[MUSIC PLAYS]

STEPHEN J.D. O'KEEFE: I shall be talking about diet and cancer with special reference to the colon, because there is increasing information that shows that diet is the driver, key driver, in the development and spread of colon cancer.

Let's see if I can get this thing right. So the World Cancer Research Fund and American Institute of Cancer Research have put together an incredible monologue on, or polylogue, on the relationship between diet and cancer in general. And they've also looked into the other lifestyle issues as well. And they base this on 20 systemic literature reviews, based on epidemiological and experimental evidence using common methodology. It was compiled by nine independent centers of scientific excellence. And it covered 20 cancer sites. So it was a massive undertaking.

And this basically summarizes where we are at the moment. What they've done is to associate the instance of colon cancer with the diets that people eat and then combine that with the experimental evidence that shows that certain nutrients can prevent or produce cancer.

So starting at the top-- mouth, pharynx, and larynx-- there's been a decrease if you eat a lot of fruit and vegetables, increase if you drink alcohol. Now, if it says one, the evidence is convincing, which means that it probably is causative. And if it's two, it's highly probable.

Esophagus, fruits and vegetables, again, and fiber. Increase with alcohol, obesity, and mate, which is a drink that they drink in South America.

With regard to lung, it's been shown that there's a decrease if you eat a lot of fruits and vegetables. Physical activity is also important. There's the increase if arsenic contaminates the water and if you take B-carotene supplements. And then red meat begins to appear.

With stomach, again, if you have a high fruit and vegetable diet, it

appears to be preventative. If you have a lot of salty foods, you increase your risk. Obesity and processed meat also increase risk.

With the pancreas, folate has been shown to be associated with-- or dietary folate-- with the decreased risks, whereas obesity, alcohol, red meat, and processed meat are associated with a higher risk.

With the gall bladder, obesity has been associated with increased risk. With the liver, aflatoxin, which is something produced by fungi in vegetables, as well as alcohol, are strongly associated, whereas obesity is also increased.

With the colon, which I'll be going into in a lot more detail, fiber, exercise, and dairy product intake appear to be preventative, whereas red meat, processed meat, alcohol, and obesity increase risk very strongly.

With regard to breast, the lactation, exercise, fiber intake, and dairy products appear to suppress risk, whereas alcohol, obesity, and red meat increased risk. With the endometrium, high exercise appears to be preventative, whereas obesity increases risk.

With the prostate, lycopene, which, as you know, comes predominantly from tomatoes, selenium, and dairy products appear to decrease risks, whereas high calcium supplements and obesity appear to increase risk. Kidney, a low alcohol intake-- that's about one glass of wine a day-- appears to suppress risk, whereas obesity increases risk. And finally, with hematological cancers, obesity is strongly associated with increased risk.

So summarizing all these factors together, those associated with increased risk of cancer are alcoholic beverages in excess-- small amounts might, in fact, be protective-- overweight and obesity, red and processed meat, salt and salted foods, and beta carotene supplements. And factors that decrease risk are physical activity-- so you see how important exercise is-- a high fruit and vegetable intake, high dietary

fiber, dairy products, and breastfeeding in women.

So based on this, the Europeans have come up with suggestions of how you can actually decrease your risk of cancer in general. Be physically active in everyday life. Limit the time you spend sitting. Have a healthy diet. Eat plenty of whole grains, pulses, vegetables, and fruits. Limit high calorie foods, particularly those high in sugar or in fat. Avoid sugary drinks, Coca-Cola. Avoid processed meats. Limit red meat. Limit foods high in salt. And if you drink alcohol of any type, limit your intake.

And if you do these things, they have come up with a suggestion that you can reduce the burden of cancer and avoid about 30% of the most frequent cancers. So that really is a major preventative factor.

So now turning to colon cancer, it's fascinating to see that the instance of cancer varies incredibly throughout the world. In green are the low incidence areas. In red are the high incidence areas. And you can see that in Africa, generally, colon cancer is very rare, whereas in westernized countries, it's remarkably high. And studies have linked dietary intake with these incidences and have shown that the increased risk is associated with high red meat, high processed meat, and animal fat intake, whereas if you have a high fruit and vegetable diet containing a lot of fiber, then you are at decreased risk.

In putting all this together, Doll and Peto, who were epidemiologists in Oxford University about 20 years ago, concluded that over 90% of GI cancers were due to differences in diet. Now you need to pause to think about that for a minute, because that's a very, very important factor. And it means that by simply changing your diet, it is possible to have a massive impact in colon cancer risk, irrespective of your genetic makeup.

So I'll go into those factors in a little bit more detail. Fiber is a complex carbohydrate in the diet from plant sources that escapes bowel digestion and reaches the colon. It is basically food for the colonic microbiota. And this is where it's key because up until recently, we didn't

really know what the microbiota were doing in the colon. But there's increasing evidence that the microbiota affect not just colon cancer, but a wide variety of diseases in humans.

It was first attributed to the local colon cancer prevalence in Africans by Burkitt just after the war, working in Uganda. He found that, in fact, Africans, in general, consumed over 50 grams per day, which is about twice what the recommended intakes are in this country and about three times as much as most of you in the audience actually consume. The epic study, which is a massive epidemiological study in Europe, has estimated that there's a 40% risk reduction in populations with low fiber intake that double their fiber intake.

When we move on to red meat and fat, red meat is potentially carcinogenic through its content of heme, the microbial metabolism of protein residues to release and form nitrosamines and polycyclic aromatic hydrocarbons associated with barbecuing, burning meat, and producing heterocyclic amines. And of note, there was a 35% reduction in cancer risk when one adds more than 160 grams of red meat or processed meat per day, compared to less than 20 grams per day.

And an initial interesting finding was that the increase in cancer risk was enhanced even higher if you add a low fiber diet, hinting at there's an association between different aspects of the diet that you're eating, again, hinting at the importance of the balanced diet.

Fat is interesting because it induces hepatic synthesis of bile acids. And colonic microbes convert bile acids to secondary bile acids, which are strongly carcinogenic in experimental studies. It also produces taurine bile acids, which contain a lot of sulfur, which stimulate the blossom or production of Bilophila wadsworthia in the colon, which produce hydrogen sulfide, which is highly geno-toxic, and therefore increases the risk of carcinogenic change.

Saturated fat has inflammatory actions mediated through prostanoid metabolism. So there's a lot of complex interacting mechanisms. So

when I was giving this talk in Argentina, they all said, but you know, I don't believe these WHO reports, you know. Meat is part of our culture, and I'd rather die than give up.

So I assured them that, in fact, eating meat in small quantities is OK because studies have shown that spinach reduces the proliferative effects of heme in the rat mucosa, so hinting that if you have a lot of salads that you eat at the same time, you can get away with eating moderate amounts of meat.

And remember, we are omnivores, you know. We're not all vegetarian. In humans, resistant starch reduces the proliferative and oncogenic effects of a high meat diet. And in a beautiful study recently published from Australia, they looked at 300 grams of meat a day for four weeks, with or without 40 grams of resistant starch, which is a type of fiber, and basically found that the fiber prevented an increase in oncogenic and microRNA observed with red meat alone. So again, bringing up what I'm trying to put across is the importance of a balanced diet.

Fruit and vegetables, so there are multiple mechanisms whereby they can suppress carcinogenesis. First of all, their high antioxidant and antineoplastic properties, due to their content of vitamins, such as a vitamin C and folate, micronutrients such as selenium and calcium, and also, importantly, phytochemicals, which are basically complex chemicals, which are contained within the cell walls. And they're only released when they get into the colon and metabolized by microbes. And therefore, they're released at a local level and have a profound effect upon the colonic mucosa.

So just to give you some sort of idea of what I'm trying to talk about, if we eat food, it's a complex mixture of bioactive molecules that go into the small intestine. We then digest and absorb of that 90% to 95%, which is extremely important because they provide food for body health. But what's been neglected is the residues that are left behind, particularly those associated with carbohydrate and protein intake. And basically, if you have a balanced diet containing a lot of fiber, they

metabolize what's left to produce short chain fatty acids, which are the key food source for the colonic mucosa.

It's remarkable that colonocytes, unlike any other cell in the body, rely on butyrate for their energy needs, whereas every other cell relies on glucose. And butyrate can only be produced by microbes and fermentation. And basically, so they produce good things that influence mucosal health.

On the other hand, if you have a high fat or high protein diet, you induce the production of toxic metabolites, which increase inflammation and cancer risk. So that's really the key part of my talk. What is fascinating about butyrate is that it's not just the energy source for colonocytes, but it has four major effects on body health.

First of all, it is a histone deacetylase inhibitor, and therefore has epigenetic effects on reducing cell turnover and reducing neoplastic change. Secondly, it's anti-inflammatory because it binds to GPR receptors and down-regulates NF-kappaB to reduce inflammation. As you know, chronic inflammation increases the risk of neoplastic change. It's important in cellular metabolism as well and decreases ischemic transcription.

And probably the most important thing is on the far right, which is an increasing area of interest, shows that it increases barrier function, and so therefore doesn't let nasty things get through the mucosa. It increases mucin synthesis, so it protects the mucosa. It increases triflora factors, which increase healing, heats up the shock proteins, and it produces anti-microbial peptides as well. So it regulates the microbiome and makes it behave itself. And all of these factors together produce anti-carcinogenic effect, and that might, in fact, comply with the reason that I said before, that you can get away with carcinogenic intakes if you have a high fiber intake.

So you know, what's fascinating is that if colon cancer varies throughout the world, and people who said, well, it's different genes and different

populations and so on and so forth-- and, in fact, it isn't. And it's proven that the environmental factor is diet and that it is an environmental disease by this beautiful study published by Le Marchand from Hawaii, where he studied Japanese migrants to Hawaii at the turn of the century and observed at that time that colon cancer was extremely low in native Japan. But within one generation-- I don't have a pointer here. Which is the pointer? Is it this thing? OK, here. So within one generation, the incidence increased to that of native Hawaiians. So this cannot be explained by genetic change. It is an environmental factor.

Colon cancer is a westernized disease. Westernization has been associated with dramatic increase in expected lifespan from 25 before the Industrial Revolution to over 80 today. This change is far too rapid to be attributed to genetic evolution, but can to the remarkable ability of humans to adapt to their environment, the ability to avoid life-threatening events, such as perinatal complications, acute infections, trauma, and war.

And the emergence of Westernized diseases, which include colon cancer, present the most serious threat to public health in the USA today. What do we know about food and food requirements? Food, from what we know of the process of evolution, dietary needs of every organism are genetically determined.

And some have argued that our health is best served by the diet that was established for Homo sapiens in the Paleolithic Era in Africa, a period that lasted from about 2.5 million years ago to 11,000 years ago. And coprolite analysis from cave dwellers from hundreds and thousands of years ago showed evidence of high grain consumption. And more recent studies looking at changes in dentine in excavated specimens from early man have shown that their intake of grain was very high by looking at the grooves shown at the bottom there in the enamel surface. You can tell whether they're high carbohydrate eaters or grain eaters.

And also by the stable isotope content, which can be either C3 or C4, depending upon what types of vegetables it came from, showing that

throughout our development, carbohydrate and grain intake has been high, and meat has been a delicacy.

And so when we compare this to what westernized diets that we eat and associated with high colon cancer risk, we can see that a classic diet is grilled or fried meats. It's got a high fat content, high simple carbohydrate. We tend to wash it down with a couple of beers. Coming from Pittsburgh, of course, we all have to have ketchup here. And where are the fruit and vegetables? Where is the fiber? So we know that we've got a problem.

And if you look at the few remaining groups of native Africans in preserved areas of the Congo, for instance, the [INAUDIBLE] average fiber intake is between 75 and 100 grams per day, which is incredibly high if you think that our average intake is only about 15 grams per day.

And the traditional African diet tends to be grain-based. There you can see people bashing up corn, making it into a powder, which is then made into porridge and cooked and eaten communally. And this process actually increases the amount of fiber in the diet because as you warm and cool carbohydrate, or maize carbohydrate, it increases the amount of resistant starch, which is one of the fibers.

So now I move on to what fascinated me, was that I had the privilege of working as a gastroenterologist in South Africa and then coming back to the USA. And what was incredible was that if you did a colonoscopy, in Africans, you very, very rarely saw polyps and very, very rarely saw cancer, whereas when I came back to Pittsburgh, nearly every other African-American had polyps, which, as you know, are pre-neoplastic.

So we wanted to investigate the reasons for this. And we chose African-Americans because of the similar genetic background. And we found, comparing the two populations, that African diet was low in meat and fat, and high in fiber, as we would've expected from those pictures that I showed you earlier. The microbiota composition and function was dramatically different, with a predominance in butyrate producers and

higher levels of butyrate. And, on the other hand, [INAUDIBLE] conjugators were high, as were bile acids. And when we did biopsies of the mucosa, we found that biomarkers of cancer risk, namely ki-67 proliferation and inflammatory markers, such as CD3 and CD68, were much lower in Africans than African-Americans.

But the problem is that there are a lot of other differences in the environment that might explain risks, such as food sanitation, housing. So we managed to get some funding from NIH to do a unique study, where we actually switched diets. So we gave African-Americans an African diet and Africans a horrible westernized diet for two weeks' period. Did endoscopies before and afterwards, collected stools for microbiota before and afterwards, and looked at biomarkers of cancer risk. And the results were absolutely astounding.

And this topic is fascinating to people because colon cancer is the second cancer killer in the world. And everybody knows somebody who's died of colon cancer. And we have an interest in what we eat. And so this was published in all the leading journals around the world, which was very nice. And Michael Pollan, who has written a book, *In Defense of Food,* and produced a video for PBS, produced this, which is quite fascinating. And we helped him with our contribution on colon cancer.

[VIDEO PLAYBACK]

- At the University of Pittsburgh, researchers are exploring just how eating mostly plants may promote health. Colon cancer specialist Stephen O'Keefe spent many years working in Africa, where he saw very little colon cancer among his patients. But when he moved to the United States, he was struck by the fact that African-Americans have one of the highest colon cancer rates in the world, even though many are genetically similar

to Africans.

- Studies have demonstrated that the factor most associated with differences in colon cancer between Africans and African-Americans is diet.

- Most Africans tend to eat more vegetables, fruits, beans, and whole grains, and less processed food than we do in the West. And those plant-based foods contain substances our bodies can't digest, which we call fiber. We understood for a long time that fiber was important. And we thought it was important to help people overcome constipation and improve what's called transit time of food through your body.

But O'Keefe's studies reveal that fiber does something else. It feeds bacteria in the colon that help to keep it healthy by producing a compound called butyrate.

- Our cells don't produce it, but bacterial cells do. If you eat enough fiber, you maintain a bacterial population that convert the fiber into butyrate, which maintains the health of the colon and prevents cancer.

- Fiber is food for these microbes. And if you don't feed it to them, they're not going to be well, and you're not going to be well. And the less fiber you eat, O'Keefe has found, the more bacteria you have that make harmful compounds that

can lead to cancer.

Since the Western diet that many African-Americans eat is low in fiber and most Africans get plenty of fiber, O'Keefe wondered what would happen if Africans and African-Americans switched diets. O'Keefe's team fed fiber-rich diets to African-Americans and fiber-poor diets to people in South Africa.

- And then we basically measured substances that are good or bad for the colon, before and then two weeks after their dietary switch.

- After just two weeks, the amount of harmful compounds increased in the colons of the Africans, while the amount of beneficial compounds like butyrate increased in the African-Americans.

- This is a butyrate. This is very great. It's very easy to see. The exciting thing is that by changing your diet, you or I can influence our risk of colon cancer in just two weeks.

- So the larger lesson is that we're not just eating for ourselves. We're eating for the trillions of microbes that inhabit us.

[PLAYBACK ENDS]

STEPHEN J.D. O'KEEFE: OK. So basically, what we showed was within two weeks. Now, what's incredibly important is that if you tell someone to change their diet at home, they go home and they cheat. They follow it for a couple of days, and then they carry on eating what they were eating before. So the critical thing is that we admitted each population to an institution and kept them there. We cooked all the foods for them, measured what was

eaten so we knew exactly what was taken.

And at the end of this period, our key biomarker was looking at biomarkers of cancer risk in mucosal biopsies done at colonoscopy. And honestly, we can't wait for colon cancer to occur because that takes 20, 30 years. So we used bar markers, which are associated with progression to cancer.

And if you look at the top here, these are proliferating cells, marked in brown here. And this is an African-American's before and then after Africanization of the diet. And you can see a dramatic decrease, as shown in bio-graph here, and the reverse in Africans.

And we looked at inflammatory markers. This was CD3 positive injury epithelial lymphocytes in Africans before and after. And again, we showed the reciprocal changes with reductions in African-Americans given the African diet and increases in Africans given the westernized diet.

And it's again shown graphically on the right-hand side. And when we looked at the microbiota composition, I mean, basically, working on the hypothesis that it's not the diet that affects colon cancer risks, but it's the effect that diet and fiber has on the microbiota to produce good things or bad things, which improve or worsen colonic health.

And this, first of all, shown at the top here is the microbiota co-occurence networks. It means different microbes working together. In African-Americans on the usual westernized diet, you change them to a westernized diet, and there's a sudden, a massive increase in interconnecting pathways mainly associated with increased butyrate production, and with the exact reverse shown in native Africans, much more complexity before. And then with westernization, you can see the microbiota are basically squashed.

And if you look at metabolic pathways with NMR, it-- I'll take you through this. This is native Africans. This is the metabolites within the colon before, on their usual diet. And you can see there's a lot of metabolic

activity. Changed some to a westernized diet, everything disappears. And the exact opposite in African-Americans on the usual diet, not much metabolic activity. Give them a westernized diet, a massive increase in metabolism.

But of course, it's not just butyrate. And what's fascinating about this-- I'm not trying to bore you with science here, but we all thought that the Krebs cycle was complicated when we were in school. And this is the Krebs cycle right in the middle here. It's tiny compared to metabolism in general.

And now with metabolonics, we can actually look at all the different metabolites produced in reaction of diet to colonic function. And we can link up these different pathways. I can't go through them, but they're color-coded to go for, for instance, short chain fatty acid production, viral acid production, fat digestion, and so on and so forth.

But just suffice it to show, if you look at what happened to these pathways in Africans afterwards, the black lines show significant shifts. And most of the lines went to the left, with an increase in bowel acid metabolism and a reduction in short chain fatty acid production, and with the exact opposite in African-Americans. So you can see the complexity of things that are going on, that it's all tightly controlled. And what we're really talking about is systems biology.

There's a lot of interest in individual microbes. We know that H. pylori is associated with gastric cancer. So isn't there a microbe that causes colon cancer? I don't think so. I think it's a mixture and a balance, which is important. But there is one called a fusobacterium nucleatum, which has been associated with polyp progression to cancer.

And fascinatingly, we found that, in fact, the microbe density associated with the mucosa increased in African-American-- in native Africans given the African diet, and reduced in an African-American that's given the African diet-- I'm doing it the wrong way around-- African-Americans given the African diet. Anyway, basically, a high fiber diet suppressed

fusobacterium.

And we showed the same with Bilophila wordsworthia, which I mentioned before produces a lot of hydrogen sulfide and increases inflammation and cancer risk. So the importance of these microbes in the mucosal surface in the propensity to develop colon cause is obviously there.

One thing that is often missed is that microbes are an extremely important source of synthesis of vitamins, such folate, biotin, B-12, and thiamine. These are studies-- we evacuated people and measured the amount of these vitamins in colonic evacuates. And the amount extracted was much higher than the diet showing that synthesis.

And it's important to note that all of these four vitamins are associated with DNA repair and suppression of proliferation. And in our diet, when African-Americans were given the high fiber diet, it increased the production of folate. So there's a lot more to find out. But we're getting there slowly.

Our latest study is looking at Yupik-- well, the Native Alaskans, which used to be called Eskimos, who have a high meat and fat intake, but a very low fiber intake and have an extreme cancer. It's the highest risk of colon cancer in the world. And basically, what's interesting is that if you look at it here, it goes right through all the different age groups, compared with US whites. But what's interesting is that it is not just colon cancer, but it's also esophageal cancer, naso-pharyngeal cancer, and stomach cancer. Again, going over those links that I took you through at the beginning, that a balanced diet is associated with reduced risk.

And what we propose is that the reason for their higher rate is that their diet is deficient in fiber and phytochemicals. They have an average intake of about five grams per day. And as I've suggested, we need to at least eat 50 grams a day. And we're just about to get NIH funding to be able to do this after a lot of sweat, and tears to show that if we fill that

gap, we can actually reduce biomarkers of cancer risk and develop programs to reduce their remarkably high incidence of colon cancer.

So in summary, microbe behaves as a community, where an inter-microbial interaction strives to produce the metabolic phenotype that supports colonic health and function. It has a genetically determined need for food residues derived from a healthy, balanced diet. Provision of an imbalanced diet leads to disturbance in structure and function, with unopposed production of metabolites that can induce inflammation and proliferation, which increase the risk of neoplasia.

And in conclusion, Mr. Chairman, from epidemiological studies, we know that westernization of the diet leads to an increase in colon cancer within one generation. Our results show that change in diet composition produces immediate effects on the metabolic phenotype of the colonic contents, associated with reciprocal mucosal biomarkers, changes in cancer risk. Our results suggest that current guidelines for the consumption of fiber-rich foods are far too low and increasing fiber to over 50 grams per day, which is easily achieved in African-Americans, and indeed, in most westernized populations, is likely to have an immediate effect on colon cancer risk and death.

There is well warn about progressive westernization of the African diet. Unfortunately, if you go to South Africa now, even in rural areas, Kentucky Fried and McDonald's are appearing everywhere. And as the income level increases, we can expect westernization. But we hope, with our studies, that we are going to pick out traditional aspects of the diet, such as a high fiber content, that can be continued, and so therefore, reduce their risk of eventually developing colon cancer.

So I will acknowledge my workers in my own lab, from South African Studies, Professor Keith Newton in Kwazulu-Natal. The metabolomics was done by Jeremy Nicholson's lab at Imperial College in London. de Vries's lab did the microbiota in Wageningen University in Holland. And Gascon's lab to look at the hydrogenotrophic organisms. These kinds of studies cannot be done by one person anymore. You have to work as a

team and provide your own expertise. And of course, we could not have done them without funding from the NIH.

[MUSIC PLAYS]