumptions of either of these fatty acids.

Now let me just mention one other issue brought up by Dr. Simopoulos in regards to the population-wide effects of such diets. First of all, having written the American Heart Association's recent dietary guidelines, I would hasten to point out two things; first, that the Lyon study actually didn't study the step one diet,

although it claimed to. There was actually a high saturated fat intake on the control diet. However, the benefits of supplementing the diet with foods containing omega-3 fatty acids, I think, are clear not only from that study, from others. And the prior population probably would benefit. And in fact, in our recent dietary guidelines, the American Heart Association has now advocated the inclusion of fish at least twice per week in the diet.

However, coming back to the gene issue, there are some reservations. We know that particularly the omega-3 fatty acids can have beneficial effects reducing heart disease risks and perhaps other beneficial anti-inflammatory actions. But they may also affect the coagulation system. And there are some concerns about how much omega-3 fatty acids, for example, are safe to take in the population. And I think the kinds of recommendations that Dr. Simopoulos put forward are certainly consistent with overall health. But they shouldn't be taken to an extreme where some individuals might be predisposed perhaps genetically to possible coagulation problems with such diets.

So we still are on the edge of learning where we should make major changes to take advantage of some of the biological benefits of these particular fatty acids,

where some of the risks might be. And this is, I think, where the era of nutritional genomics, particularly understanding fatty acids effects on gene expression, might be very, very valuable.

DR. SIMOPOULOS: I think I ought to continue the discussion. The omega-3 fatty acids have been used, particularly EPA and DHA, in the form of fish oil for the past 15 years. And in many situations, it involved following patients for at least three and a half years. But during the time period, there has never been a single case of clinical bleeding, number one. Number two, what they do is people who are obese, people with diabetes, hypertension, heart disease, and the elderly, they have a decrease in bleeding time, which the omega-3 fatty acids in the diet bring it within the upper limit of the normal range so that the increase in bleeding time is not an abnormal time.

Thirdly, there have been a lot of patients that have been given omega-3 fatty acids two to three weeks prior to angioplasty or even quadruple bypass, and these people have not bled. So I think it is very important to understand the difference. There is no evidence of clinical bleeding with omega-3 fatty acids. The dosages that have been used by physicians -- and these are people followed by physicians -- have been between 3 and 5 grams

of omega-3 fatty acids per day.

In the GISSI study, where they followed the patients for three and a half years on a Mediterranean diet, they used close to 1 gram, basically 850 milligrams of a ratio of EPA to DHA of two to one. These patients did not manifest any abnormalities whatsoever.

So I don't think you should worry about that. The safety, I would say up to 3 grams per day of omega-3 fatty acids, has certainly been shown. In patients who are followed by psychiatrists, they use higher levels, but these are followed very closely by physicians.

So what is the adequate intake of omega-3 fatty acids? When we had the meeting of a workshop a year and a half ago at the NIH, we recommended an adequate intake of about 650 milligrams per day. This is considered safe for anyone.

DR. ANAND: Dr. Krauss?

DR. KRAUSS: Yes. I think it is important to keep those numbers in mind. One of the concerns, I think, in talking to the general public, and our experience in heart disease I think exemplifies this, is if you pick a particular nutrient and you responsibly give guidelines for certain levels of intake, there is a tendency for people to say, well, if so much is good then a lot more would be better. We're dealing with a

tremendous problem in this country of people focusing on one particular magic bullet for disease prevention. And when we identify something important like omega-3, for example, and we give responsible recommendations, we must reinforce the message that that doesn't mean that people can then multiply that by a factor of two or three and reduce their risk even further. And that is, I think, one of the messages that we want to convey in terms of responsible nutrition.

And if we ever hear that there may be some genetic subgroups that might benefit from megadoses of a particular nutrient, that should not be taken to indicate that the entire population would benefit from very high doses. And I don't think we need to discuss what that should be. I think the data that Dr. Simopoulos described certainly holds up well. But some people don't consider those numbers. They just think about the foods. And we really have to be careful that this is a quantitative science.

DR. ANAND: Next question, please. Go ahead.

DR. FERRARI: Hi. Serge Ferrari at Harvard, and I work in the osteoporosis field, which is another common disorder, of course, with a big implication in public health and has many related issues to the disorders we are talking today.

So I want to go back to the gene and particularly to that question of how good predictors those polymorphic genes we are all finding in our specific disorders are going to be. The parallel between osteoporosis and cardiovascular disease, that in both cases we have an endpoint. For us, it is the fracture; for you, it is the myocardial infarction. And in both cases, we have pretty good predictors or risk factors that you call hypercholesterolemia or high blood pressure, and we call low bone density.

So the question is, knowing what we are knowing now, which is that all those disorders are going to be determined by tens, dozens of genes with polymorphic variance that is going to count for maybe less than 5 percent of the variance of those traits, what is really our hope or what are the thoughts that underlie our hopes that those variants are going to be a better predictor than the simple measurement of what we already have, bone density for fracture or blood pressure for cardiovascular disease? How much of a progress can really do at the individual level with those alleles versus simple measurements that we can already make?

DR. ANAND: Dr. Krauss?

DR. KRAUSS: Yes. That is something that I think we have all thought about quite a bit. And it is

certainly true for some of the traits we have discussed this morning: lipids, cholesterol, blood pressure, that one can readily determine whether and how much one responds to a given diet in a rather short period of time by simply trying that diet and measuring the response. And I would actually endorse that approach in trying to optimize diets for many individuals.

However, it is a little bit of a scatter shot type of thing. And what we're looking towards in the future with more advanced technology and a lot more data is being able to use multigene profiles that consider, for example, the important issue of gene-gene interactions to be able to add up some of the variances that may be individually rather small and come up with a composite risk.

Now that is a futuristic view that is based on genomics and genomic technology and lots of data that we hope would be forthcoming. But the current application of what we are talking about clearly does translate to looking at the genetically influenced traits themselves as endpoints for some of our dietary experimentation, so to speak. Each individual represents an experiment with an end of one. How is that individual going to respond to diet? If we measure the right things, we can certainly tell that individual a lot. We hope that one

of the messages of this whole program is that the public can understand that there are such differences and that there are genetic factors, even though we are not yet ready to put the formulas together to put that into practice.

DR. ANAND: Dr. Murray?

DR. MURRAY: I'd like to just expand a little bit on Dr. Krauss's comment about the genetic profile. For some years, we have known that if you -- you can take a dominant disorder, a gene, and let us say we use something like the mouse because we can't do appropriate genetic manipulation in humans, and that that gene will express itself to varying degrees from absolutely no expression to very severe expression based upon what we call genetic background of the mouse.

Of course, we now know that that is the composite of genes contained in the genome of the mouse of a particular strain, and that one genetic background leads to severe expression. The other modifies the expression so that it is barely expressed at all.

To some extent, we have that exhibited in sickle cell anemia. We have now identified some genotypes that are related to something called restriction fragment length polymorphism profiles, and so that we can predict, based upon a particular profile whether a patient will

have a very severe form of disease within some parameters, of course, or whether they'll have very mild disease, or whether they'll have hardly very little expression of the disease until very late in life.

So people from different parts of the world may have the same SS genotype, but in one case they hardly are sick at all, or they may get sick only occasionally, and others are sick from the time they are a few months of age, so that that kind of information suggests very strongly that having a profile of genetic markers, not any single one, not any one SNP, for example, but a series of these will give us a stronger predictive power. And as a statistician, you speak like one, you know that any prediction you make is subject to limits based upon how strong your data are.

And the more such variance we have, the stronger, the more reliable our predictors will be. And when we can take into background the whole of the genetic background of the particular individual we're looking at, then we'll be in a much better position to say, yes, you will get the disease if you live long enough; or, no, don't worry, you'll be okay.

DR. ANAND: Next question or comment, please.

DR. TOBIN: Brian Tobin, representing Mercy University School of Medicine, and also the 21 academic

institutions involved in the nutrition academic award program. Secretary Glickman hit on two comments that I think is probably very near and dear to most of us, and that is, one, that the public is really crying for good information on nutrition; and second, that the biomedical community has been very slow to react to advances certainly in nutrition, and also advances in diet-gene interactions.

The good news is that there are 21 medical schools currently involved in putting together a unified curriculum document for the training of nutrition to physicians, and that curriculum document will include some 20 different areas where nutrition needs to be incorporated in the academic community of physician training.

It has recently been decided that diet-gene interactions need to be incorporated into the document. And I would like to ask Dr. Krauss and Dr. Hunt, Simopoulos, and Murray what their perspective would be on effective ways to incorporate the information that you have into the training of our student physicians because it is my opinion that it is sorely needed.

DR. ANAND: Okay. Go ahead, Dr. Krauss.

DR. KRAUSS: Well, student physicians will be practicing medicine well into the genome era. And I

think the best thing that we can do is prepare them for the avalanche of knowledge that is going to be forthcoming from the Genome Project. I think it is probably premature to give them particular information about specific genes and how they might be used in clinical practice. But I think from a training standpoint, this is the time to get them tuned into how to use the material that will be coming along.

In the meantime, I think there is an important message, again dealing with the one size doesn't fit all approach to practicing dietary medicine, just as we are applying it to pharmacological medicine. And I think it is a conceptual message perhaps rather than right now burdening them with information that is probably enough in a state of flux to make it obsolete in two weeks sort of thing.

So I think it is an orientation and it is an approach to the individual patient that gives an understanding of biochemical and genetic individuality and the need to follow parameters that don't assume that a given diet is going to have a given result on blood pressure or cholesterol, but follow that patient. All too often, we are seeing my colleagues in the medical community prescribing drugs that are supposed to reduce the risk of heart disease and writing a prescription is

thought to be the end of it.

In fact, that is just the beginning because there has to be follow-through and a determination that the desired effect is forthcoming. And I think that approach to the practice of medicine for physicians really ought to carry over into diet as well.

DR. ANAND: Dr. Murray?

DR. MURRAY: I can't say too much about diet, but I can about genetics. The primary care -- the Center for the Education of Primary Care physicians has established a program of training centers in which they will provide modern genetic training for primary care physicians. This is becoming a part of their board requirements for certification.

But prior to that being introduced, they are setting up these centers. And I was just part of a large committee that helped set up some of the criteria for the content and which content was included, genetics and nutrition. But it was only a small part of the whole picture. So we are not just waiting for medical students to come out, but they are trying to deal with primary care physicians who have been out in practice and who will practice for the next 20 or 25 years so that they can also provide appropriate information and counseling to their clients, their patients, and so forth.

In the Washington Post on Saturday, some of you may have seen an article about disorder alpha-1 antitrypsin deficiency. This is not necessarily a nutritional deficiency, but has certain implications for nutrition and diet. And I'll refer you to that for some of the things that are being done to try to upgrade the knowledge of the practitioners and other health care givers in the community.

DR. SIMOPOULOS: Actually, I'm involved with a panel that has developed a video for primary physicians emphasizing the importance of genetic variation, genes and chronic disease, and of course genes and nutrition. But, going back to the medical school setting, I think it is important to develop concepts that relate genes to nutrients the same way we have departments of pharmacology where they have developed very good concepts relative to drugs and genes. And I'm hoping that this approach might be able to actually elevate of nutrition education in medical schools. It seems to me that you should grasp at the opportunity to point out the importance of molecular nutrition and gene nutrient interactions. I think these concepts are to be included in medical education. And at the same time, the primary physicians and others who are in practice also are to be educated in this area. I don't think we should continue

with the universal dietary recommendations for the prevention and management of chronic diseases because they are not appropriate.

DR. ANAND: Dr. Krauss, you had a comment?

DR. KRAUSS: I was just going to say that this is entering the medical mainstream, this whole area. In the journal JAMA, read by most practicing physicians, in this next week there is going to be a report appearing about the importance of familial factors influencing the cholesterol lowering response to diet from some of my colleagues in Dallas. So I think physicians do need to be educated as this field advances. And I want to strongly echo what Dr. Simopoulos said about using molecular insights to elevate the role of nutrition in the medical community.

I think nutrition has been set aside in general, in part because the science behind nutrition has never really seemed to be on a par with many of the other rapidly advancing areas in modern biology and medicine. I think that is wrong, and I think that marrying nutrition to molecular genetics and biology is a way to present it in its proper context.

DR. ANAND: We have time for one more question or comment, and then in the afternoon again we have set aside time for discussion and questions. Please go

ahead.

AUDIENCE MEMBER: Yes. Where can people get tested for these various genotypes, and how?

DR. MURRAY: Where can they be tested?

DR. ANAND: Yes.

DR. MURRAY: You can go through a medical genetics center or genetics clinic, which is usually set up with a comprehensive counseling, as well as access to such testing. Such centers are listed centrally through the American Society of Human Genetics, which is actually based out in Rockville here in the Washington area, through the March of Dimes, National Foundation March of Dimes, and most major medical centers now have divisions and/or departments of medical genetics or clinical genetics.

You can also contact companies that do such testing. Most of these companies are on the Stock Exchange. So if you see a company that has gene or something like that in its name, it probably does genetic tests.

What you have to be aware of, however, is the fact that getting the result of the test is one thing, but having it interpreted is important, that the implications of the test differ according to your specific family history, your specific health status, and

a variety of other factors that need to be taken into account when you get the test result.

Some of you know of horror stories of women who got the BRCA-1 gene test, got a positive result, and then had their breast amputated, only to find out they were at low risk, if at risk at all, because they had the gene. There was no family history, and therefore they were not at increased risk. But they were not properly advised about the significance of the test in their case.

So counseling is an important part of any genetic testing that may go on. Also, be leery -- again, I refer you to this Washington Post article -- of who is giving you the counseling. Lots of physicians out there don't know what they are talking about. And there is the story of a woman who was given bad information about a particular genetic test, and so she is suffering the consequences of it.

DR. ANAND: Okay. We are going to break here now, and we'll reconvene at 1:30 this afternoon. Thank you very much.

(Whereupon, at 12:05 p.m., a luncheon recess was taken.)

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A F T E R N O O N S E S S I O N

(1:35 p.m.)

DR. SIMOPOULOS: Good afternoon. We are ready now to begin the post-lunch session. The first speaker is going to be Dr. Anne Molloy. Anne is the senior experimental officer at the department of clinical medicine, Trinity College, Dublin, Ireland. And we are very pleased that she took time to come and speak at our meeting. The topic of her presentation is, "The Role of Genetic Variation in Establishing Nutritional Requirements: Folate, a Case in Point."

Anne has contributed enormously to the understanding of the role of folate in neural tube defects. Anne?

DR. MOLLOY: Thank you very much. And first of all, I would like to say thank you very much to you and to the USDA for inviting me here to give this talk on something that my group -- I work with Professor John Scott in Trinity, and a lot of you may have heard of him. And we have done a lot on this topic over the past few years.

I'd like to start by showing you more or less the conventional view, which I think has been said this morning. One would have a conventional view of nutrition

that a good mixed diet provides an adequate supply of all nutrients, except in certain circumstances such as pregnancy; and secondly, that while nutrient requirements vary for different groups, such as infants and elderly, that within any groups the requirements are the same.

Well, what I would like to do today is to show you that in the case of folate, at least, that this may not quite be true. And I want to focus on a variant in a folate metabolizing enzyme, which is common in the population and which has been shown to alter folate status.

Folate is needed for two really big important cycles in the human cell, or in any cell. And the first cycle has to do with DNA synthesis. It is required to provide purines and pyrimidines, and thus it is involved in cell proliferation. And the second cycle it is involved in is a cycle of methylation, where it methylates DNA, protein, neurotransmitters -- a wide variety of molecules in the body. And in that circumstance, it is involved in gene expression.

And thirdly, it is required to maintain low levels of homocysteine, and that you probably have heard about homocysteine in the last few years. And I'll tell you a bit more about it.

So what I wanted to do very simply here is to

build up a picture of what folate does in the cell, just so that you'll see exactly where this enzyme is that I'm going to talk about and what its place is in relation to folate nutrition.

So first of all, dietary folate is absorbed and enters the bloodstream as 5-methyltetrahydrofolate, so it has a methyl group attached to it. And to get into cells and to be retained in cells, it has to go through this enzyme system, methionine synthase, which is a B-12 dependent enzyme system, in fact, which is a little bit interesting, but I'm not talking about it. But it has to give up this methyl group to homocysteine. And then it's in the cell. And as tetrahydrofolate, it can be retained in cells. It can't be retained as 5-methylfolate.

So the next thing, once it is in cells, is it picks up one carbon unit. It is a carrier of one carbon unit. So you can see these one carbon units as formal groups or as methylene groups. And those then are given up into the synthesis of purines and the synthesis of pyrimidines. So they are involved in DNA synthesis.

But the other side of the coin is then is where does that homocysteine come from? Well, in fact the homocysteine comes from methionine, and it is the other cycle of folate metabolism. So if you look at the other cycle, you see that methionine is converted to this high

energy molecule, S-adenosylmethionine, which gives up that methyl group to a variety of acceptors, and the coproduct of these methyltransferase reactions is S-adenosylhomocysteine, which is converted to homocysteine. So there is a cycle there that involves homocysteine and the resynthesis of methionine via a folate dependent enzyme.

So if you look at the overall picture, you can see that homocysteine has a key place in this entire system. And you can also see that when cellular folate is replete and no more uptake of dietary folate is required by the cell, these methyl groups are sequestered from the DNA synthesis side of the picture to remethylate homocysteine, and the enzyme that does this is MTHFR. This enzyme is a very important enzyme in folate metabolism because it specifically channels the one carbon units away from DNA synthesis and up into the remethylation of methionine.

If that enzyme isn't working properly, or if there isn't enough folate in the diet, or if other systems go wrong within that methylation cycle, the level of homocysteine begins to rise, and then it begins to spill out into the blood. It can be catabolized via the cystathionine synthase enzyme. But that only happens in the liver.

So you can see here that there is complex system, which is there to maintain homocysteine at the correct level, and also to maintain the proper functioning of folates. If you have something going wrong with the system, and if homocysteine levels do rise, they can cause problems. And we now know that moderately elevated plasma homocysteine is associated with increased risk of coronary artery disease, peripheral artery disease, cardiovascular disease, complications of pregnancy, including miscarriage, preeclampsia, and other adverse pregnancy outcomes, renal disease, diabetic retinopathy, a variety of other types of vascular problems. And also, more recently, it has been shown to be associated with neuropsychiatric disorders, cognitive dysfunction, et cetera.

So clearly, anything which promotes higher than normal plasma homocysteine levels may increase risk of disease. And so I want to look now at the enzyme MTHFR because this is the enzyme where we have found this variant. As I said before, it is a very important enzyme in folate metabolism. It is cytosolic. It is a homodymer. You probably wouldn't be interested in some of this. But in case anybody is, it is localized in chromosome-1, and in vivo it carries out this irreversible reaction which commits folate bound, one

carbon units to methionine synthesis.

As it turns out, this enzyme is the most commonly found inborn error in folate metabolism. That is not to say that it happens very often. But about 50 cases worldwide of severe MTHFR deficiency have been found. And these children who are born with this disease usually have a variety of problems, including neurological problems, developmental delays, and they also have cardiovascular disease.

Very many different mutations have been found, and some patients have been found to have three or four mutations. These are the severe mutations. And generally, they have less than 20 percent activity. So we are not dealing with those because they are a specific event and a specific problem.

What I am going to talk about is this C677T variant. And this variant was discovered about five -- in 1995, insofar as it was identified. But it was postulated to be present for about ten years prior to that. It is missense mutation converting an alanine into a valine in the enzyme. It is thermolabile in vitro. And really, that just says that when you analyze it in the laboratory and you heat it first, it has less residual activity. And what we would say is it is a slightly more wobbly enzyme when you look at it in vitro.

But when you look at people who are TT homozygote for this variant, they have mild enzyme deficiency, and they have mildly raised plasma homocysteines, so that clearly the enzyme is not functioning as well as the normal or wild type enzyme. And in fact, it varies widely throughout the world. So, there would be a smaller number of people who would be TT homozygotes, and we would say that people who are TT homozygotes are really the ones who are at risk of these problems.

It has a very high incidence in South America. You would find TT among South American Indians to be in the region of 35, 40 percent. It is very low in Africa, and you would find that people would have sometimes -- in some populations, they have found no TTs. But it is very low prevalence all together.

In Europe, it is somewhere between those. In the Irish population, which we have studied, it is about 10 percent. In the Italian population, it is about 16 percent. So it is even varying within the communities of the EU. And you can see here and throughout the world it is in fact quite widely varying.

A number of studies have looked at persons with mildly elevated homocysteine. This is just one example of a big study where they looked at a large group, large

population. These were men. And there were 280 CCs, 273 CTs, and 72 TTs. And when you look at the serum folate, the serum folate is slightly lower in the TTs than in the other CCs. When you look at the homocysteine, the plasma homocysteine is higher in the TT group than in the CCs. And what this group has done, which is what a number of groups have done, is they have taken the median folate level and they have looked at the homocysteines below and above the median plasma folate level. And they found that really this high homocysteine problem seems to be all associated with those who happen to have folate below the median level.

So if you are a TT person, and if you are in the bottom half of folate status, then the homocysteine will be higher, much higher than it should be compared with other groups who are in the bottom of their folate status, whereas if the folate level is reasonably high, then appears to be no difference between the TTs and other groups.

So we asked the question, do the 5 to 15 percent of people who are homozygous for this mutation have inadequate folate status as evidenced by the red cell folate? And to answer this question, we took two groups of people originally. We had a group of nonpregnant women, 318, and we had a group of pregnant women, 242,

who subsequently had normal babies, or babies that were not affected by NTDs.

And in fact, when we were answering this question, we had this population already analyzed. We were looking at other aspects of neural tube defects. And so we just asked the question, well, how does their folate status look if we just categorized them by this genotype? We measured our folates by microbiological assay, which has traditionally been known as the gold standard method of analysis. And looking at the genotypes, there was no difference -- there wasn't a major difference in terms of the genotype distribution. There was a slightly higher difference, but it was not significant, and there were two normal population groups anyway of women.

So when you look first of all at the plasma folate, you find that in the pregnant mothers we found that the plasma folate was significantly lower in the TT group, in the women with this variant compared with wild type. We didn't find this among our group of nonpregnant women, who were all staff of one of the big maternity hospitals. And that is not -- it is not surprising. It is something that we often do find is that the plasma folate may be lower, may not be lower. It depends on the person's day to day folate status.

But when we looked at the red cell folate status, we found in fact that when you looked at the wild type, the TT group had a significantly lower red cell folate, both in the pregnant and in the nonpregnant group. And this was highly significant in the pregnant group. It was significant against the CCs, as well as against the TTs when you did a post hoc test, a post nova test.

We showed that the TT group had a lower folate cellular status than the other groups, than the CC or the CT group. When you look at that by distribution, you find that the group who have the TT genotype have about 20 percent lower folate status than the other two groups. And if you look at that in terms of the population at the time, about 30 percent of the women who were TT had red cell folate status less than 200 micrograms, which would be regarded as a borderline of sufficiency/insufficiency. So it was drawing those people down into quite a low folate status.

Now since then, a lot of work has to be done on the MTHFR, and another function and the aspects of it. And I have just put up two studies here, one of which was done by Quere et al, and the other by Jacob Selhub's group in Boston. And what this group here did was they didn't look at the overall red cell folate status. They

looked at the methylfolate status in red cells. And red cells, when they mature, all of their folates tend to be converted to methylfolates. In normal people, you would not expect to find anything other than 5-methylfolate in the red cell of a person or in the plasma, indeed.

And when they analyzed the red cell folate content of the 5-methyltetrahydrofolate content of the red cells, they found that it had dropped to almost 50 percent in the TT group. And also, they found that this correlated very strongly with the increase in plasma homocysteine.

So this was another way of looking at the same thing that we had seen. And later on, Jacob Selhub's group had a look to see whether there were other types of folate there. And they showed that normally one finds only methylfolates in the red cells. You don't find any form of folates. But in these TT groups, they found that there was about 29 percent of the folates present as formyl folates rather than as methylfolates.

So in other words, there was a shift in the distribution of folate. Normally you would find in the plasma and in the red cells all of the folate present as methylfolate. What Bagley and Selhub were finding was that some of the folate was left over as the formyl folate rather than all being converted to methyl folates,

which was just another way of saying the enzyme clearly isn't functioning as well as it should do.

Very recently, in a paper that came out this year, a Norwegian group looked at the riboflavin involvement. Now why would they want to look at that? Well, MTHFR involves FAD. And FAD, there was a very nice paper last year on the structure of MTHFR by Martha Ludwig and Rowena Matthews. And they showed that in fact this amino acid is not near the active site of the enzyme, but it is in such a position where it involves or where it affects the binding of the flavin to the protein, and that in people who have valine instead of alanine, the FAD is more loosely bound and tends to dissociate more easily.

So this was a very nice reason for looking at the riboflavin, the possibility that riboflavin status might be involved. And when they looked at the riboflavin status, they found indeed the higher homocysteine levels. They found that folate was lower, but not significantly so. And indeed, they didn't find that the riboflavin was significantly lower. But when they looked at correlations or associations between homocysteine and riboflavin and folate, they found that it was inversely associated with plasma homocysteine only in the people who had the T allele, but not in the CC

subjects.

So suddenly there was an interaction here of vitamins with this enzyme that one normally didn't see in the wild type enzyme. So the next question that I was trying to look at was do subjects who are homozygous for the mutation have a different plasma response, plasma homocysteine response, to supplementation with folic acid than other individuals.

So there is a lot of work being done on this in the literature. And really, to sort of summarize the type of work that has been done, and to try and get from the literature any evidence that it might be affecting it -- and the summary that I have put together is that TT homozygotes certainly have a higher plasma homocysteine for a given folate status. So it is not just that they have lower folates, but for a given folate status, they have a higher plasma homocysteine than CC homozygotes.

And there is also a stronger relationship between plasma homocysteine and blood folates in the TT than in the CC homozygotes. So in the case of this particular genotype, it seems to be strongly explaining the variation in red cell folate, or the variation in homocysteine that is due to folate, whereas in other groups the association is strong enough, but isn't quite so strong.

Conversely, TT homozygotes have a greater percent reduction in plasma homocysteine in response to folate supplementation. Their homocysteine levels are higher, and if you give them folate supplementation, they drop lower.

Now I haven't actually teased out whether that is a real effect of the TT homozygotes or whether it is a fact that people who have higher homocysteine anyway respond more to folate because that has also been found. And then you have this problem of regression to the mean, whereby if somebody has a high level and you do something, their level will drop lower -- further anyway. So that is a tricky one to kind of say, is this something that is happening with respect to the TTs or whether it is something that would happen anyway. But certainly they do respond very well to folate supplementation.

So to summarize in that part of it, the variant is a cause of low folate status in the general population. And a substantial minority of the population may have a higher folate requirement because of the specific gene, and current dietary reference values do not reflect this requirement.

So to look at the emerging, just to back off a bit and to look at the emerging view of folate nutrition,

it is now considered from a lot of work in the last number of years that inadequate folate status -- and I do not mean deficient folate status; I mean less than optimal folate status -- has been regarded to be an important risk factor for a number of chronic diseases, as well as certain congenital malformations. And these include neural tube defects, cardiovascular disease, some cancers, and neuropsychiatric disease.

What does this mean in terms of MTHFR? Well, there have been hundreds of papers in the literature in the last few years looking at the MTHFR variant in all of these different problems. And to summarize it, I think the general consensus would be that MTHFR is not in itself an independent risk factor for any of these diseases. But it is a cause of high homocysteine and low folate, which are risk factors. So it is another problem that one might have which might lead to higher homocysteines or lower folates.

And I'll just give you the example of neural tube defects very briefly. We know that neural tube defects of incomplete closure of the neural tube, that you can have spina bifida, or other defects, and that it happens early on in pregnancy. We all know that folate can prevent neural tube defects. And we also know that most women carrying affected fetuses do not have blood

folates in the deficient range. But they do have lower folate and B-12 status, and they have higher plasma homocysteines than control mothers. And a lot of us who are working in this area are looking to see if congenital -- if genetic abnormalities in folate related enzymes might account for this.

We have looked at a very large study. We have probably done the largest study in the world in Ireland because it is such a prevalence country for NTDs. We have looked at 218 complete families. And we have confirmed that in the Irish population at least, the TT genotype is a risk factor for neural tube defects, and that the risk is mostly residing in the cases the mothers and fathers have intermediate risks, which is expected if they are carriers. It is not a risk factor -- this TT genotype does not appear to be a risk factor in some other populations, but it certainly is in the Irish population.

So the risk factor, accounting for about 13 percent of the population attributable risk, suggests that the case, rather than maternal genotype, is crucial in conferring the risk. So we asked the question, could the MTHFR variant be explaining the low folate status in our NTD mothers? So we had a group of NTD mothers where we looked at the cases and controls. There were 82 NTD

affected mothers. This was early in pregnancy during an NTD affected pregnancy. We had 261 controls. And there was a slightly increased proportion of TT mothers among the NTD mothers than among the pregnant controls. But this is what is expected because we know it is a risk factor for NTD in our population.

And to summarize that result, we had previously shown that red cell folate is a risk factor for NTDs and that there was a graded risk of NTD, which is inversely proportional to the red cell folate status of the mother. And this is early pregnancy red cell folate. The lower a woman's red cell folate was, the higher the risk of having an NTD affected birth was.

So we re-analyzed those mothers then having excluded our TT genotypes because we didn't have enough to do the analysis on TT mothers alone. But having excluded TT genotypes, we found that really the risk wasn't changed that much, so that although TT is a cause of low folate status in the population, although it is a risk factor for NTDs, it is not contributing that much to NTDs, and there are other factors presumably out there.

So it is more common in NTD affected mothers, but it is not a major cause -- and it is a major cause. It is not a major cause of low folate status in these NTD mothers. There may be other genetic variants which cause

NTD risk interacting with the embryo or the mother's folate status.

And just to show up this work by Mitchell et al., which was published in 1997, they looked at red cell folate analysis on monozygotic and dizygotic twins. They had quite a large number of pairs of each group of twins. They concluded that virtually all repeatable variation in red cell folate is attributable to genetic factors. They found that 46 percent of the variants in red cell folate was attributable to additive genetic effects.

So really, our nutrient status is under an enormous amount of genetic influence. A lot of other people are looking at other folate genes, the methionine synthase gene, the methionine synthase reductors gene, the CBS gene. They are looking at the effect. These are the changes which are known. They are polymorphisms which have now been discovered. They are looking at the effect which these have on homocysteine status, and they are not finding very much, to be quite honest. They are only a very small effect. And really, at this stage, the MTHFR one is the only one which is significantly affecting folate status.

So finally, just to say the future, while a good mixed diet will probably prevent overt signs of folate deficiency, it may not optimally reduce the risk of

certain events such as neural tube defects, cardiovascular disease, and colorectal cancer. And altered nutrient status may eventually be shown to be a common result of genetic variance.

And I would just like to acknowledge my collaborators. I work with Professor John Scott, who is an international folate expert. We collaborate with the Health Research Board in Dublin, and we have had a long time collaboration with Dr. Jim Mills and the NICHD and Dr. Harry Brody in the Human Genome Lab. Thank you very much.

(Applause)

DR. ANAND: Our next speaker is Dr. Donald B. Jump. Dr. Jump is a professor in the department of physiology and biochemistry and molecular biology at the Michigan State University, East Lansing, Michigan. Dr. Jump received his PhD in biochemistry from Georgetown University in Washington, D.C. Please join me in welcoming Dr. Donald B. Jump, who will speak on nutrients and gene expression. Dr. Jump.

DR. JUMP: I would like to thank Dr. Simopoulos and Dr. Anand for inviting me to present, and also the USDA for sponsoring this session.

Well, my job or my assignment today was to basically talk to you about nutrients and gene

expression, and give you an overview of the recent progress in our understanding of how particular nutrients influence gene expression.

What we are dealing with here for the most part is nutrients as promoting adaptation. And for many cell systems, adaptation is an important facet for survival. And we have known about adaptation as a component of changes in gene expression of bacteria and yeast for well over 30 years. And there are a number of clear cut models for them, lac operon being one of those examples.

But in higher organisms, the nutrient effects on gene expression are oftentimes obscured by the effects of hormones. The recent progress in both molecular biology and the ability to culture certain cell types in vitro has enabled investigators to sort out the nutrient effect from the hormonal effect. You will see that these mechanisms are very complex, oftentimes involving changes in metabolism and changes in gene expression.

What is meant by gene expression? Basically, we have a biological response in the cell. A cell changes its metabolism, its growth, and differentiation. This is usually brought on by a change in the activity of a protein or the abundance or both. If it is an abundance issue, it is due to a change in the mRNA encoding that protein, and oftentimes due to a change in transcription.

That implies that there is some factor within the nucleus of a cell that has undergone some change in its capacity to either turn on or turn off transcription, and that is really what I'm going to focus on today. And basically, there are two general classes of nutrients that will do this, micronutrients -- clearly vitamin A and vitamin D use nuclear receptors to mediate their effect -- and the macronutrients, carbohydrate, cholesterol, and fatty acids.

Today I will talk primarily about cholesterol and fat, two of our major problems in human health, and their effects on gene expression.

I just want to remind you about cholesterol. We had heard a lot from Dr. Krauss this morning about cholesterol and its pathogenic effects. I just want to remind you that there are good facets about cholesterol serving as components from membranes, making the regulatory steroids, as well as bile acids without which we would have a hard time absorbing fat from the diet. It gets to be a problem when we have high cholesterol in the diet, which turns out when it is packaged into lipoprotein particles is a risk factor for arterial disease, atherosclerosis.

And so what I would like to do first is to tell you a little bit about how cholesterol regulates its own

metabolism.

We now have a fairly clear understanding of how cholesterol is regulating its own metabolism. And this is basically the work of Brown and Goldstein. And essentially, what goes on here is that in situations of low cholesterol, there is a tendency of a cell to upregulate its capacity to take cholesterol from the circulation by increasing the capacity or number of LDL receptors, as well as the capacity to synthesize cholesterol. And there is a very complex sequence of reactions. One of the enzymes is HMG CoA reductase.

And we know now that the effect of cholesterol in low cholesterol is to induce transcription of these genes, and high cholesterol to repress transcription of those genes, so that implies that some place back here in the nucleus, where we have the genes encoding these particular proteins, there is a gene where there is a response element that is binding a protein. And that response element is identified as a sterol response element (SRE) that binds a protein called the sterol response element binding protein (SREBP).

The idea here is that what cholesterol is doing, it regulates the nuclear content of SREBP in the nucleus, and it does so by essentially regulating a proteolytic cleavage event that occurs with a precursor that is

tethered to the endoplasmic reticulum in the golgi. And there is a protease -- actually, there are two proteolytic steps that are involved in which cholesterol is regulating those events, so that with high cholesterol, this proteolytic event is inhibited, and as a result you can't make this form of SREBP that moves to the nucleus and stimulates transcription of genes.

In low cholesterol, the protease is activated, and therefore we can make this protein. It moves to the nucleus, and we turn on transcription.

Now this is a wonderful mechanism that again Brown and Goldstein worked out that provides us with a novel insight of a nutrient sensing system for a cell. There are problems, though, when this system gets messed up in that there is either disregulation of this system, or there are problems with overproduction of HMG CoA reductase, or a down regulation of the LDL receptor, as we heard about earlier with saturated fat diets.

I should also remind you that this particular enzyme, HMG CoA, which is at the top of a sequence for making cholesterol in cells, is really the target for the statin drugs. And this is the mechanism that is used pharmacologically to control the production of cholesterol.

Now I want to turn our attention to dietary fat,

and this is the area that we spend most of our time with. I just want to remind you of the big three, saturate, mono-, and polyunsaturated fats, and the kinds of fat that we see in the diet. And when you have a problem with dietary fat is an issue of both quantity and type. When there is too little, particularly of the n-3 and n-6 fatty acids, we have essential fatty acid deficiency. And when we have too much saturated fat and n-6, which is the common problem we have in western societies, we have the onset of chronic disease.

The other problem is that in terms of type, where there is an imbalance of the saturate, mono-, or polyunsaturates, where there is not enough polys in the diet, and too much saturates, that is a contributing factor in the control of the LDL receptor and plasma cholesterol.

Well, getting to this chronic disease notion of dietary fat, there are now a number of situations where dietary fat has been implicated in the onset of progression of chronic disease, insulin resistance, which is involved in the onset of a type-2 diabetes, obesity, which is a risk factor for a number of diseases, certainly coronary artery disease, atherosclerosis, hypertension, and certain types of cancer, which is probably the most controversial area of dietary fat and

chronic disease.

Now I want to start here and just try to remind you of some facets of how dietary fats are doing things to cells. And I want to tell you about the cost of the production because this provides us with the notion of essential fatty acids, 18:2, linoleic acid and 18:3, alpha-linolenic acid.

Linoleic acid, 18:2, humans cannot make this particular fatty acid. We obtain this through our diet through the ingestion of vegetable oils. And that can be elongated and desaturated to a more complex fatty acid called arachidonic acid. And arachidonic acid, which goes into membranes, can be released from membranes, activated, and metabolized by a variety of enzymes. One of those is cyclo-oxygenase that gives rise to compounds that are referred to as eicosanoids. Eicosanoids are oxidized forms of 20-carbon fatty acids. These are released from cells and released locally into the interstitial fluid. And they can react with receptors -- these are G protein linked receptors on the surface of cells -- and change second messenger levels. And in doing so, they can either turn on or turn off various cytocine cascades that have effects on existing proteins or nuclear factors, and nuclear transcription factors.

So one of the common events that you see with

this sequence, particularly if it is involved in inflammation, is the production of cytokines, inflammatory factors like TNF-alpha, or changes in metabolism, or changes in the production of vascular cell adhesion molecules.

Another interesting facet that is seen here is particularly with N3 fatty acids. Now we can take alpha-linolenic acid and elongate that to eicosapentaenoic acid (EPA) through our metabolism, or we can obtain EPA directly in the diet in the form of the fish oils which were mentioned earlier.

This particular fatty acid is a competitive inhibitor of the cyclooxygenase system. However, it is possible to generate eicosanoids of the PGE3 class that have a potential ameliorating effect, or as we heard earlier, an anti-inflammatory effect on many processes.

Now I want to also introduce you to another factor that is in this sequence of events, and that is called PPAR gamma. Now PPAR gamma -- we'll hear a lot more about that in a few minutes -- is a nuclear receptor for fatty acids of oxidized type. So there are a number of oxidized fatty acids that can affect binding. Some of the eicosanoids can bind. Some of the HETEs can bind. And in fact, perhaps variants of EPA can bind.

And what is actually rather interesting here is

that binding and activating this particular receptor leads to events that also appear to be anti-inflammatory in its response.

A couple more issues about PPAR gamma in both human and rodent physiology is that it is a major player in adiposite differentiation, and there are a class of drugs, such as troglitazone, that are used to activate PPAR gamma. Those particular drugs are playing a major role in dealing with this problem of type-2 diabetes because it is a so-called insulin sensitizing drug.

The other facet is that this particular receptor has effects on lipid metabolism through the induction of lipoprotein lipase. This is an enzyme involved in clearance of fatty acids or triglycerides from the circulation. And there is a notion that it may be helpful as a target receptor in dealing with cancer. Basically, the idea is that PPAR gamma activation leads or pushes cells to a differentiated state, which is what you want to do, and get them away from a growth state.

Next I will talk about n-3 and n-6 polyunsaturated fatty acids and their effects on hepatic metabolism. The liver plays a central role in whole body lipid metabolism. And basically, as we heard earlier today, we get fats from the diet. They came in the form of chylomicrons. Fatty acids are in the cells that can

be incorporated in the complex lipids that are used within the cell or sent out in the form of very low density lipoproteins.

It turns out that n-3 and n-6 fatty acids have unique effects on hepatic metabolism. One of those is that there is an effect on oxidation. So we can take fatty acids and oxidize them, and the n-3 fatty acids are particularly potent in doing that, more so than the n-6 fatty acids.

The other thing that the liver can do, particularly in rodents and not so much in humans, but it can happen in humans as well, is the synthesis of fatty acids de novo. And both n-3 and n-6 fatty acids do that. The other thing that is pretty clear that is a very dramatic effect of N3 fatty acids is their suppressive effect on the production of triglycerides in terms of the production of the LDL. And this accounts in large part for the hypolipidemic effect of the long chain or 20-carbon omega-3 fatty acids.

We are dealing with two basic events, suppression of fatty acid synthesis and induction of fatty acid oxidation. We're shifting metabolism of fatty acids. And we're going to use the N3s to keep things simple, and we're going to look at two different transcription factors here. One is PPAR alpha, and the

other one is a family member of that one we were talking about before, SREBP-1-C. And what is going to happen here is we are going to either change the activity or the abundance of a transcription factor in the nucleus that can account for these major changes in metabolism.

Well, PPARs are peroxyzome proliferator activated receptors, and they are essentially involved in whole body lipid metabolism. They are major players in this process. They are members of the steroid receptor supergene family in which there are many, many types of receptors. But these are basically fatty acid receptors. And there are three types. There is an alpha, beta, and gamma. And the alpha type is the one that we are going to be talking about now because it is the predominant form that is seen in the liver.

PPAR alpha is a class two nuclear receptor. It binds the DNA in association with another receptor, the retinoid X receptor (RXR). RXR is a variant of the vitamin A receptor. PPAR/RXR heteradime bind DNA, at a response element, a so-called DR-1, upstream from a regulated gene. Two PPAR-regulated genes include acyl CoA oxidase and Cyp4A. They are involved in nonmitochondrial fatty acid oxidation.

So certain fatty acids can be bound to PPAR. Certain hypolipemic drugs, such as the fibrates also bind

PPAR. Ligand binding to PPAR activates transcription of these oxidation genes, which in turn elevates fatty acid oxidation.

We now understand, through the work of Frank Gonzalez and his collaborators around the world, that PPAR alpha is essentially involved in nearly all facets of fatty acid metabolism in terms of how you get a fatty acid into a cell, the fact that it binds to proteins within cells, so-called fatty acid binding proteins, and its oxidation in the mitochondria, the peroxyzome, and the microsome. So this is a major player in orchestrating whole body fatty acid metabolism.

Several years ago, because of this involvement, we interacted with the Gonzalez group here at National Cancer Institute and asked the question of whether PPAR alpha could account for all of the effects in the liver, and we found basically that it could not. We used what is referred to as the PPAR alpha null mouse, which was developed by Frank and his colleagues, and basically that is a genetic manipulation where you inactivate this receptor through a homologous recombination. And what those studies showed is that PPAR alpha was required for the oxidation mechanisms, but not for the fatty acid effects on the synthetic processes making fatty acid such as the generation of fatty acids and the enzymes involved

in that process.

So that brought us to another player that was emerging as we were in the process of dealing with PPAR. The Brown and Goldstein group had described the potential effect of SREBPs on fatty acid metabolism. They had already done the work on cholesterol metabolism. And so we jumped into this field and asked the question of whether this could be a potential target for fatty acid manipulation. And what we basically found was that SREBP is a major target, and this actually is for me to point out that there is a division of labor in cells on how these SREBPs work.

There are three subtypes in cells, but in the liver we see predominantly this one, 1C, and 2. Two is a major player in cholesterol homeostasis. And we have already heard about that. And 1C is a major player in the synthesis of fatty acids and triglyceride synthesis. And again, like we saw with PPAR, this transcription factor is orchestrating the transcription of a whole slew of genes, a large number of genes involved in glycolysis, fatty acid synthesis, and triglyceride synthesis. It is induced by insulin and is repressed by leptin. And leptin, as you know, is that hormone that comes out of our adipose tissue, the so-called satiety hormone, and it functions as an inhibitor of SREBP.

Well, to make a long story short, what we found was that in dealing with lipogenesis and low PUFA containing diets or a mono- and saturated fatty acid containing diet, you see an induction of lipogenesis, and you put polyunsaturates in the diet, and it goes down. And this is due to a transcription mediated process. And if we work our way back to the genes, we identify so-called fatty acid response elements. And it turned out that in a number of the genes, these response elements would contain a site that would bind this particular transcription factor, SREBP-1C.

So what was going on, basically, polyunsaturated fatty acids were suppressing the nuclear content of SREBP. But in contrast to what we saw with cholesterol metabolism, the prominent effect did not seem to be here at the protease step, but it appeared to be down here at the mRNA step. And so fatty acids basically were regulating what appears to be the rate of turnover of the mRNA. That is not a transcriptional effect. It is an mRNA turnover effect.

So what you find now with the SREBPs is that we have this division of labor that one sees involved in the control of lipid metabolism. And it is a target for polyunsaturated fatty acid suppression. It acts at the pretranslational level, it is very rapid, and that it

basically suppresses the precursor in the mature form. And one can go through and demonstrate those studies. The SREBP-2 system in cholesterol is acting at the post-translational level, and it basically is a conversion of a precursor to a mature form of the protein.

Now it turns out that in some cell types, SREBP-1C is over-expressed. It can be seen in obese mice, the genetically obese mice, which is associated with an induction of fatty acid on triglyceride synthesis, and fatty liver. And the interesting observation is either in a hepatocyte model like ours or a transgenic model, where you over-express SREBP, you no longer are able to suppress SREBP by fatty acids. So there is some change or distortion that occurs.

This is actually fairly important because of a concept developed by Roger Unger and his colleagues at Dallas dealing with lipotoxicity, and that is the over-accumulation of unoxidized lipids in tissues, such as the liver, skeleton, muscle, and so on. And this may be related to insulin resistance and heart dysfunction.

So what you want to get from the macronutrient story is that they are doing multiple effects on cells. We are having clearcut effects on cell function. But you cannot forget that they are playing a major role in energy production, major component for structure,

structural elements, and also the generation of certain signaling molecules.

The take-home message is just to remember that it is an adaptive response. The macronutrient control uses many of the same kinds of regulatory mechanisms that have been described previously for endocrine system. And it is a mechanism to integrate these dietary signals with internal networks. Macronutrients such as dietary fat essentially are interacting with the genome through certain transcription factors like PPAR and SREBP. Glucose, which I didn't talk about, acts with insulin to help its assimilation into lipid. Cholesterol and fatty acids are feedback regulators of their own synthesis. And fatty acids enhance their own oxidation.

Under novel therapies, I think one of the things you should recognize is that the fibrate drugs, which have been in use clinically for many years are now known to have a molecular target. That target is PPAR alpha. It turns out that n-3 fatty acids also utilize PPAR alpha as a target. And so there is a method for n-3 PUFA to modify hyperlipidemia through the regulation of PPAR.

The PPAR gamma binds the glitazones, e.g. the troglitazones. PUFAs also bind to PPAR gamma and may modulate insulin resistance. There is no drug that I know of that targets the SREBPs directly, but there are

drugs that target cholesterol synthesis, such as the statins. But I think that this is an area that needs to be developed, using new approaches to target these particular transcription factors.

And finally, I just want to tell you who is doing some of the work on the dietary fat side of things for us. Michelle Mader, Bing Ren, and Annette Thelen were the principal players from our laboratory; Frank Gonzalez and Jeff Peters from National Cancer Institute. And I can never forget the funding opportunities that were given us by National Institutes of Health and the USDA. And thank you.

(Applause)

DR. SIMOPOULOS: Thank you, Dr. Jump. The next speaker is Dr. Savaiano, PhD, who is the dean of the School of Consumer and Family Sciences and professor of foods and nutrition at Purdue University, West Lafayette, Indiana. Dr. Savaiano will speak on dietary management of lactose intolerance and environmental adaptation to genetic variation.

DR. SAVAIANO: Thank you for inviting me here today and for hosting this symposium. I'm going to take quite a different tact from some of the talks you have heard, and I'm going to give you an example of environmental and biological adaptation to a genetic

situation. It is a very old genetic situation that has been fairly well described in the historical literature.

The issue of lactose intolerance, though, as a genetic area of study in the last 10 to 20 years has been really quite devoid. What has been done in the last 10 to 20 years is the fuller realization of the ways that we can manage a high calcium or a dairy based diet if one is lactose intolerant. But let's start first with some of the genetics and some of the information we do have.

From human biopsy studies, it is clear that there are two populations. There are individuals who after the age of three to five maintain a high level of intestinal lactase. They are about 25 percent of the world's population, about 75 percent of the U.S. population. We can call them a variety of names: lactose digesters, lactose tolerant.

Then there are a group of individuals, the vast majority of humans, 75 percent of the world's population, all other mammals, cats, dogs, mice, rats, et cetera, who lose 90 or 95 percent of their intestinal lactase activity sometime after weaning. In humans, it is about three to five years of age. It varies across species. But that evidence is fairly convincing.

And so we have two distinct populations. Listening to the talks today, you might ask what is the

variation within those populations? What is the variation in terms of enzyme activity or enzyme expression among the maldigesters or the digesters? Very good questions, which we don't have answers to.

We do know that, though, the lactose persistence is a dominant trait, and the lactose nonpersistence is recessive. We do know that, and that it is simple Mendelian genetics, two alleles, a single gene locus. At least that is our best guess. And we have come up with a variety of terms. Somebody who does not maintain their intestinal lactase activity could be called lactase nonpersistent. Scientists are great at this wordsmithing. The reason we use that -- we don't want to call them lactose intolerant because as you'll see, they are not lactose intolerant. I'm going to convince you by the end of this talk that people who you thought are lactose intolerant really are not. In fact, hopefully, I'll convince you that lactose intolerance is not an issue at all, and that we really ought to ignore it. There aren't too many scientists who want you to ignore their research area, but I'm convinced, and I hope to convince you of that by the end of the talk.

There are also individuals who acquire lactose or lactase nonpersistence who become lactose intolerant due to some secondary situation, whether it be

malnutrition, the flu, traveler's diarrhea, et cetera. Anything that takes the mucosal lining off the intestine will pretty much give you an acute case of lactose intolerance. And then there are very rare occurrences of a congenital deficiency, which we really won't talk about today, but they are very rare.

Okay. So with that background, if we look across the world's population at individuals, and we put them into those two categories of digesters and maldigesters, or persistent or nonpersistent, what we find is that in northern Europe, the vast majority of individuals are digesters, 90 plus percent, maintain high levels of intestinal lactose and digest large amounts of lactose.

Of course, lactose, I should remind you, is the only real carbohydrate found in dairy foods. It is the sole source of carbohydrate in dairy foods. It is a disaccharide, and it is split, of course, by a lactase or a betagalactylsidase. If we move to Asia, we would find theoretically 100 percent of Asians to be lactase nonpersistent, 100 percent of Native Americans to be lactase nonpersistent. We find about 70 percent of Africans and about 70 percent of African Americans.

We talked about the Masai group earlier, the Masai tribe. It turns out if we look at individual

populations -- and this is where the genetics actually gets very interesting, and I'm not sure I believe the party line. But there are three groups in the world, northern Europeans, the Masai tribe of central Africa, and the Bedouin tribes of the Near East, who all maintain intestinal lactase activities. If you look at biopsy data, indeed you find that. And the belief is independently, these three groups had genetic changes that took place, some thousands of years ago when they began using milk as a food for adults. And they gained some evolutionary advantage to using milk as food for adults, whether it allowed women to bear children because they had stronger bones and would not have breakage of bone during childbirth, or it prevented diarrheal disease -- there are a number of theories as to why there might have been an evolutionary advantage.

But at least the best theories of the day are these three populations separately evolved with similar kinds of genetic changes. I find that hard to believe. I think with what we know about modern genomics, there are differences between those three populations. We don't know what they are yet, but there may well be differences.

If we look in the U.S. population, and we calculate based on 1990 census data the number of

individuals who are lactose maldigesters, we come out with a number of about 29 percent. If we move forward to the projected populations for 2025 and do the same calculation, the number is up to a third of the population. And, of course, American dietary habits evolved to a great extent out of northern Europe, and we are great users of dairy foods and lactose containing foods. So what we have in the United States is a growing group of individuals who are lactose maldigesters and a long established culturally accepted use of dairy foods as a source for calcium and other nutrients.

The question is, are those two things at odds? And I think I am going to hopefully convince you they are not by the end of this talk.

Why all the concern? Well, the concern I think very much relates to a number of the issues we have talked about today related to optimum diet. What is the optimum level of calcium in the diet of a human? If one were to go back to look at some of the early data on the hunter-gatherer diets and do calculations, and those data have been published, it is estimated that with a plant-based, meat-based, bone-based diet that our ancestors were getting 1,500 milligrams of calcium a day.

Americans -- at least American women from Hanes-III survey -- are getting somewhere between 500 and 800

milligrams a day. One can look at dietary standards in the United States, and those numbers, of course, range depending on age and gender and so forth anywhere between 800 and -- well, depending on who you believe -- 1,400 or 1,200 milligrams a day. Nonetheless, there is by most experts' agreement a significant calcium shortage in the United States and around the world.

If we think about how this might have evolved, most food sources that occur naturally the hunters-gatherers might use, would be quite good sources of calcium. And the development of grains and agriculture as a way to provide much of the food supply really reduced calcium intakes. Grains are one of the few poor sources of calcium. And in fact, we consume high amounts of grains.

That was really the beginning of reductions in calcium consumption among populations throughout the world. The United States and other developed countries have certainly maintained those low levels for some time now. So the concern is adequate calcium. And the question is, as I'm going to address it, can dairy foods be used by individuals who are lactose maldigesters, who have the potential for intolerance without symptoms? This has become a public political debate. I'm going to show you some data, and we'll stay out of the political

debate.

What is the concern? What is the risk of using lactose sources as someone who is a lactose maldigester? The risk is that a portion of the lactose may reach the large intestine, and in the large intestine it will be fermented by bacteria. The fermentation products will include short chain fatty acids, hydrogen, methane in some individuals. And flatulence is something, at least in this society, we look at as a negative. If we were in France, it might be quite different. There are cultural norms about flatulence, as I think you are quite aware.

If excessive doses of lactose reach the large intestine, there can be acute diarrhea. It is self-limiting, and once it is over, there is no further health risk. But it can occur. Those are the only two downsides to the question of lactose intolerance. Of course, individuals might consider those substantial.

Let's look at some of the evidence. There are a variety of factors that influence whether or not an individual who is a lactose maldigester will have symptoms. And they are very simple factors. We are going to do GI physiology for the rest of the presentation, which is quite far removed from genomics, very simple GI physiology.

Dose is a critical response, and I'll show you

some data on dose. It is amazing how little data there is on dose, and the data that is there suggests that individuals who are maldigesters can have a cup of milk, an 8 ounce glass of milk, and hardly even know they have had the glass of milk. And if you put that with a meal so that you slow down transit of that lactose through the intestine, it is completely tolerated and can't be recognized in some double blinded trials.

Lactase activity, not just the activity of the mucosa, which we don't know in maldigesters in the majority of the population what the variation is in that activity -- there has to be some -- and how much that variation influences tolerance. We really don't know the answer to that question. But what we do know is additional lactase activity from microbial sources, whether they be a purified source or a yogurt that contains lactic acid bacteria that have very high levels of lactase can substantially supplement lactase activity and improve tolerance.

Colonic fermentation turns out to be a key biological adaptation to preventing any symptoms from lactose intolerance. And I'll show you some data that demonstrates that we can make digesters out of maldigesters by adapting their colon bacteria.

And finally, there are psychological issues

here. We have learned to avoid certain foods. Mine is broccoli. Back in the days of the former president Bush who didn't like broccoli, I empathized with that. My mother used to destroy broccoli in a frying pan, and I wouldn't eat it. And for years, I could look at broccoli and almost throw up. Well, I am arguing that there may be individuals who see milk in the same light.

Here is some dose response data. It is double blinded, it is randomized. Basically, what it shows is that when you take lactose maldigesters and give them a half a glass of milk, they don't even know they have had it. They don't make any gas. The symptom data looks very much like this. At 12 grams, which is a cup of milk, an 8 ounce glass of milk, 12 grams of lactose, they start to see some symptomatology on an empty stomach. This is actually lactose in water with no other nutrients. So this lactose is screaming through the intestine as fast it can to maximize the potential for symptoms. If you do the same experiment -- and I'll show you some -- with a meal, there is no gas and there are no symptoms.

I said that there was 12 grams of lactose in a cup of milk. One of the simple things that is so misunderstood about this area is how much lactose is in foods, a very simple food analysis in communication. The

amount of lactose in hard cheese is so small it is in the 2 to 3 gram amount per serving. It cannot cause symptomatology. We're eating lunch, and somebody told me that their son or a friend of theirs was eating a cookie and keeled over with intestinal discomfort. Well, those of you who are clinical scientists know that people keel over with intestinal discomfort for a variety of reasons, but attributed it to lactose that was in the cookie! I guess they can attribute to what they want, and that is part of our issue.

The only real major sources of lactose are fluid milk products with about 12 grams per 8 ounce serving. All other dairy foods, whenever you separate the curds from the whey, the whey has the lactose, and it is generally in very low amounts in the other dairy foods. So ice cream has about half as much lactose as fluid milk, processed cheeses 2 to 3 grams, hard cheeses about a gram, sour cream, 4 to 5 grams. Yogurts don't have a fairly high level of lactose, but I'll show you some data that shows that it is very well digested in lactose maldigesters.

In fact, that lactase activity in yogurt is an interesting biological adaptation. I started studying this in this area because a graduate student of mine had come from Morocco. And he said, Dr. Savaiano, I want to

study in your lab; I have come with money. And I was a young assistant professor, and if you know what that meant, it was a good deal. And I said, fine, study purine metabolism, which one of my colleagues -- is what I used to study. And he said, no, no, I can't do that. I have got to go back to Morocco and do something relevant to my country. He said, I want to know if Moroccans are lactose intolerant. And I said yes, they are, now let's do something else. He said no, but it makes no sense to me because Moroccans eat a lot of dairy foods. Moroccans eat a lot of yogurts. They eat a lot of other kinds of dairy foods. They are traditional foods in their culture.

So he started looking at the literature on yogurt. And lo and behold, it turned out -- and this is a study we did in '83, so it is getting quite old, but sometimes the first experiment works. Here is milk, and here is lactose in water. And you can see the typical response. There is a lot of gas produced and symptomatology. And this is about 20 grams of lactose, so it is just under 2 cups of milk. If you take the same amount of lactose and you put it in yogurt, and you feed that, you get much, much less gas, about one-third as much gas produced, the reason being that yogurt cultures contain high levels of two lactic acid bacteria,

lactobacillus bulgaricus and streptococcus thermophilous.

The lactase these organisms produce during the fermentation of yogurt is phenomenal. The levels are incredibly high, and the bacterial cell wall and cell protects these organisms during gastrointestinal digestion so that you end up with a natural pill that contains a lactase, the yogurt bacteria, a natural pill that contains lactase that helps digest the lactose from the yogurt in the intestine.

If you do the aspirations, you find lactase activity from the yogurt. If you measure distal ileal, you'll find that lactose has been digested. And when you compare that to milk, the numbers match these numbers in terms of gas formed.

You can also feed these subjects almost as much yogurt as you want, and they never have symptoms. Now why yogurt? Think about the Bedouin tribes person 3,000 years ago saving their milk. They put it in what? A bag, an animal skin. The animal skin had yesterday's milk in it, and before that the milk the day before. And over time evolved a group of organisms that learned to live off of that back, and actually in a very symbiotic relationship.

The shelf life of yogurt is actually quite amazing. If you ever go overseas and want to eat a safe

food, yogurt is probably the safe food to eat because pathogenic organisms cannot outcompete yogurt bacteria, so that we have a natural food processing or food safety mechanism that was developed, evolved thousands of years ago. The organisms protected the milk from being spoiled. People who drank the milk didn't get sick from the milk. And in addition to that, they developed tons of lactase activity, which result in an in vivo digestion of the lactose.

And this just tells you that when you measure symptoms, you never find any. We have done hundreds of subjects. Others have repeated this in many countries. And it is quite an amazing food, actually.

This is to remind me to tell you that not only can you do this with a natural yogurt, you can also do this with a pharmacological or pharmaceutical approach. One can, and there are in this country and other countries, lactase preparations that when added to milk in vivo digest lactose, and one can reduce gas production and symptomatology. There was a clinical trial some years ago of a variety of brands, some of which aren't even on the market. Vitamin E was the placebo. And they work, and they work effectively. Whether they are necessary or not is a different question, but they are effective.

So one of the genetic -- one of the adaptations, the biological adaptations, is this natural yogurt experiment that took place. The next one I want to talk about that is really very interesting, and maybe more interesting, is colon bacteria adaptation. We talked about the lac operon a little earlier. Colon bacteria are incredibly adaptable to carbohydrates that enter the colon, particularly lactose. And this is a study of human subjects that were adapted, that is, they were given either lactose or dextrose in water three times a day. And we measured their colon's ability to metabolize this lactose. And this was during the ten day period.

We started them at about 40 grams a day, so that is about three and a half cups of milk equivalent, which is a fairly sizable dose of lactose, and we increased them up to about 70 grams a day, which is -- what, a quart is 50 grams, so it is a quart and a half almost of milk a day. And over that period of time, their symptomatology didn't budge a bit. They maintained very low levels of symptomatology.

Interestingly, this is data when we challenged them, when they were adapted to dextrose, or when they adapted to lactose. So we fasted them overnight and gave them a challenge, which is the classic way to do lactose tolerance tests. And this is the gas they produced when

they were challenged with -- when they had been adapted to dextrose. They are challenged here with lactose. But when they have been eating sugar water three times a day with no milk in their diet, and you give them lactose, their colon bacteria have not adapted to this substrate. The result is excessive hydrogen production, gas, and symptomatology.

The same subjects -- it is a crossover trial, so they get adapted and off both substrates. When you adapt them to lactose and challenge them with lactose, they don't even make gas. They look just like a digester. We have adapted environmentally an individual who was a maldigester and made them a digester because their colon bacteria are now making up for their small bowel lactase that is low. And they have much less symptomatology.

I think I have a real world example of that next. This is 17 healthy African American girls that we did in a real world, free living trial of this kind of experiment. They were in our labs for a calcium balance study, and we dovetailed this study on top of them. We gave them a diet that had 1,200 milligrams of calcium per day, 33 grams of lactose a day, and we did these challenges at the beginning and at the end of this.

At the beginning, they were probably partially adapted. This is day one here. They came to us from

their normal living environment. We had not changed their diet in advance of the study. We had not taken it to a low level of lactose. But even though with 21 days of this high dairy food, high calcium diet, you can see the hydrogen reduction that took place. They digested the lactose challenge better at the end of 21 days, and their symptomatology, to be perfectly honest, was modest at the very beginning and was nonexistent or very modest at the end as well. You can see that abdominal pain didn't change significantly. Bloating went to zero. Flatulence went to zero. But to be perfectly honest, the symptoms were almost zero at both the beginning and at the end.

This strikes in tremendous contrast to the public outcry that has taken place about African Americans and their inability to digest lactose and how the dietary standards should not include dairy foods. So my data at least is not consistent with that view.

Well, you might be asking yourself at this point in time so this scientist goes out and selects people who are maldigesters. But maybe there is a range of maldigesters. Maybe there is enough genetic variation that we can find individuals that are really lactose intolerant. You all know one, I bet, somebody who says I'm really lactose intolerant.

So we went out searching, and this study was done in Minneapolis-St. Paul, a population of over 2 million. We went out and tried to find people who came to us and said I'm really lactose intolerant; I can't eat dairy foods. And we found -- actually, we found about 70 of them. We enrolled 30 of them in the study. We enrolled them if they avoided dairy foods, and they showed us with dietary records that they really were avoiding dairy foods.

And interestingly, of the 30 we enrolled in this study, 9 of them were digesters. They had no right to even think they were lactose intolerant, yet they did. We put all 30 of them in a double blinded crossover study, and all we did was give them lactose hydrolyzed milk or 240 milliliters of regular milk daily with breakfast for one week. They all believed if they had this they would be in the bathroom all morning. They couldn't tell the difference. They couldn't tell which was hydrolyzed and which wasn't.

Here is bloating. Here is abdominal pain. Here is perceived flatus severity. Here is diarrhea. Absolutely no difference. They didn't know which was which. We masked them for test and did sensor tests and so forth, so it was truly double blinded.

But what about 2 cups? Two cups starts to get

us close to what the dietary recommendations are. And so we did another study, a little bit different, looking at the same kind of issue, but the dose was a cup at breakfast and a cup at dinner. And given transit, they really shouldn't influence each other. They should be independent. And you can see that it didn't make any difference in either stomach discomfort or diarrhea. Abdominal pain and bloating were very low. Perceived flatus severity -- we also put these subjects into categories of whether they believed they were symptomatic or they were asymptomatic. And you can start to see that those that believed they were symptomatic, there was a trend here toward a difference.

We actually counted flatus. And the difference in flatus per day here was about -- that's 10 and that's 15. So it is about five a day. How is that from genomics to counting flatus? There is variation in scientific presentation. The reality is these subjects were not all discomforted by this process at all.

Well, here is the summary. Lactose intolerance really depends on dose. You can make anyone have intolerance symptoms if you give them enough. But under normal dietary conditions, that is very unlikely. Symptoms are minimal when milk is eaten with a meal. Yogurts are well tolerated. Maldigesters adapt to

lactose through their colon bacteria. And even severely intolerant individuals can drink two glasses of milk per day without appreciable symptoms.

I thank you for the opportunity to come here. It is quite a different talk than the ones you have heard earlier. But I really believe it is an interesting example of how the environment adapts to differences in genetics. We have individuals who are living in a milk-based food culture who through colon adaptation, who through use of appropriate quantities of food and through use of foods like yogurts can do get -- at least some of them can; not enough of them do -- get adequate calcium in their diet.

Thank you.

(Applause)

DR. ANAND: I hope you're writing down the questions because we will have a question and answer period at the end of this day. Now we are going to take a break, a short break for about 15 to minutes. We'll reconvene at ten minutes after 3 o'clock. So take a walk.

(Recess)

DR. ANAND: First of all, I want to thank you very much for those of you who have stayed so long. I hope you're enjoying it. Our next speaker is Dr. Bruce

Watkins. Bruce Watkins is professor and university faculty scholar of food science at Purdue University and adjunct professor of anatomy in the department of anatomy and cell biology. He is also director of the Center for Enhancing Foods to Protect Health at the Purdue University. He is going to give us an overview of the Center for Enhancing Foods to Protect Health at Purdue University.

Please join me in welcoming Dr. Bruce Watkins.

DR. WATKINS: Thank you, Dr. Anand. It is a pleasure to be here. I want to express my thanks to the organizers of this symposia and also thank Drs. Simopoulos and Anand for this invitation to speak. It has been a wonderful day. I have learned a lot, even though I got up at 4:30 to fly here. But it was comfortable on a university plane.

Unlike the other presentations, I am going to give you an overview of another dimension of how foods may impact genes to influence risk of chronic diseases.

And actually, when you think about functional foods, it might be Mission Impossible because the components that make functional foods, providing a component that lowers chronic disease risk or benefit health includes thousands of different compounds. It is an undiscovered country, so to speak. So we have a real

challenge ahead of us.

I want to cover several different topics in this brief presentation, and I will be concise. But I want to give you a brief background and history about foods and how nutrition knowledge developed to why we are at this point of talking about functional foods. I want to give you the historical perspective of diet, how that has really changed in the past couple hundred years to maybe impact risk of chronic disease, and how nutritionists, food scientists, dieticians, and the medical community ought to be working together to reevaluate how we formulate foods and what kind of fats we put in those foods.

Next, I want to give you some of my definitions of functional foods. I teach a course on functional foods. I think it is imperative to have an understanding of what you're talking about to understand and elucidate how a chemical works, how it functions. So you have to pigeonhole it and give it a proper definition.

Next I want to talk about foods and health claims. What is this interaction that is consumer driven, a response by industry and perhaps action by government to provide health claims? Are we going to see more of those? I think we will. We need to take a prudent approach to that, though.

I want to tell you a little bit about the functional foods research at Purdue University. We have the Enhancing Foods to Protect Health Center. We also have a botanical center at Purdue. And in the past few months, both of those centers combined have received about $10 million for research.

I want to talk to you about some new functional foods. Perhaps you have tried some of these. So there are some successes out there and foods that deliver that something beyond nutrients to help improve health and lower chronic disease risk. That I want to get into this subject of what this conference is about, and that is genetics, and my own twist to omics in this lexicon of new terminology of how we describe gene research, genomics, the proteins that are derived from genes that affect structure and function, which is proteinomics, and what I would like to call nutriomics and phytomics, if we want to consider nutrients and phytochemicals in that area of research that seems very exciting and promising.

And then I want to tell you with just one slide what we are doing about the education on functional foods. I think this is a crucial component of doing research, communicating to the public, educating our students about functional foods, and we need to create not only textbooks but probably interactive CD-ROMs to

learn about this subject because it crosses so many different disciplines.

When you think about the development of nutrition knowledge, we have had a very rich 100 years. We started out discovering the essential nutrients, identifying requirements to avoid nutrient deficiencies, and we have moved into this area of developing dietary guidelines in the hopes and with the intent of lowering chronic disease risk. But where is that leading us with respect to foods? My view is as follows, "Health related research includes efforts aimed at discovering the components in foods that act as health protectants."

I think under this large umbrella of health protectants you could probably put nutriceuticals and phytochemicals because they are not quite nutrients, but they seem to afford some health benefit and may lower chronic disease risk as we understand more about these in this undiscovered country of studying phytochemicals. This is a new area of exploration in food and nutrition research.

So this is why we have programs, centers devoted to functional food research. Functional foods are what I perceive as a new focus for health related research. Why is this? Well, because we are looking more into what is contained in foods that may lower our risk for disease.

We have examples of clinical trials done with individual nutrients that were proposed to have a health benefit, but it turned out that there was no effect, or an opposite effect in some of these studies.

So there is something more than just the nutrients that are in foods that help to lower our risk of chronic disease and perhaps influence gene expression and these polymorphisms. And the interaction or interplay between nutrients, phytochemicals, and these polymorphisms, I believe, is going to influence genetic susceptibility for chronic disease.

There was a USDA report last year indicating that $250 billion was spent on or lost to the U.S. economy due to diet related chronic disease. I don't think this is all due to just nutrients or a lack of nutrients, but I think it is this interplay of other components in foods that aren't truly classified as nutrients, which sustain life.

In addition, when we look at our health care costs, they have been escalating since 1995. A recent report in Science indicates that by the year 2015, we are going to be spending $2.3 trillion on health care. Now obviously, there is tremendous interest in the Human Genome Project, how genes influence risk for disease, how pharmaceuticals may be better developed based on this

knowledge of genes. But I think it is time for nutritionists, dieticians, and food scientists to look at the interplay of nutrients and phytochemicals in this process to lower health care costs. Maybe time can press that chronic disease to the last two years of life, which is known to cost less than treating someone for a chronic disease over a period of decades.

You have seen this or a form of this graphic presentation from Dr. Simopoulos' talk. This came from a publication from Alexander Leaf, and Weber. It is a modification of this, but I want to point out to you that changes have occurred in the formulation of foods in the past 150 years in an attempt to try to lower chronic disease risk. However, in the past 100 years, we have seen an increase in total fat intake. We have seen a plateauing of saturated fat, but we have seen this big increase in n-6 fatty acids relative to n-3 fatty acids. And n-6 fatty acids seem to have some undesirable effects, as well as good effects on such things as risk for cardiovascular disease. But perhaps we need to reconsider balancing the types of essential fatty acids in our foods to lower chronic disease risk. And this is where new information about omega-3 fatty acids is so important. And this maybe affects not only biochemical pathways involving metabolism and physiology, but at the

molecular and gene level, which is really exciting.

So making changes in how we formulate foods and what we feed our food animals, how we may use traditional breeding methods or GMOs to modify plants, requires the interdisciplinary discussions between food scientists, animal productionists, nutritionists, dieticians, and the biomedical community.

Well, here are some of my definitions of components of functional foods. And there isn't agreement on this in the United States, but I think the way in which I approach it provides a different view that may be more acceptable of recognizing these compounds as new components that contribute to our diet and our health.

Nutriceuticals, if you look at these, which are used in formulating new functional foods, which I would describe as processed foods, would include components such as nutrients, of vitamins. Beyond satisfying a vitamin requirement, there may be a health benefit. This has been the topic of numerous conferences and symposia. And nutriceuticals would include physiologically active compounds, for example, eicosapentaenoic acid (EPA), which has a very powerful effect on eicosanoids and perhaps gene regulation, as we learned today.

On the other hand, phytochemicals are compounds

produced by plants. Some of these are categorized as secondary plant metabolites. But we don't have an absolute requirement for some of these, but yet they have some kind of potential health benefit that may lower the risk of chronic disease. But they are a separate group of compounds.

Examples of the phytochemicals are genistin in soy beans, lycopene in tomatoes, phenols in olive oil, anthocyanins in some berries and fruits. And of course, plant materials contain a wide variety of phytochemicals as you look across the different types of fruits and vegetables we consume.

Well, another way of viewing these compounds that are components of functional foods or even designer foods, are raw agricultural products. The nutriceuticals and phytochemicals used in these new food formulations -- and you could view these compounds as health protectants following under this big umbrella of compounds in foods that are not necessarily nutrients but afford some kind of health benefit.

Now in my definition of a phytochemical, which may be something you haven't seen before, I view it in a different way other than a phytonutrient or using the word nutrient in a way to describe its activity based on its chemical structure and what it does biologically. I

view phytochemicals as limiting dietary components with respect to the individuals dietary practices, lifestyles, and genetic risks for disease.

For example, I don't think we need to go on and fortify every food with a single phytochemical because if you eat two to three tomato based meals a week, you probably get enough lycopene. And if lycopene shows to be effective in lowering risk of certain types of cancers, your diet would be adequate.

I think as we have been discussing genes, we have been thinking about tailoring or making the diet individualized for the person. And in this respect, my definition of phytochemicals being limiting dietary components with respect to the individual's dietary practices, lifestyles, and genetic risk for disease or susceptibilities would fit that.

Now it is a different way of viewing these compounds. Now everyone has heard the saying you are what you eat. I can remember my major professor at Colorado State saying, Bruce, don't you know you are what you eat when he saw me wolf down McDonald's hamburgers during lunch. But I think this is turning around to become a saying of you become what you eat because of how foods and their components affect gene expression.

Really, when you look at functional foods,

consumers out there want something that is healthier. There have been surveys done by IFIC to show that there is more and more interest in foods that promote health. We have an increase in the aging population. So there is some consumer issues that are driving the industry to come up with these products that are beneficial to health.

You see that the consumer in the United States spent $4 billion on herbal products last year, and $15 billion on supplements. I don't think this is going to go away. But as the consumer wants and desires more of these foods, the industry is going to respond. And I think government is going to take action, evaluating the data and look for evidence that a food could be provided with a health claim, or perhaps an individual nutrient.

In 1999, the FDA provided a health claim that foods containing soy protein at 6-1/4 grams per serving would reduce the risk of coronary heart disease. Functional foods represented 15 billion in 1998 sales. And if you are watching what is happening in Washington, as many of you are, and looking at the evidence on omega-3s and reductions in chronic disease, I think the FDA in reviewing new evidence that omega-3 fatty acids reduce the risk of cardiovascular disease may provide a health claim. And this may change the way in which we formulate

a lot of new foods because as our RDAs are developed for omega-3s, we are going to have to include these. And if there is a health claim, people will want those sorts of foods. And we can't feed everyone salmon. There just isn't enough salmon. So we need to look at alternatives, and the alternatives are new functional foods.

In 1999, we established a center at Purdue. The center is for Enhancing Foods to Protect Health. There are four centers now on functional foods in the United States. Our center received support to create a center of excellence involving 50 different faculty from Purdue University, five schools at Purdue, Indiana University, their medical school, and other participants. And our mission is to conduct research on these phytochemicals and nutriceuticals and to develop delivery systems.

I think we're one of the few centers where our final objective or our long-term goal is to deliver these components in foods to benefit society. And there is also a keen interest in nutriceuticals and phytochemicals in lowering chronic disease risk in companion animals which have problems like cancers and joint disease that we suffer from.

It was necessary in an effort to communicate between faculty at different locations and to do it more effectively to create this web site. And it is a unique

web site that is flash driven in HTML versions. And it is a good way to communicate, and it is also a nice way to provide information to industry that can have access to the web site to find out what research is going on and get definitions of certain terms with chat rooms and other features.

There are four major research focuses for the center, or four major aims. And we are currently conducting research in all four of these aims. The first one is the discovery of novel phytochemicals and nutriceuticals. We're actually looking at a new type of phenolic isolated from crabapples that may have antioxidant activity in vivo. We are testing and validating the benefits of health protectants, looking at immune function, lipid metabolism, and carcinogenesis. This is with nutriceutical fatty acids and phytochemicals. We are actually developing or commercializing some functional foods and health protectants.

Here we are making design dairy products with the help of a biotech company in Colorado developing fish that have higher levels of maybe CLA and omega-3 fatty acids, looking at the role of resistant starch in reducing obesity and perhaps problems with diabetes, and process engineering to create these products. And the

final research area is enhancing the health protectant capacity of foods through biotechnology, traditional breeding practices for plants so we are using a variety of approaches to create designed oils, designed proteins for more crops, and create better tomatoes.

As I mentioned, Purdue University also has another center that was recently developed. It is called the botanical center. This is a collaboration of Purdue with the University of Alabama-Birmingham. Investigators at Rutgers, IUPUI, which is in Indianapolis, the medical school, and the University of Illinois. Their focus is to study the effectiveness and actions of polyphenolics. And they have projects in cancers, osteoporosis, and cardiovascular disease. And this particular center is directed and led by Connie Weaver in the foods and nutrition department at Purdue.

I want to give you a couple of quick examples of what I perceive as effective functional foods. One is a cholesterol reducing margarine. You have probably heard of Benecol and Take Control. And the other one I want to talk about generically are designer eggs.

Plants contain plant sterols, plant stanols. These compounds are very similar in structure to cholesterol. But these are a minor component in the diet for an omnivore such as us, most of us here in the room.

Probably have an intake of about 250 milligrams per day. In a vegetarian diet, it would be about twice that, 500 mgs per day.

Well, these products, these spreads contain plant stenols that lower blood cholesterol by interfering with the absorption of cholesterol in the gut. And there are two products that are out there that consumers have been using that have been shown to reduce blood cholesterol, especially LDL cholesterol. Benecol is one. Take Control is another one. The recommended intakes are a couple of tablespoons per day. One you can only use as a spread; the other you can use in cooking. So there are some functional food examples out there that you can try in your local supermarket.

Another area of interest in this area of functional foods are the nutriceutical fatty acids, fatty acids that have some kind of beneficial effect on metabolism and physiology, and yet we have not determined a requirement for these fatty acids. When I think about supplements and functional foods, the two categories would be omega-3 fatty acids and various isomers of conjugated linoleic acid.

We have talked a lot today about the omega-3 fatty acids. And I was pleased to see that the American Heart Association a few weeks ago recommended that we

consume fish as a part of the diet. And in fact tuna packed in water would be a good source of these long chain omega-3 fatty acids, EPA and DHA.

Something that has come into the limelight more recently are conjugated linoleic acids. Conjugated linoleic acid is the structure on your right. It is very similar to linoleic acid except that the double bonds are joined. They are not separated by a methylene group. And that change in structure has a profound effect on how that fatty acid is metabolized. It doesn't act as an essential fatty acid like linoleate (ph) for us. And there is all kinds of potential benefits that have been demonstrated in animal models, and yet we don't know very much at all about how it affects the human, although you can find supplements of CLA in a General Nutrition Center.

Some of these, while the naturally occurring CLA is found in ruminant products, cheese, milk, red meat, beef, lamb, but some of these supplements are chemically isomerized vegetable oil to make CLA, which contains more than the 9,11, isomer, a variety of isomers.

Another area of functional food work is designer foods. This has received quite a bit of success in Canada, the United States, Australia, and Europe. And designer foods to me is a raw agricultural product. It

could be eggs or tomatoes, where we have modified the nutrient and our phytochemical nutriceutical content of that food to make a functional food that would deliver a health protectant capacity, either benefitting health or lowering chronic disease risk.

You know, we used to feed chickens in this country fish meal, which is a source of omega-3 fatty acids. But yet the diets now are much less complex, and they are practically devoid of omega-3s, so it is not unusual that we have very, very low levels of omega-3 fatty acids in these foods.

I did say I wanted to talk about this concept of genetics and how functional foods may relate to that. There has been a proliferation in the terminology of omics research. I think not only for functional foods, but nutrients in general, the targets are a lot of very important regulatory controls in metabolism as well as chronic disease risk. If you look at HMG CoA reductase, which is a very important enzyme for synthesizing cholesterol, anomalies of this enzyme are associated with cancer risk. And some recent research that has been done in Wisconsin showing that isoprenoids or compounds related to the family of carotenoids have an additive effect of downregulating the activity of this enzyme.

So is it possible to look at these

phytochemicals as being synergists working with a very expensive cholesterol lowering drug, statins, to control this enzyme? I might add that statins have recently been shown to increase bone formation in laboratory animals.

Signal transduction apoptosis is another area of important research where phytochemicals and nutrients can interface with genes to affect apoptosis and cell-cell communication and, of course, gene regulation. And to give you a feel for this, in my view, the way to look at it is that approach would be studies on diets and nutrients that are associated with genetic susceptibility of chronic disease. And I would call this nutriomics for nutrients and phytomics for phytochemicals.

So if we studied SNPs, these polymorphisms that are associated with one base pair change that can relate to protein synthesis from translation or transcription, identifying SNPs that impact cancer or bone disease in relation to dietary habits or nutrients of phytochemicals, I think this is where a real opportunity is available for scientists interested in nutrition research and improving health.

In my own area of research, the discovery of the CBFA-1 gene just a few years ago and soluble ligands that regulate osteoblasts and osteoclast differentiation is a real target opportunity for looking at how nutriceutical

fatty acids can affect risk for osteoporosis. We know the mature osteoclast resorbs bone. Mature osteoblast form bone. And chondrocytes are important in early bone formation and modeling of the young, but very important in joint disease in the adult. But we know now that certain transcription factors and these signal transduction factors that influence differentiation of progenitor cells appear to be influenced by nutriceutical fatty acids.

So it could be that we have been eating the wrong type of fat as mature adults, such that we have fewer progenitor cells becoming osteoblasts to remodel bone when bone is resorbed. Likewise, another potential target is in controlling catabolic events in bone that lead to an overall decreased bone mass. And that target is the control of CoX-2, an enzyme that has been the target for new drug development in decreasing inflammatory joint disease.

What we do know now is that certain nutriceutical fatty acids may downregulate the activity and perhaps decrease the mRNA, the message from genes, to make this enzyme.

The area of education on functional foods consists of the following projects: A USDA higher education project funded to make a CD-ROM on functional

foods with Sci Media, a consortium at Purdue. We have chosen three interfaces to communicate this very difficult subject area where you can explore the human body and find out what nutriceuticals, phytochemicals impact chronic disease risk, read appropriate papers, see a potential mechanism, and look at where in the world functional foods, botanicals are produced. And you can explore the plant cell and learn about the biochemical pathways that lead to the synthesis of phytochemicals. We think this will be a great tool for the college student and industry.

Let me quickly summarize what I have told you in this brief period of time, is that functional foods are processed foods, processed foods that help to reduce the risk of heart disease, cancer, a variety of chronic diseases once we have adequate knowledge and demonstrated clinically that these are effective compounds. Designer foods can be animal and plant products containing a modified nutrient content, usually enriched with phytochemicals and nutriceuticals. Nutriceuticals and phytochemicals are used to make the functional foods, fortify them so that they will have a health benefit. And what I have hoped that I have tried to do -- what I hope that I did in this short period of time is tell you a little bit about the potential for looking at

phytochemicals, interaction with genes, and the susceptibility of chronic disease. And I think we might, although other people have indicated different terminology today, we might call this nutriomics and phytomics.

Thank you very much.

(Applause)

DR. SIMOPOULOS: Thank you, Dr. Watkins. The next speaker, and the last speaker, is Dr. Frank Booth, who is an exercise physiologist. He is professor in the department of veterinary biomedical sciences, College of Veterinary Medicine at the University of Missouri, Columbia, Missouri, who will speak on physical activity and gene expression. Dr. Booth.

DR. BOOTH: Thank you, Dr. Simopoulos, for the introduction. And I want to thank the staff here today for their hospitality. Many people have been very kind in their assistance.

As compared to a century ago, most Americans have less physical activity today. This has major health consequences in the relationship to contribution to the obesity epidemic, to the epidemic of type 2 diabetes, where I'll show you some data suggesting that physical activity has an independent effect on obesity, on producing diabetes, and then at the end just briefly talk

about how physical inactivity contributes to physical frailty in older people.

So the first part of the talk will be about physical inactivity and its relationship to obesity. Basically, everybody in the room sort of understands this concept, that if the calories you eat and expend are equal, you have a constant body weight. What happened in the past number of years is that we expend less energy and the size of the portions of our meals has increased. Americans are eating more food. We eat Big Macs, Big Values, those types of things. And physical activity has decreased, so this is out of whack now. We're taking in more calories than we are putting out, and this would tend to increase body fat.

How physical inactivity has increased is shown in a study that was published in 1999 in which 13,000 adolescents were studied relative to hours of watching TV per week. It is about two hours a day. So kids are watching TV two hours a day up to about -- I'd say the late 1980s. And then what happened is we put on computer games and web searching and so forth. And what we have now when we add up TV watching and computer surfing, it is about three hours a day in these age groups. The kids are sitting, are not active.

And this is correlated, not cause and effect, to

the following information, which is by the CDC. If you look, there is a one time point here around 1980 and the next time point around 1990. Childhood obesity has doubled. This is the percent of children that are obese, and these are the various time points they measured. And there has been a doubling of obesity that is coincident, not cause and effect, with this increase in the time that kids have gone from two hours a day watching TV to three hours a day TV, video games, and so forth.

If you take the basic information you learned in your classes and you apply to how much inactivity you have to do a day in order to gain a pound of fat per year, this is just sort of a simple calculation that what you basically have to do if you just decrease your walking 600 feet less a day, you'll burn 10 less calories a day. You repeat this for the year, that is going to be equal to a pound of fat.

So it is a very, very small amount of change in physical inactivity that over a long period of time will accumulate to an increase in body fat, in a pound of fat. And this is another example. One of the major ways to burn fat is to walk up stairs. And of course, most buildings have elevators and people prefer taking them. But if you only climb stairs one minute a day, you add that to your lifestyle, you'll burn at the end of the

year one pound of fat.

So it is the amount of inactivity and activity we're talking about, it is not major changes to get a big change in body mass. Now this is a very complicated area, I understand, because there can be changes in basal metabolic rate if you are obese and you try to lose weight. Your basal metabolic rate will go down. So this is a very simplistic view. But it does point out the fact that physical inactivity can have a major role on body fat.

A calorie in the blood has two choices, basically. If you are inactive, the calorie is going to go into the fat cell. The muscle doesn't need to have the calorie because it is not active. You are sitting playing your computer game.

Let's look at some of the data that looks at the effect of a single bout of exercise in humans -- nd all of the data I'm going to show today is human data -- on what can be a fat tolerance test. In other words, you give a meal of fat, a measured meal of fat to the person, and you look at blood lipids. And how does exercise, one bout of exercise, effect this? Basically, what this study was is they had a 90 minute treadmill walk that was in women that was 17 hours before they gave this meal. And here is the meal. And it is a pretty -- it would be

a meal just for the sake of our discussion here. It would be like having a Big Mac and a shake and all that stuff right at this point.

And what we have here is the plasma triglycerides. When you eat this meal, which has this amount of fat and this amount of carbohydrates, you can see the rise in the blood triglycerides. So these subjects did this three times. One time they did it without exercise, and you can see they had the highest rise in plasma triglycerides. But when they did this exercise bout 17 hours before they had this fatty meal, it is the lower curve. And so they had an improved tolerance to the fatty meal.

In other words, the improved tolerance means their body was able to clear these triglycerides out of the blood faster. And what is the advantage? Well, there is fats around your blood vessels for a shorter period of time, and therefore theoretically there might be less atherosclerosis.

The third experiment they ran was the control group, where they had no exercise, but the people at less calories to equal to the amount of calories they did when they did the minus 90 -- when they did the 90 minute treadmill run in order to control for the amount of calories that were burned in the exercise bout. So there

is something going on that is independent of calorie intake here that is causing you to get rid of your Big Mac in your blood that faster from the exercise bout.

Here is another study, and the reason I show this, it is almost an identical study, but in this case they were looking at chylomicrons. Again, this is the fats that when you absorb it in from your stomach, the fats carried in the blood is chylomicrons. And also the same results were found for the LDL, which are the fats that are coming out of the liver. And again, it is the same basic trend. Here is the inactive group. And in this particular study, they were looking at artery and veins.

Essentially what we are saying here, then this calorie, when you eat the Big Mac, sitting in your blood, and it has to go somewhere, and if you are inactive, it is going to go into the fat cell. And this is due in part to the fact that when you are inactive -- and this has been shown in basically animal studies, that if you measure the lipoprotein lipase activity in skeletal muscle, it is decreased within hours of inactivity. The calorie in the blood or the triglycerides are going to even go more into the fat cell if the muscle is inactive.

But it has also been shown in human studies -- and basically, these human studies I am going to present,

what they do is they have the humans exercise, and they take pieces of the muscle from their skeletal muscle. It is called a muscle biopsy. They put a local anesthetic in the muscle. They take sort of a needle in there and pinch out a piece of skeletal muscle, and they take the skeletal muscle and do biochemical measurements on this human muscle. And so what they found is that immediately following the exercise bout -- and I'll show you data on that in a second -- there is an increase in lipoprotein lipase activity.

Lipoprotein lipase is an enzyme that breaks down triglycerides in the blood into fatty acids. So it is breaking down this triglyceride that is on the VLDL of the chylomicrons and allows the fatty acids to go into the skeletal muscle. And the single bout of exercise in humans is increasing this, so now after you have the Big Mac, I have the arrows going to the right because the exercise bout, the muscles used a lot of substrates, and it needs to replenish its fuels that it used up during the exercise. We don't know exactly the signal how a lipoprotein lipase activity is increased, but we do know that it can clear more fat from the blood into the muscle.

And here is some actual summary of the data that was published in a review article. And basically, what

this is, this is lipoprotein lipase messenger RNA, again from all human data, from the muscle biopsies. And these are the times post-exercise. So this would be zero hours post-exercise, 5, 10, 15, 20. Here is the mRNA. Here is the starting value. You can see it goes up transiently post-exercise, and by 24 hours post-exercise, it is down. So this is the mRNA. This is the protein. It follows a little later. So the messenger RNA is making the protein. And I'm not going to show you the activity data, but the activity data in the blood shows that usually post-exercise you have an increase in lipoprotein activity in the blood when you use a heparin injection to reduce it all from the capillaries.

This is an experiment that shows you that if you look at people before they exercise and after they exercise, and the exercise bout would be a two hour run, three hours post-exercise. And what they are looking at is the arterial venus difference or chylomicron triglyceride clearance. In other words, they put catheters in the femoral artery, femoral vein, and they can measure the uptake of the chylomicrons. That is the particle that is carrying the fat you had in the Big Mac. And you can see after exercise you clear a lot more of that chylomicron from that muscle that had undergone this two hour run three hours earlier, again showing you more

directly that the muscle itself -- before we were showing you whole body triglyceride clearance. It is definitely there is triglycerides, more triglycerides, going into the muscle. And the lipoprotein lipase activity is probably playing a role in that.

Now here is a more recent study, and I'm going to show four slides like this, so I'm going to sort of go slowly through them and explain them. Again, this is human data. Part of this data was published in October of this year. And basically, what they did is they did two things in these pieces of human muscle they took out. Number one, they were able to measure what is called transcription rate. What they did is they were able to isolate DNA from these muscle biopsies and finish the synthesis of transcripts of messenger RNA that were being made in these people post-exercise at various time points. So these would be control groups, and then they had zero time post-exercise, 15 minutes post-exercise, 1 hour, 2 hours, 4 hours post-exercise. And there was a significant increase in the amount of the transcription rate of the lipoprotein lipase mRNA in about one hour post-exercise.

They didn't see any increase in the mRNA here, whereas other studies have. One of the hypotheses is sometimes you have to have a couple of bouts of exercise

on different days to really get this to increase. But and also, this time point of four hours, remember I said it was transient, and it was just starting to rise at four hours in that earlier one and peaking around 12. So they may have measured this a little too early.

But again, the point here is this is human data after exercise showing you that the genes are responding immediately post-exercise to the exercise bout. These genes I'm going to show you are metabolic genes. These are genes involved in taking the stuff you eat, putting it into the muscle for fuel for exercise.

This is sort of looking at a correlation. This is lipoprotein lipase activity that they measured in various groups of humans and compared it to capillary density in the skeletal muscle. And you can see that the higher the capillary density, the greater the lipoprotein lipase activity. One of the adaptations to aerobic training is as you increase the number of capillaries in your muscle -- it is called you increase your capillary density. And related to that is that the higher the capillary density, the more the lipoprotein lipase activity you have.

I want to show you some human data to show you another mRNA. This is called VEGF mRNA. VEGF is a growth factor that causes capillaries to proliferate.

And one bout of exercise in untrained people -- this the level of the mRNA -- increased to here. And this was a one hour bout where people just did a kicking exercise sitting. And after they were trained, they had less of an increase of the mRNA, but still it is a substantial increase. But remember, these people already had a lot of capillaries compared to this untrained person. So they probably didn't need as much of an increase.

So one of the signals for the increase in the capillary densities increased immediately post-exercise. And the more capillaries you have the more LPL lipoprotein lipase you'll have in the capillaries, and the greater your insulin sensitivity will be.

Another fact that has been shown in the human study is the side effect of the reaction of having more lipoprotein lipase activity in human skeletal muscle is that the muscle itself will produce HDL. And this was shown in this next study, where in this particular study there was a bicycle where you bicycle with one leg. The other leg doesn't cycle, okay? And so you were able to use one leg as the control that didn't exercise, and the other leg you used that did the exercise. And the exercise, what you were going to see here is the nonexercise leg, the difference between these is the arterial venus or the blood going in and the blood coming

of HDL. The trained leg, this would be the vein, the vein actually had more HDL than the blood coming in. So the muscle that it exercised is actually producing HDL. And again, this is human data.

So where do we stand? Well, we know that after eating a Big Mac we can reduce the level of these in the blood, maybe not your resting, fasting levels, but after postprandially after a meal, yes. It is probably related to the increase in LPL. We know that there is going to be an increase in HDL from the muscle. One of the effects at rest is an increase in HDL in the blood.

I'm going to show you some data on an enzyme, carnitine palmitotransferase I (CPTI) that is involved in transporting fatty acids into the mitochondria. This is the same study I showed you earlier. This is the rate limiting enzyme for fatty acid transfer into the mitochondria. You can see its transcription rate was up an hour post-exercise. And at all time points post-exercise, there is an increase in the mRNA of this particular enzyme, CPTI.

Just to sort of summarize this, again how fast does this effect go away? These were people who were trained. Fifteen hours after the exercise bout, after a fatty meal, this is what their blood looked like. If you just detrained for two and a half days or six and a half

days, you can see that you immediately revert back to the nontrained state, where you have the fats in the blood for a longer period of time.

So one of the points I am trying to make today is the gene responses to exercise are very rapid. They occur very, very fast.

I want to briefly talk about the role of physical activity in type 2 diabetes. The people that had been fit, had a threefold reduction in the incidence of type 2 diabetes. Same thing for women, a threefold reduction. Women tended to have more type 2 diabetes than men.

So physical inactivity is causing a greater incidence of type 2 diabetes by about a threefold factor. Now in another study we compared people of two different body weights. In one group body mass index is greater than 27, so includes overweight and obese people. The rate of diabetes is reduced about twofold when you go from low fit to moderate fit.

But what is interesting, are the data on normal weight people, people whose body mass index is less than overweight. They still showed the same effect. Yes, being overweight increases the incidence of diabetes. But you can still get an exercise effect if you are moderately active or highly active, at least a twofold

decrease.

So this leads to my point about the fact that exercise itself has an effect on preventing type 2 diabetes independent of body weight because these people were normal in body size.

So if we think about it sort of teliologically, you know, sort of say your muscle that isn't doing anything and just sitting around, that muscle doesn't need to take up as much fuel. If you're there as a muscle, and you are not contracting, you're sitting or at the computer for 13 hours a day, you don't need fuel. You don't need to take -- if you don't need fuel, you don't take up as much glucose. And this is sort of related to -- I'm going to show you next, there is a very rapid development of insulin resistance with physical inactivity. And insulin resistance is a player in syndrome X, which then is associated with a higher incidence of these chronic diseases.

So again, back to our calorie example, if you are physically inactive, and the calorie is sitting out here, and the muscle is inactive, the only place the calorie has to go is to the liver if this is a fat -- or this in this case is a sugar calorie. It goes to the liver and can be converted to fat and then leave the liver as VLDL or the glucose or sugar can go into the fat

cell and be converted to triglyceride in the fat cell.

The converse would be true. If you are an active skeletal muscle, then you would expect to have greater glucose removal because the muscle uses up its blood -- I mean it uses up its store of sugar called glycogen. And therefore, post-exercise, you would expect more sugar to move into the muscle. And this is essentially what happens after, and it is a single exercise bout effect, just like the fatty meal. You are going to see the same effect. It is going to be after a single exercise bout, and it is going to go away fairly rapidly.

Again, a human study. These people were trained. So what they did is they gave them a sugar meal, and then they looked at their blood sugar post-the sugar meal. And if you are trained, the body is removing the sugar fast. After they were allowed to detrain for ten days -- and this is tough to do, to take people that like the exercise. They don't let them exercise for ten days. They were becoming more diabetic-like, just after ten days.

These people then were asked to exercise once, and they got back to this lower glucose tolerance curve, away from the diabetic tolerance curve. The blood insulins were quite similar. The blood insulins showed

the same effect. Here was the trained people. They detrained. They had more than a doubling of the area of the curve. And then when they did the single exercise bout, they came back to here.

So this is a very acute response. What are some of the chemicals that are going on in the muscle that are bringing the changes? We know that there is an increase in GLUT-4 protein with a single bout of exercise. And this is related to helping increasing the amount of glucose going to the muscle. Here is the correlation. As you increase the GLUT-4 protein, you increase the amount of glucose that is taken up into the muscle by looking at glucose AV difference. So there is a direct correlation between these two. And if we look at it at a more molecular level in humans, these people at a 60 minute moderate exercise bicycling, and then here is their value of GLUT-4 and mRNA immediately post-exercise, an hour post-exercise, four hours post-exercise, you are going to see that the body is immediately responding to this increase in physical activity by increasing this protein that is involved in transporting the blood sugar from the blood into the muscle to store as glycogen for the next exercise bout.

And this is just sort of a summary. In human skeletal muscle, there is going to be an increase in

hexokinase and an increase in glycogen synthase in muscle post-exercise. The transcription rate of hexokinase-2 is increased post-exercise, as well as one hour to four hours. There is an increase in the mRNA. Again, these are in pieces of muscle from the human biopsies. Same thing for glycogen synthase, an increase in transcription rate. And in all time periods post-exercise, an increase in the mRNA. Glycogen synthase is an enzyme. It takes a sugar, converts it into glycogen in the muscle.

So to sort of summarize what we have shown here, we have shown that there is less of an increase in blood glucose and insulin after a meal. This is beneficial because it is lowering insulin resistance. If you have a rapid removal of these from the blood, you are lowering insulin resistance. And insulin resistance is related to getting these chronic diseases so that this some of the underlying basis how humans lower the incidence of these chronic diseases.

High intensity bicycling in humans increases an enzyme that is newly found in exercises circles called AMP-protein kinase. What is important about this is people have known for a number of years that exercise signals the uptake of glucose independent of the insulin singling pathway. And so they have now identified this other pathway, at least one other pathway, that is now

being found to alter gene expression, this enzyme AMP-protein kinase.

What is important here is that maybe there are other pathways that can be used to get around the diabetic state and allow drug companies to potentially have drugs in case people can't exercise.

I'd sort of like to end up by examining the question of this old lady who is in an institution. You can sort of tell by the surroundings. She is in a nursing home, and she is using a cane. I always say she is not using the cane because her bones are weak. She is using a cane because her muscles are weak. And this is sort of a nutrition exercise paradigm as far as I'm concerned because when you have a chronic disease and you're old, you are probably going to have a nutrition deficit, and you are going to go to bed. Both of these cause a loss of muscle mass. This loss of muscle mass leads to a reduced physical activity. The reduced physical activity then causes more of a loss in muscle mass. And it is a vicious negative cycle, and you end up like that lady.

And the question is since more people are going to be getting older -- and it is a gender specific thing; it is going to be more females -- how do we break it? Well, one of the things is that during these events of

the disease state, there has to be concern about the proper nutrition. And then maybe post-disease state, we need to think about actually having resistance training for these older people to break this cycle because this cycle leads to two very bad things. If you lose skeletal muscle, you eventually are going to have weaker bones, and you are going to have the loss of the independence of living, which is going to increase health care costs.

And there is a lot of data. The best data, I think, is the data from space flight. When you go into space flight, you don't have any gravity there. It is like lying in bed. And people in space lose bone mass at ten times the rate as people on earth in a year, the lower bones. So when you do go to bed rest, you lose bone mass. So it is very important to try to prevent the loss of bone mass in this cycle by trying to increase the amount of exercise in this particular population. Thank you.

(Applause)

DR. ANAND: Could you have the lights on, please? I think you'll agree with me that we had excellent presentations and great speakers. Would you please join me giving a big round of applause to all the speakers?

(Applause)

DR. ANAND: I also want to acknowledge the help of the staff from this Jefferson Auditorium. Kerry Goland, Kerry Goland is here some place. Kerry Goland and Melvin Wiggins. Melvin, come outside. So here. Hi, come on in.

(Applause)

DR. ANAND: Thank you very much. Now it is your time to ask question or comment on any of the speakers. So, please, there are two microphones here. Identify yourself and ask any question or brief comments.

MS. MINSKY: My name is Bonnie Minsky. I'm president of Nutritional Concepts. We provide individualized nutrition evaluations through Internet software, and we do incorporate genetics, functional foods, and nutriceuticals into our evaluations. I have two questions of Dr. Savaiano. Are you there? I have two questions.

DR. SAVAIANO: I'm here.

MS. MINSKY: Oh, there you are. I'm sorry. The first question is with regards to the perceived lactose maladaptors, were those individuals shown in your studies to have increases or decreases in their blood or cellular calcium levels when their lactose was increased?

DR. SAVAIANO: Not measured.

MS. MINSKY: Ah, now that could be an important

measurement. From what we see, we often do see very, very low levels of calcium in the blood.

A second question, I see in my private practice many clients who cannot digest casein, the milk protein. Have any studies been done to assess the prevalence of this in the human population or in the problems that it has occurred?

DR. SAVAIANO: There is a fairly good literature on milk allergy, and in particular casein protein allergy that exists in the literature. Most of that data shows that infants have maybe a 5 to 10 percent allergic response to bovine milk proteins, and that number is dramatically reduced as those infants mature and their intestines become really less open to those proteins moving through the intestine. And so by the time those infants are a couple of years old, that level is very low.

However, there still are in the literature, obviously, reports of adults with allergic responses to milk proteins. I should point out they are very, very low in their frequency.

DR. ANAND: Go ahead, please.

DR. SAVAIANO: The question of calcium in the blood is an interesting one. Traditionally, that has been a very poor measure. Bone mass is the measure one

wants to look at in terms of calcium status. Blood calcium is highly regulated, despite poor bone mass. And the literature is very full of data that shows that blood calcium is maintained. I would not expect to see differences in blood calcium levels.

Really, I think what you want to measure is bone density. And the question is a much longer term question. I think the question is over a period of years, can one demonstrate with increases in calcium in the diet stronger bone mass. And that data is accumulating. At both the early ages, around adolescence, where much bone is deposited and additional calcium in the diet appears along with exercise to be important in maximizing that bone density, and in the older years, where along with hormonal treatment and exercise, calcium is a factor in maintaining bone mass. So it is obviously multivariant, but dietary calcium is one of those factors.

DR. ANAND: Go ahead.

MR. JONES: My name is Edward Jones. I'm a PhD student in nutrition at Cornell University. And my question -- my first question is about the sponsorship for the research that you did, Dr. Savaiano, on the black females at Purdue.

DR. SAVAIANO: That study actually was sponsored

by no one. We piggybacked that study on top of an NIH trial that Dr. Weaver was conducting. And we had no funds to do that. I have had sponsorship from pharmaceutical companies, the federal government, the Dairy Council. In my career, I have had sponsorship from about every organization, private and public, you can imagine.

MR. JONES: Okay. Well, my question was just about the whole premise of that research and that some of the older research on lactose intolerance, like by Kretchmer. I want to read one particular sentence. He says that this general adult lactase -- adult deficiency in lactase has come as a surprise to physiologists and nutritionists can perhaps be attributed to a kind of ethnic chauvinist since the few human populations in which tolerance of lactose has been found to exceed intolerance include most northern European and white American ethnic groups.

I don't see how your research is anything other than this, meaning that we accept that lactase persistence into adulthood is a minority issue in this country and most parts of the world. And what I don't hear you state is what is the objective of trying to get black females that you sequester in a fraternity house, after three weeks subjecting them to high doses of

lactose, to basically be able to tolerate one or two glasses of milk. And what does this contribute to the literature?

My concern is that if you take a population that is largely lactose intolerant, and maybe there are other compounds in milk as well, and there is a certain kind of knowledge, there is a cultural knowledge about that -- it is also in Asians as well. And it looks like your research is saying our objective, rather than to focus on the nutrients calcium and vitamin D and the protein and the nutrients in milk are to promote milk in a population that recognizes lactose intolerance and maybe other forms of intolerance of other compounds of milk as a nonissue that perhaps these people are crazy. They really -- you know, they say they lack tolerance, but they don't know what they are talking about.

And I'm just really, really disturbed by what seems to be unstated premises of chauvinism in that research.

DR. ANAND: Any comments?

DR. SAVAIANO: I'm an Italian-American who is a lactose maldigester. I live in the United States of America, where I walk into a supermarket, and one of my food choices is this gigantic dairy aisle. The question is should I choose those foods, should I not choose those

foods? If I choose those foods, what is the nutritional gain that I might get from them, and what is the downside of choosing those foods? That is how I have approached this.

MR. JONES: What about soy? What about calcium --

DR. ANAND: Okay.

DR. SAVAIANO: There are lots of good choices for calcium in the diet. We have such a calcium shortage in this country, in my view, if one is trying to get from 600 milligrams to 1,200 milligrams a day, it is going to take a variety of good food choices. It is going to take probably some supplementation. There are companies out there today putting calcium in every food that you can imagine, and you have seen these. That is one strategy. Dairy foods is another strategy. Soy is another strategy.

If you actually go back to our ancestors and look at what they ate, that is another strategy. More green leafy vegetables and fruits is something I think all of us need to eat in probably a lot more than five servings a day, probably seven and nine servings a day. If you actually look at the evidence for how many people eat that, we can't even get them to five.

We spent a lot of time talking today about what

the optimal diet is. I think that we are still evolving to try to understand it. I don't disagree with the fact that one can avoid dairy foods and get enough calcium. It can be done. One can avoid supplements and get enough calcium. But nonetheless, calcium in dairy foods is 75 percent of the calcium available in our diet from food disappearance data, and it gets very hard in this culture to avoid dairy foods.

Do you have to avoid dairy foods? My evidence would say you don't have to avoid dairy foods.

DR. ANAND: Go ahead, please.

DR. ENIG: Mary Enig, a consultant in nutrition here in the Maryland area. I have found the last questioner kind of interesting because over the years I have been accumulating information, and it is obviously not published because it has been sort of anecdotal. I'm an expert in lipids, and I have done a tremendous amount of work in lipids and consequently have had an interest in the lipids, the natural lipids that we have lost in our food supply. And along with that has come some of the information on milk.

And I have found in some of the information that I have been accumulating with some people, where we have been doing some writing that the black population in the United States by and large was a very good user of dairy

products in the farm areas. They were using mostly clabbered milk because this was fresh milk. It was fresh milk from the farm.

So my experience has been that the African American population -- and I worked with George Mann, who did the research with the Masai -- were dairy users certainly when that dairy was the equivalent of yogurt because the clabbered milk was the equivalent of yogurt. And my experience in talking to people who had asked me for information is that a lot of the problems with dairy that we see in the United States is because of the additives to the highly processed dairy products that a lot of people are eating.

I found people who did not have any problem with a brand of ice cream that didn't have additives, but had problems that they attributed to lactose intolerance with a brand of ice cream that had carrageenan and all sorts of various sundry gums added to it.

So I found your research very interesting. And as a matter of fact very much an echo of things that I have heard and known for many years during my research and the type of information that I have accumulated.

DR. ANAND: Thank you very much.

DR. ENIG: So I thought your presentation was extremely good. I have a question for you, Bruce, with

respect to the functional foods. I think that we have a lot of functional foods that are real foods, that are whole foods, that don't have to have things added to them to be functional foods. And one particular functional food that I have been involved with some research on in the last number of years is the coconut, the coconut oil, the coconut milk, the lauric oil source, which is a functional food. It has a particular functional property that you don't get from other foods, and you don't have to add anything to that to turn it into a functional food. And there are a lot of other foods out there.

Now I know that the industry doesn't like to not be able to make things different. And as a matter of fact, during my period involved in foods and nutrition, I have heard many times nature didn't make good foods, but we the food industry can improve on nature. But I think we have got a lot of things out there that as whole foods are good functional foods that we have ignored that we haven't really utilized as much as we could. And I'd like to see some looking into that type of thing.

DR. WATKINS: I would just comment. I would agree with you. Depending on your definition of functional foods, and looking at a metabolic response or physiologic response, there are foods out there that have very potent effects. Licorice is a good laxative. Is

that a functional food? I guess you could view it in that way. And is the health benefits of orange juice really due to vitamin C or the flavonoids that are contained in the orange juice?

DR. ENIG: Right. But we don't necessarily have to add more flavonoids to the orange juice --

DR. WATKINS: No.

DR. ENIG: -- to get the functionality of the orange juice. And when it comes to coconut, coconut oil, the lauric acid has a functional property such that it is antimicrobial. That is completely above and beyond any of its caloric value.

DR. WATKINS: Foods are a complex matrix of many different things. And I think there is opportunities to make more of these.

DR. ANAND: Dr. Miller?

DR. MILLER: Hi. Greg Miller with National Dairy Council. My question is targeted towards Bruce, too, and I guess it goes along that same framework in terms of defining functional foods. When is it nutrition? When is it nutriceuticals? And when is it pharmacology? And I guess the example I would use is aspirin is a compound that is derived from a plant. If I put it in margarine and use margarine as a delivery vehicle, is that now a functional food, or are we in the

arena of really we use aspirin as a drug. Is it still pharmacology? So the question is how do we define that?

DR. WATKINS: That is out of the realm of my area of expertise in determining legal aspects of what a functional food is. And I think that is part of the reason why we haven't come to agreement with that yet in this country. I wouldn't foresee of aspirin, knowing what its function is chemically, to put it in a food and call that a functional food.

I think of a potential bad approach in this endeavor was the attempt to add calcium to potato chips to make it a source of calcium that someone could seek out and consume and perhaps link it to a functional food. I think is not the right approach.

DR. ANAND: This side here. Go ahead.

MS. FOX: Tracy Fox, nutrition and policy consultant. Kind of a basic question for any one of the panelists in terms of how do we translate a lot of the technical information we have heard, both this morning and this afternoon, to kind of messages to consumers. And does some of the information you presented or the research that was presented this morning really change some of the basic messages, and that is exercise more, eat more fruits and vegetables, eat less fat?

You know, it sounds like from what we have

heard, none of these basic nutrition messages would be compromised genetically, so to speak. In other words, if you exercise more, that is going to express the good genetic components. If you eat more fruits and vegetables, that is going to -- we are going to derive benefits, you know, genetically from that as well. So I guess I'm sure I'm not using the right terms. But it seems to be nothing of what I have heard is going to change the basic nutrition messages. In fact, that almost makes it stronger. And I just want to kind of get the panelist's view on that to make sure I'm sort of on track.

DR. ANAND: Who would like to take this?

DR. SIMOPOULOS: I don't think actually I heard everything you said very clearly. But let me try to respond, and then you can ask your question again. You're interested in how the information that we have discussed here today does or does not change the general health message relative to nutrition.

There are two aspects. I think when you look at the effect of genetic variation on dietary response, that very clearly tells you that based on the genetic variation, not everybody handles food the same way, number one. And number two, that this genetic variation has been studied much more extensively relative to

lipids, so that if you were to give dietary advice, you need to take into consideration who should lower their total fat and who should lower both saturated fat and cholesterol in order to get a healthful cholesterol level.

That message -- that information and the way you are going to present it will depend whether the individual, for example carries ApoE4, or ApoE3 or ApoE2. So in other words, the genetic variation determines dietary response rather than a universal recommendation, everybody in this room should lower their fat and cholesterol intake. This is one area.

The second area relative to the exercise, what was discussed this afternoon is how physical activity influences, for example, lipoprotein lipase, mRNA, and that can bring about a change in triglyceride levels or glucose levels, or a change in insulin level. That tells you the mechanism. If you were to present information how genetic variation in the individual influences the level of physical activity to obtain endurance, that would be an entirely different approach to it.

So we have two aspects. One is genetic variation in dietary response, or how nutrients influence gene expression Dr. Jump presented. And the other is how physical activity influenced gene expression. What we

did not cover is how genetic variation in the individual influences his ability to physical activity and endurance attainment.

So the message does change, and the mechanism is better understood.

DR. ANAND: Go ahead.

DR. TOBIN: I didn't intend to address that question. Brian Tobin from Mercy University. But it does occur to me that it does in some way, as you said, Dr. Simopoulos, change things. It may not change the public statements we make about what the criteria of an adequate diet are, but it certainly does change the way we look at individuals. It changes the way we look at an individual who may come in for dietary counseling who despite an increase in physical activity, who despite a decrease in caloric intake, who despite keeping their dietary fat less than 30 percent still has high triglycerides and high cholesterol. They may fall into that LDL subtype B that Dr. Krauss was talking about.

And so I think it takes us from the population to the individual, which is where the future of nutrition education and future of nutrition efficacy will be. So in mind, the conference today has changed and convinced me that we will be in an era of moving from population based strategies to individualized based strategies.

There were a couple of questions that I wanted to ask. And I have three separate questions for three of the different presenters. Dr. Molloy, it was fascinating research that you presented. To your knowledge, are there any societies in Europe or North America -- and by that I mean medical societies such as obstetrics and gynecology -- who have clinical practice recommendations that promote the consideration of a genetic individuality?

DR. MOLLOY: Not that I know of. It is a very difficult issue because once you get into the situation of where you are talking to a person about their genetic background, then you have got all of this counseling that you have got to consider. And, you know, so nobody has really addressed that issue, or if they have addressed it, but they haven't been prepared to go forward. We certainly don't -- in all of the genetic studies that we do, we anonymize and we certainly don't want to tell people because there is no particular reason in our instance. And then if you came up with something that was dangerous or that was life threatening, it is just a very difficult issue to deal with.

So the answer to that is we haven't, and I don't know of anyone who has.

DR. TOBIN: So in terms of clinical practice

recommendations, do you sense that there is an enhanced sensitivity to recognizing that a given individual won't respond the same way that the population based study says that they should response?

DR. MOLLOY: Well, I think in a way answering your comment, I completely agree with what you said a few minutes ago, that what we are in the business of is looking at individuals and looking at small groups of people. But when you are looking at burden of disease, you are looking at population. So it very much depends on what way -- what questions you want to ask.

It certainly looks as if -- I mean, from what everything I have heard today, it looks as if people are individually responding differently, and one can deal with that on one to one basis. But I don't think you can deal for that globally. I don't know whether that is quite answering your question.

DR. TOBIN: No. That does. I think that is a very good answer.

Dr. Booth, I think the research that you presented on up regulation of LPL is very intriguing. And maybe I'm missing the literature, but this is the first time I actually saw data that said that VEGF goes up after exercise. I hadn't read those articles. Does anybody know the mechanism of action for the increase in

VEGF? Is it generalized sympathetic nervous system activation? Can you duplicate it in a cell with the addition of catecholamines or something like that?

DR. BOOTH: Hypoxia inducible factor (HIF) induces vascular endothelial growth factor (VEGF) expression. There is an animal study where they ran the animals in hypoxia in normal room air, and they didn't get the increase in normal room air, but they got the increase when they put in hypoxia.

HIF-1α (Hypoxia Inducible Factor one alpha) binds to the hypoxic response element.

DR. TOBIN: And the increase in LPL is particularly intriguing. One of the sort of difficult facts to sort out is that insulin, increases in insulin concentration also do that. So you can have a hyperinsulinemic individual who in fact has syndrome X that has increased LPL activity. But at the same time, you also increase that LPL activity following vigorous physical activity.

DR. BOOTH: There is some human literature where they have infused insulin into people and find that skeletal muscle and fat cells respond in opposite directions --

DR. TOBIN: Yes.

DR. BOOTH: -- LPL. You know that. And they

don't know the reason why for that yet, why they have the identical promoters and why the same stimulus would cause one to go up and the other to go down. I don't think that is resolved.

DR. TOBIN: And my last question is for Dr. Watkins. I think it is highly congratulatory that you are putting together a CD-ROM program with the sponsorship of USDA. You said that that is targeted towards nutrition students. Do you have any interest in targeting that towards medical education because I think that would be a hugely successful piece of educational information.

DR. WATKINS: Well, that's a good question. In fact, there has been interest from faculty and medical schools at IUPUI and the University of Kentucky on the content. And this is part of the reason why the interfaces have expanded. But the original intent was to develop something for an undergraduate level that could cut across different disciplines if you are a student in agriculture or economics or nutrition. But yes, I think it could be a product for medical schools. I would be glad to talk about a possible collaboration.

DR. TOBIN: Yeah. I'm convinced that one of the challenges we have in the future is to find the unique collaborations and educational routes to make the

transition from the basic sciences, the molecular biology and the genetics, and then convince the consumer population that these are really good things. And I just hope that we don't find ourselves in a position that the general population is interpreting nutriomics and phytomics and then winding up with the case of genomophobia.

(Laughter)

DR. TOBIN: We have to prevent that somehow.

DR. WATKINS: Is there authoromics then?

DR. ANAND: Dr. Booth, I have a question. Just as when we are placed in a very cold environment, we spontaneously start to shiver, is there any mechanism in the body that when we have very, very high calorie intake, it makes you run around the block? Is there any genetic mechanism, any mechanism?

DR. BOOTH: I'm not sure I understood the whole question. I'm sorry.

DR. ANAND: My question was that we eat a lot of calories, we eat a very heavy Thanksgiving dinner, is there any mechanism in the body that makes you run around the block?

DR. BOOTH: No, no.

(Laughter)

DR. ANAND: I wish there was.

MS. STRUMBO: I'm Phyllis Strumbo from the University of Iowa, and I had a question for Dr. Booth. A few years ago I remember that at Tufts University in some of the research they indicated that people after age 65 could increase their muscle mass through exercise. And I'm wondering, is there a plateau or is there a threshold that if you fall below -- I'm thinking at the time -- I thought, oh, good, I can wait until I'm 65; I don't have time to exercise now.

But I'm also thinking of my mother and mother-in-law, frail women. Is there a threshold that you go below where you could not expect to have improvement from exercise?

DR. BOOTH: Yeah, there probably is. And I think it is when you get people that are to the point where they are barely able to be mobile. For example, I was just home this weekend with my father, and he is in that particular case. And despite my pleas, he refuses to do the weight machine, and he has trouble getting in and out of the car. And what another physician has told me is one of the long range things is for people when they get to that degree, where they reach that threshold you talked about, we need to come up with some kind of a drug. That is really where the drug is needed, and the nutrition, the proper nutrition at the same time. But is

basically going to be the exercise that does it, but without the nutrition it won't. To give them that drug, it may be in the muscle, to cause it to grow.

Now we have some data we just published where we had some old animals where we tried to mimic what happens to people in bedrest. And their muscles would not regrow from the limb mobilization. The muscles atrophied with the limb immobilization and would not grow. But we took another set of the same animals, and we dripped on -- we actually had a substance called insulin-like growth factor put onto the muscle, and the muscle could regrow. And that followed up a study that did adenoviral injections into old animals and prevented muscle wasting in old animals.

So one of the potential drugs that could be used is insulin-like growth factor-1 into old muscle. The major problem is how do you get it there. We have over 600 muscles. Which ones do you hit, you know? And we used a drip technique where we had a catheter dripping it on it for two weeks, and we got a lot of muscle mass back. And so what that told us is what is missing in old muscle that can't regrow is some unknown growth cocktail, and IGFs -- pharmacological doses of IGF brought those muscles back.

Then back to your question, that maybe we get

the muscles past that threshold, get those people moving around, and then we can have them lift weights.

DR. ANAND: Any other question or comment? Well, our undersecretary is not here. So I think she would like to thank all of you. I would like to thank all of the speakers for wonderful presentations and wish you good luck, have happy holidays, and don't eat too much. Thank you.

(Applause)

(Whereupon, at 4:40 p.m., the meeting was adjourned.)

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