

Reversing Aging Rapidly
with Short-Term Calorie Restriction

Continued from...

L.E.: Here's a difficult question. We know that long-term calorie restriction extends life span, but since short-term calorie restriction only reproduced some of the changes that long-term calorie restriction produced, how can you predict the effect of short-term restriction on life span?

S.S.: My assumption is that the effects of calorie restriction are linear. By this I mean that if you are able to improve the expression of 70% or 60% or 50% of the genes and return them to the level of expression that life-long calorie restriction produces, then my assumption is that you're going to get 70 or 60 or 50% of the effects of long-term calorie restriction. My reason for thinking this is that it seems that what matters is general gene patterns rather than specific genes. Between different tissues, you don't find that the exact same genes change, but you find that genes that are in the same kinds of pathways and that are involved in the same kinds of responses change. And so my guess is that calorie restriction creates beneficial patterns of gene expression. At this point, though, it's just an opinion. Ultimately, we'll have to do more studies and follow up to find out whether or not these short-term changes really do also delay the onset of age-related diseases and extend life span.

L.E.: This starts to get us into the area of how you can apply your findings clinically and what the implications are for clinical intervention. Short-term calorie restriction is obviously more attractive to most people than long-term calorie restriction, but we've also heard that yo-yo dieting, in which you repeatedly get rid of a lot of weight for a while and then gain it back again, might be harmful.

S.S.: My current understanding is that the studies say that it's not harmful to do that. My personal opinion is that you should do whatever you need to do to get the weight off, short of something that would hurt your health. I don't think that you should try bulimia or drugs that could harm you, but I think that people would be well advised to do whatever they can to get the weight off.

L.E.: That's very encouraging. I'm sure you've just made a lot of people happy.

S.S.: A recently published study indicates that if people will lose ten pounds, regardless of what their weight is before they start the diet, then many of their physiological parameters of health will improve. It improves your glucose sensitivity, lowers your blood glucose, lowers your blood insulin levels, improves your heart rate, improves your blood pressure. So, even losing weight for a short period of time has beneficial effects.

L.E.: Okay, so is it theoretically possible to use short-term calorie restriction to partially reverse aging in very old humans?

S.S.: Well, I can't tell you for sure, but my guess is yes. Not necessarily to reverse aging, but to improve health and physiology and to slow aging.

L.E.: Based on your mouse study, wouldn't that almost necessarily involve a partial reversal of age-related changes?

S.S.: Yes, it would. I hadn't thought about that, but I suppose that's true.

L.E.: Of course, there might be hazards associated with asking 95 year-olds to eat less. They may have trouble staying alive as it is. Some people who are really old need more calories.

S.S.: I've had people ask me about using caloric restriction for cancer patients or for very elderly people, and my advice is always not to try it. Calorie restriction is something that's very well characterized in animals and rather poorly characterized in humans. We are not animals in a vivarium. We have to go out and cope with a very complex world, and we have to have energy and strength to do it. There's no question that under-eating improves our health, but I don't think that you should take sick people and try to improve their health by under-feeding them.

L.E.: Including a person who might be considered sick precisely because he or she is extremely old and debilitated?

S.S.: Yes.

L.E.: So there may be limits beyond which you can't go?

S.S.: There may be.

L.E.: This suggests the need for a more practical alternative. Since almost nobody wants to be on calorie restriction anyway, and since it does have its safety issues and inconveniences, there is a desire on the part of many people to develop what are called calorie restriction mimetics, in other words, drugs that imitate the effects of calorie restriction. You refer to that in the paper, and the fact that your results provide an opportunity for screening drugs and for finding the magic pill that would simulate calorie restriction.

S.S.: To the best of my knowledge, gene chip analysis would be the fastest way of doing a first screen for drugs and treatments that mimic the effects of caloric restriction.

L.E.: Are you in fact screening any potential candidates at this time?

S.S.: Yes, and we're preparing to screen others, too. We're also preparing to screen compounds for their effects on gene expression for other companies. I think the gene expression assay for the first time provides a method for demonstrating the extent to which a given agent is capable of reproducing the effects of caloric restriction on these genetic expression biomarkers of aging and thereby predicting the agent's ability to reproduce the beneficial physiological effects of calorie restriction. So, I think it's very exciting. Subsequent studies can be done to verify that compounds that pass this biomarker screening are in fact effective in preventing the onset of age-related diseases and extending life span. Excitingly, gene chip biomarker studies can be done in humans after the preliminary studies are done in animals. The screening of humans for delaying the onset of age related diseases with these compounds is a very real possibility.

L.E.: So if you find a compound that's effective in a mouse or in a monkey or whatever, you can find out right away if it has the same gene expression effects in a human.

S.S.: Yes, you can.

L.E.: So not only do you have a technique here that can give you a comprehensive look at virtually the whole genome, but what's even more revolutionary is that it can do so for aging intervention tests in a short time.

S.S.: That's really the contribution of our technology-it makes the initial screening rapid.

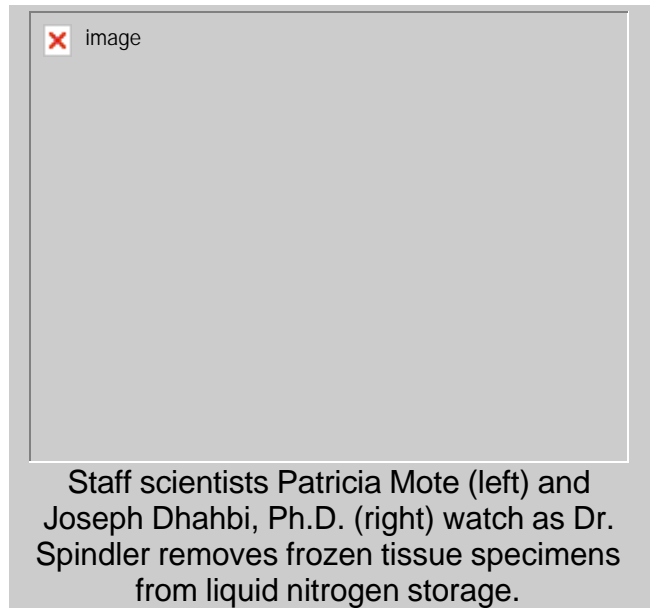
L.E.: This has really been the bottleneck that has held up the entire field of interventive gerontology: it's just not practical to test somebody for their whole life to see if they live longer or not. Now you have a solution to that problem.

S.S.: Even to test an animal for an effect directly on life span takes more than three years in a mouse. Others have produced screens that they say would take a year or ten months. But if you can screen in four weeks, the number of screenings that you can do increases enormously, and the cost goes down dramatically.

L.E.: Talk a little about the company you are working for that is developing the commercial applications of your work.

S.S.: We founded a company called LifeSpan Genetics, which is currently funded by the Life Extension Foundation. LifeSpan Genetics has licensed the commercial rights to the three seminal patents that have been applied for in the field, two from the University of Wisconsin, one from the University of California. We are testing drugs for their calorie restriction mimetic effects and are continuing to look at the effects of different periods of caloric restriction in many tissues in both mice and monkeys, and we are also planning studies in humans.

L.E.: It used to be that the Life Extension Foundation would quietly support research in labs that produced no fanfare, or would report research from other labs that was not funded by LEF. So it's very gratifying that now we're able to report on research that was funded by the Foundation and that has not only made it into the prestigious Proceedings of the National Academy of Sciences, but has also been singled out for special attention by that journal. I understand that stories on your work are appearing on CNN and Science Now as well as in the Wall Street Journal, The Washington Post, the journal Nature, the Reuters News service, and other media sources. Congratulations! It's very exciting that the Foundation is now helping to advance what are probably the most powerful anti-aging tools ever available!



S.S.: For many years the Life Extension Foundation has funded studies of ours and of others that were published in scientific journals. But now through LifeSpan Genetics (www.lifespangenetics.com) we have two papers, not only the PNAS paper but also one soon to be published in the Journal of Nutrition, which represent work that is patent-pending and licensed by the company for work in identifying anti-aging CR mimetic compounds and treatments.

L.E.: From a practical point of view, how would you approach clinical application of your observations? I assume it's hard to take liver biopsies from humans, so what would you do?

S.S.: There are tissues in humans that you can assay readily. And I think there's a tremendous amount of information that can be gleaned from gene chip assays of those tissues, especially since studies are being done in both monkeys and mice. We perhaps won't have to be as thorough in humans once we have this background of knowledge from our non-human primate relatives.

L.E.: What are the prospects for using gene expression profiling to develop a biological measurement of aging? In other words, a way of giving an individual a reasonable assessment of what his or her biological age is.

S.S.: Gerontologists have been interested in developing a biomarker system for measuring biological age for at least twenty years, that I know of. And they want to do that for precisely the reasons we've talked about in this interview. One would like to be able to know when a treatment or compound is affecting aging without having to look at life span. Also, just from every human's point of view, we'd like to know how close we are to death. I don't know how well gene expression profiling will serve as a yardstick for biological age. It's a little early to tell yet, but I think because we're looking at so many things, gene expression levels eventually of 30,000 genes and 3.1 splice variants each, some of which will change with age, I can't help but think it's likely we'll find highly reliable biomarkers of aging.

L.E.: Agreed. The magnitude of the problem of dealing with aging may depend on how many genes are involved. Can you speculate about how many genes you think may change with aging in a whole mouse or a human? Based in part on the numbers seen so far in your lab, and based on the fact that there is overlap between the genes that changed with age in your study and the genes that change according to results in other labs, it seems the total number of changes could be very low.

S.S.: It is an amazingly small number. Most gerontologists agree that there are on the order of tens to hundreds of genes that are involved in changing the life span of an organism from shorter to longer, based on selection experiments (directed breeding to produce long-lived animals). It has to be a relatively small number of genes because you're able to select for long life over a relatively short period of time.

L.E.: In the initial Weindruch and Prolla paper on muscle, the number of genes whose expression went up with age by more than a factor of two was 58 out of the 6347 genes examined, or 0.9 percent. The number of genes whose expression went down by more than a factor of two was 55, or also about 0.9 percent. The total percentage of genes that went either up or down by over 2-fold was 1.8 percent.

S.S.: You have to remember that that was 1.8 percent of the total probes that were on the chip. I don't know about those studies in particular, but normally in gene expression studies done with micro-arrays, the number of genes that actually report a signal (are active) is significantly less than the total number of genes on the chip. I don't know what their results were in terms of active genes, but I think the number of genes that change with age is probably a larger percent, several percent, of the genes that are active.

L.E.: OK. How many of the genes were active in your study?

S.S.: Oh, around 4,000, or about one third of the total looked at.

L.E.: OK, now let's try to compare the muscle results with your liver results. First, you examined over 11,000 genes and found only 46 known genes that went either up or down with age.

S.S.: Yes. I was surprised to find, looking at the liver, that in control mice there were only 20 that went up and 26 that went down. And there were far fewer changes when we imposed calorie restriction.

L.E.: It's astonishing, isn't it?

S.S.: It was astonishing to me.

L.E.: On a percentage basis, those 46 genes represent 0.9 percent of the known genes on the chip, around 1.1 percent of the active genes on the chip, and just 0.4 percent of the total genes on the chip. These age-related changes in liver appear to be considerably fewer than what was found in muscle. At the rate of 1.8 percent of the genes on the chip, one would expect a total of 198 total

genes, or perhaps 109 known genes, to change in expression with age in the liver. However, you saw only 46, which is less than half of this predicted number. And yet you considered a change as small as a 1.7-fold change to be meaningful, whereas Weindruch and Prolla used a more restrictive detection threshold of over 2 fold (though they also reported some results with thresholds as low as 1.5-fold). Therefore, if anything, you would have found even more than 109 changes in known genes if aging had affected both tissues to the same extent, but you did not.

S.S.: I thought that we would see more transcription factors. I thought that we would see more fundamental regulators in gene expression changing. Aging, at least in the liver and in the muscle and in the brain, has a fairly subtle effect on gene expression.

L.E.: On the surface, everything seems to change with age, but at a deeper level, relatively few things change. As you said, aging is subtle.

S.S.: On the gene expression level it looks subtle. Now on the protein level it may be different because there's a lot of regulation that involves phosphorylation cascades and other kinds of modifications of proteins, and we know very little about that.

L.E.: On the other hand, calorie restriction may take care of a lot of that. In your study, for example, only 3 out of the normal 20 age-related increases in gene expression (15 percent) escaped correction by either long- or short-term calorie restriction! Two gene expression increases were reduced about 50 percent, and 15 increases were totally abolished by either long or short-term restriction. Of the 26 decreases in gene expression, 13 (50 percent) were blunted by long-term calorie restriction and 18 (69 percent) were blunted by short-term calorie restriction, leaving only 5 decreases (19 percent) unaffected by either long or short-term restriction. These results are amazing.

S.S.: Yes. I agree.

L.E.: Going further, it seems that a large fraction of the changes you saw with aging don't necessarily represent actual liver aging per se. Instead, they largely appear to represent changes that increase disease susceptibility. This means that the liver actually showed really very few true aging changes.

S.S.: We did see changes that look like the changes that you see in the development of age-related diseases. This has been found by other workers conducting micro-array gene expression studies in other tissues as well. I think our results are very consistent with theirs in showing that gene expression profiles in tissues begin to resemble profiles of tissues that have age related disease processes going on in them. Our tissues looked healthy-we could slice them and look at them under the microscope and see no signs of liver fibrosis for instance. But when we looked at gene expression in these tissues with age, we found changes that more and more resemble those that you see in diseased tissues. So, I think that's part of the development of age-related diseases-a drift towards gene expression that resembles the gene expression of diseased tissues. Calorie restriction reverses much of that, short and long-term.

L.E.: For example, a major fraction of the changes brought about by calorie restriction in your study had the effect of helping to prevent cancer from getting out of control.

S.S.: Most mice living under laboratory conditions die of cancer.

L.E.: So it seems that another possible practical spin-off of your work could be in the area of cancer prevention.

You point out in the paper several specific examples of how changes produced by calorie restriction might block cancer.

In fact, those insights might allow the development of anti-cancer drugs that, on the face of it, have nothing to do with calorie restriction per se.

S.S.: I don't know if our agents will be anti-cancer drugs in the conventional sense of drugs for treating pre-existing cancers, but what I can see clearly is that we're going to be able to develop preventatives. Not just for cancer. Of course, it would be a major step forward if we developed prophylactics for the development of cancer. But we can also attack other age-related diseases, because caloric restriction delays the onset and reduces the incidence and the severity of many age-related diseases. So I think we have the potential, in searching for treatments that reproduce these biomarkers of aging, of finding mimetics that will affect the rate of onset, the severity, and the incidence of many diseases.

L.E.: The beauty of CR is that it has such a broad effect against aging, hitting so many different pro-aging systems. But after you figure out what each specific CR gene effect is, you may find that you want to tailor a variety of drugs that are specifically targeted at those individual effects, rather than trying to replicate all CR effects at once.

S.S.: That's right. We don't really know yet if we'll find mimetics that will reproduce all of the effects of CR in all tissues. I think it's

probably more likely that we're going to find treatments that reproduce some of the effects and are tissue-specific in doing that. I think we'll probably end up having to combine mimetics in order to achieve the full effects of caloric restriction.

L.E.: In your paper, you only reported changes in genes whose functions are known, except for maybe one case, which was the major urinary proteins, which were not really discussed.


S.S.: We didn't report what are called ESTs (expressed sequence tags).

L.E.: Did you see any EST changes?

S.S.: Oh, yes. But we just don't know what they mean. First of all the function of the ESTs is not known. Very often it is not known whether different ESTs even represent different genes. Some certainly represent the same gene.

Some ESTs are constant regions of antibodies.

L.E.: This points up the disadvantage of not having the complete Rosetta stone of biology - the complete genome-on a chip yet. In other words, there may be a lot of genes that are even more important than what's been observed, but they're just not on the chips yet.

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S.S.: They're coming. It will only, I think, be a year or maybe two before we have a whole genome set on chips that will give you the gene expression levels of all of the genes and all of the splice variants. There may be 30,000 genes in the mouse and the human, but each one of those genes gives rise to, on average, 3.1 different messenger RNAs (mRNAs), the splice variants. So in many cases with the probes that are available now, we don't even know which splice variants, which of those three possibilities, we're assaying. But the commercial chips are getting better and better, and we're learning more and more about the genes as the Genome Project is continuing to yield information.

**Dr. Dhahbi and Patricia Mote
prepare to measure gene
expression on an agarose gel.**

L.E.: Will the complete genome and variants chips be available for both humans and mice?

S.S.: For humans as well as mice.

L.E.: Fantastic.

S.S.: And then the field of proteomics (large scale protein surveying) is growing and expanding, and the technology is improving dramatically there. So I think the next ten years are going to give us a rich picture of what's going on during aging in different tissues and how that's affected by caloric restriction.

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