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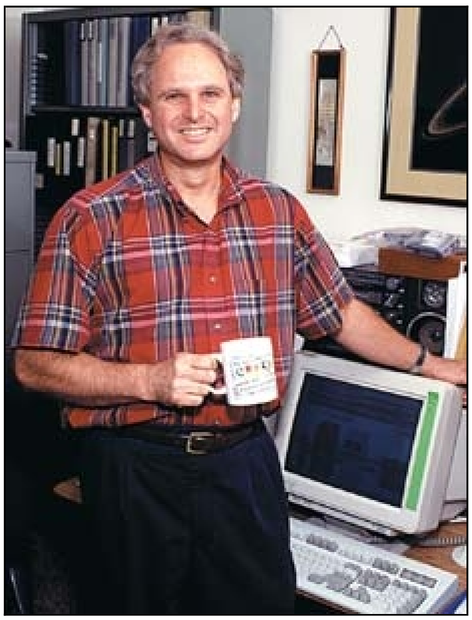
Cover Story

Reversing Aging Rapidly With Short-Term Calorie Restriction

Life Extension Foundation-funded Research Breakthrough Published
in the Proceedings of the National Academy of Sciences
An Interview with Stephen R. Spindler, Ph.D.



On Tuesday, September 4th, 2001 the Proceedings of the National Academy of Sciences' (PNAS) web site featured a paper from the laboratory of Dr. Stephen Spindler, who has been probing the life-extending effects of calorie restriction using advanced gene chip technology. (For an explanation of gene chip studies of aging, please see our interview with Drs. Tomas Prolla and Richard Weindruch in the November, 1999 issue of Life Extension magazine.) Dr. Spindler examined aging changes in the expression of 11,000 genes and the modification of these changes by calorie restriction. The major conclusions from this study are that many of the life extension effects of calorie restriction happen rapidly, and that these effects can be shown not only in young animals, but also in old animals not previously on calorie restriction. Calorie restriction not only slows aging and extends maximum life span, but it partially reverses aging changes as well! On top of that, the fact that calorie restriction acts rapidly means that, for the first time, it is possible to test anti-aging interventions in weeks rather than years, which should drastically accelerate the search for anti-aging treatments. Dr. Spindler, who is a professor at the Department of Biochemistry at the University of California at Riverside and works for a company called Lifespan Genetics, was interviewed about his results by Dr. Gregory M. Fahy and by Life Extension Foundation founder and president Saul Kent on August 17th, 2001.

Life Extension: Dr. Spindler, what is the essence of your new observations, which are just coming out in PNAS?

Stephen Spindler: I think the conclusion you can reach from the paper is that even in very old animals, caloric restriction will very rapidly produce most of the gene expression effects that you see in long-term calorie-restricted animals. That means, I think, that even in the short-term, older people may be able to benefit rapidly from switching to a calorically-restricted diet, and that fits with some of the information that has been in the literature for years. For instance, type II diabetics improve when they start under-eating. Their blood glucose levels improve. Their insulin sensitivity improves. Their general health improves, even before the fat mass, for instance, is depleted. So, there have been some hints that underfeeding could produce positive effects rather rapidly, but this research that we are publishing shows this for the first time, directly, using gene expression profiles as biomarkers for the effects of caloric restriction.

L.E.: Are you the first to actually look at the biological effects of calorie restriction using gene chips?

S.S.: No, the first studies were done by Drs. Weindruch and Tomas Prolla at the University of Wisconsin. Our interest has been the rapid effects. We are interested in the transition from one state to the other. Our contribution here has been looking at how rapidly the effects of caloric restriction are established in animals that have been allowed to eat almost all that they wanted for their entire lives, like most people do.

L.E.: Could you please describe how the short-term calorie restriction experiment was actually done?

S.S.: We took a group of animals that had been allowed to eat almost all they wanted their whole life and we intervened when they were quite old-34 months of age. These mice would be the equivalent of people who are probably 80 years old or older-I'm just guessing at the human equivalent age. We took a group of them and said okay gals, the party's over, it's time to diet. We under-fed them first for two weeks by 20%-that is, 20% less than they had been eating previously-and then for two weeks after that we fed them an additional 20% less so that for the second two weeks they were eating 40% less than they had been eating most of their lives. At the end of that time, at 35 months of age, we sacrificed all of the animals. We then compared the gene expression profiles in the livers of these mice to those in four other groups of mice. The old controls were mice that always ate almost all they wanted until being analyzed at 27 months of age. The long-term calorie restriction mice were those mice who had spent their whole lives being under-fed by 40% until the age of 27 months. Finally, the short-term calorie restricted mice were, as I mentioned, switched from fully fed to under-fed for just four weeks, and even at that only two weeks with "full strength" calorie restriction. We also had a young (seven month-old) control group and a young long-term calorie restricted group (also seven months old) so we could look at calorie restriction independently of aging.

Dr. Spindler in the UCR vivarium where the research animals are cared for.

L.E.: Since you started your short-term calorie restriction experiment at 34 months and let the mice run out to 35 months before you checked them, they were actually eight months older than your long-term calorie restricted animals, which you checked at 27 months. And on top of that you allowed only two weeks with full strength calorie restriction. That hardly seems fair to the short-term calorie restriction group, and yet you saw a lot of beneficial changes anyway.

S.S.: One of the problems with doing experiments of this kind is that it is very hard and expensive to get very old groups of mice. So sometimes we have to make comparisons between old mice that are of slightly different ages, but I think the results are still valid. It's true, though, that because the short-term restricted mice were 35 months old, we might not have been able to appreciate fully all of the effects of late, short-term calorie restriction.

L.E.: What fraction of animals would normally be alive at 34 to 35 months of age in your population?

S.S.: I would guess we're probably down to 35 or so percent of the animals surviving at those ages. We've actually taken two inbred lines and crossed them, so that we get a vigorous mouse that has no genetic defects that cause it to have a shortened life span. They are the longest-lived mouse strain of which I'm aware.

L.E.: So if you see an anti-aging benefit in these mice, it's a true anti-aging benefit, not just a correction of some life-shortening genetic defect. Now, let's attack this from a slightly different angle. Since the animals were already extremely old when you imposed short-term calorie restriction on them, and since their gene expression profiles appeared more like those of young animals after the short-term calorie restriction, it seems inescapable that calorie restriction is not only able to slow age-related changes, but that it is able to reverse age-related changes as well. And it is able to do so over a remarkably short period of time.

S.S.: I think that may be our most significant contribution here.

L.E.: Has anyone else ever suggested that calorie restriction could reverse aging, not just slow it? Or is your finding truly unique?

S.S.: As far as I know, there had been no suggestion in the literature before our study that calorie restriction could reverse age-related changes in gene expression. I think the assumption has been that it prevents deleterious age-related changes in gene expression. It had been our assumption as well, and we've published a number of papers on gene expression where we just assumed that calorie restriction was preventing deleterious changes. What these studies showed for the first time was that in fact that assumption was incorrect. Calorie restriction can reverse the majority of the deleterious age-related changes in gene expression that we found.

L.E.: That's revolutionary and very interesting.

S.S.: There's another issue here too, and that is that we only did two weeks of extreme caloric restriction and two weeks of mild. We're now looking to see if we can find early responding genes, late responding genes and genes that may be in the middle, or genes that may require life-long caloric restriction in order to prevent a change.

L.E.: So, for example, if the short-term calorie restriction were made a bit longer, it might work even better.

S.S.: That's true. I think there's the chance too that if we do this in younger animals, it is possible it will be even more effective, since they will not have accumulated damage throughout their lives beforehand.

L.E.: There would be less damage to reverse.

S.S.: Yes.

L.E.: In general, is it true that caloric restriction started earlier in life, if done properly, leads to longer life span extension and stronger anti-aging changes?

S.S.: There are papers in the literature that indicate that it is true that calorie restriction earlier in life has a bigger impact on lengthening life span and decreasing the onset of age-related diseases than even longer calorie restriction imposed later in life. Nevertheless, our study shows that very late in life in very old animals calorie restriction will rather quickly start to reverse bad changes in gene expression and send them back to youthful levels.

L.E.: So it's not too late for the older folks out there, for people who think they're over the hill, to do something about aging. There's still hope.

S.S.: That's the best news for me.

L.E.: For me, too. What is the oldest age at which caloric restriction had been previously found to lead to an extension of maximum life span?

S.S.: In mice, the oldest study I know of was started at 15 months of age. In fact that was part of the study being supported by the Life Extension Foundation that was done by Richard Weindruch and myself. We've started another study at the University of California even later in life, and we'll find out whether starting mice on calorie restriction even much later in life will have a life span extending effect.

L.E.: There is a fascinating paradox in your paper. According to some of the tables in your paper, starting calorie restriction in old animals did not reproduce all of the benefits of long-term calorie restriction. Nevertheless, some of the benefits of short-term calorie restriction in the old animals were actually stronger than with long-term calorie restriction! That's a rather striking result. Short-term calorie restriction was even more powerful than long-term calorie restriction in some cases. Can you explain this?

Staff scientists Patricia Mote (left) and Joseph Dhahbi, Ph.D. (right) watch as Dr. Spindler removes frozen tissue specimens from liquid nitrogen storage.

S.S.: I think we'll understand better what those many early effects mean when we determine whether they're maintained after acute onset of calorie restriction or whether they're only transient. I think we need to know more about later times. We've looked at four weeks of calorie restriction, and we're looking at other shorter and longer durations of calorie restriction.

L.E.: So the super-protective effects of short-term calorie restriction may recede as you go out longer, returning to being more like long-term calorie restriction. But short-term calorie restriction actually had some aging reversal effects that did not show up with long-term calorie restriction at all. Four cell cycle genes that did not respond to long-term restriction were totally corrected by short-term restriction, and the same was true for five genes in your "others" group, including the anti-atherosclerosis gene, apolipoprotein E. Short-term restriction reversed increases in two stress genes (HSP-25 and stress-induced phosphoprotein 1) that were not touched by long-term restriction, but failed to correct two expression increases in your "inflammatory response" category that were completely reversed by long-term calorie restriction (CR). It's remarkable that you actually saw a reversal of many age-related changes with short-term CR that were not prevented with long-term CR.

S.S.: Yes, that is true. And we saw that even with shorter times of calorie restriction.

L.E.: Even shorter than you showed in the paper?

S.S.: Yes.

L.E.: Incredible. Is it possible that even though shorter term calorie restriction doesn't produce the same total number of anti-aging changes that long-term restriction does, it nevertheless causes the most important such changes?

S.S.: We don't know yet, really, which are the most important gene expression changes. But we do know that the changes produced by short-term calorie restriction apply to the same categories of genes that are affected by long-term restriction and affect the majority of the same genes that are affected by long-term CR.

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L.E.: How reliable are the magnitudes of the changes you reported? If you saw a 4-fold change in a given result with short-term calorie restriction and a 2.5-fold change with long-term calorie restriction, how likely is it that these changes are really different and don't just differ because of random statistical fluctuations?

S.S.: We found that the changes we detect with Affymetrix gene chips (the ones used in the PNAS study) are really pretty reliable. It is true that you can't do very good statistics with these Affymetrix chip studies. The reason for this is that the chips and chemistries for the chips are so costly. Also, old animals are expensive and difficult to get. So we've been in a situation where we're able to measure 1,000 to 10,000 times the number of genes we used to be able to measure, but we don't routinely do as many samples as we used to do. So traditional statistical tests are difficult. We've used a technique called Northern blotting in order to validate our changes. What we found was that at least 95% of the changes that we detected with the Affymetrix chips were reproduced using Northern analysis. So, my guess is that the results we found are reliable.

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. So, even losing weight for a short period of time has beneficial effects.

Any gene chip study is really an initial screening event. It can show you that things that you might never have expected to change do change, and it can point you in new directions that you might never have known about, because it's an unbiased screening of a large percentage of the total genes that the organism expresses. When you have those changes, then you can be pretty sure, if you're looking at changes that are 1.7-fold or greater, as we did, that the changes are real.

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 percent 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.

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.: 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.: 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. 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, and then 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.

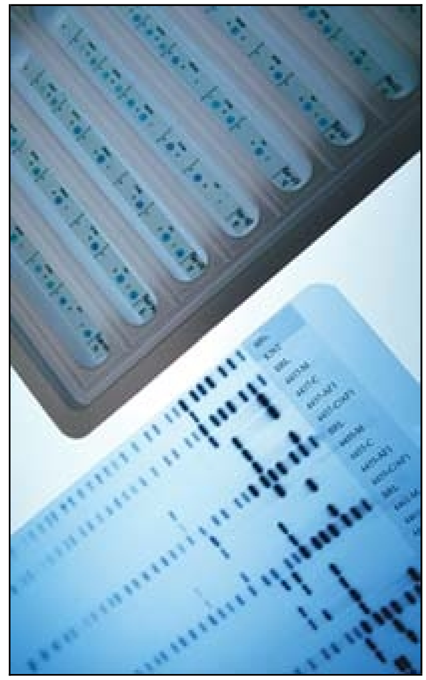
S.S.: We founded a company called LifeSpan Genetics, which is currently funded by the Life Extension Foundation. LifeSpan Genetics has licensed the commercial rights to two seminal patents that have been applied for in the field, one from the University of Wisconsin, one from the University of California. 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 other labs that produced no fanfare. 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!

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.



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?

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 original 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%. The number of genes whose expression went down by more than a factor of 2 was 55, or also about 0.9%. The total percentage of genes that went either up or down by over 2-fold was 1.8%.

S.S.: You have to remember that that was 1.8%. 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.

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L.E.: On a percentage basis, those 46 genes represent 0.9% of the known genes on the chip, around 1.1% of the active genes on the chip, and just 0.4% 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% 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).

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%) escaped correction by either long- or short-term calorie restriction! Two gene expression increases were reduced about 50%, and 15 increases were totally abolished by either long or short-term restriction. Of the 26 decreases in gene expression, 13 (50%) were blunted by long-term calorie restriction and 18 (69%) were blunted by short-term calorie restriction, leaving only 5 decreases (19%) 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 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.

Might those insights allow for the development of anti-cancer drugs?

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. 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.

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.

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.

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

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

L.E.: Do you have in mind any hierarchies of the relative importance of different gene effects that you would use to distinguish a better calorie restriction mimetic intervention from a less good one?

S.S.: Yes. I think we have to look at each tissue and use what we know about the physiology of the tissue with aging and the effects of the change in gene expression that we see and compare that to the effects after long-term calorie restriction. In every tissue the effects are different, but there is a vast literature about the physiology of the tissues with aging and with caloric restriction. This can provide enormous help in interpreting the results that we get.

L.E.: Have you compiled all of that literature into some rules of thumb?

S.S.: Certainly we're working on that. It's a complex process and one that takes studies in a lot of tissues because every tissue is different so far. There are even differences between species that we've found, and that have been found by others. So, it's going to take some work to establish clear rules. But, yes, they're coming along. We have a good idea of what the effects are in liver, and now we're looking beyond that into other tissues as well.

We look at various times after the onset of caloric restriction: at the changes that happen early, at a middle time and late. Changes that appear early and disappear, and changes that appear early and are also present in the long-term state. I think that these kinds of studies are going to give us a better feeling for which may be primary changes and which may be secondary changes

L.E.: Your task would also be simpler if you could place the changes you see within some kind of hierarchy of cause and effect as well, so you would be able to say which age-related changes are primary and which ones are secondary. Do you have any feeling as to whether you can identify such a hierarchy of changes?

S.S.: This is a difficult question. One of the ways we're trying to get at the answer to that question is to look at various times after the onset of caloric restriction: at the changes that happen early, at a middle time and late. Changes that appear early and disappear, and changes that appear early and are also present in the long-term state. I think that these kinds of studies are going to give us a better feeling for which may be primary changes and which may be secondary changes. We can use bioinformatics to cluster the genes that change and start to ask which regulators are likely to be responsible for those changes. That will allow us then to do fundamental experiments that will ask more directly, do these specific genes affect health span and life span?

L.E.: That's very exciting. Another problem for interpretation of gene profiles might arise from the fact that very similar proteins can react in different ways to the same condition. For example, heat shock protein number 70 (HSP-70) doesn't seem to be affected by aging in your experiment, whereas HSP-86 and HSP-25 both go up with age.

S.S.: Proteins like these don't act alone, they act in groups. For HSP-70, where we didn't see an effect with age, it may be that there is a rather subtle effect. When we look at the livers of starved and fed animals, we find that HSP-70 responds strongly to feeding. After you eat, the level of messenger RNA for HSP-70 goes up about three-fold in a spike (peak), and then that decays after the meal. How many of those spikes you have and how high they are and how often they occur will affect the time-averaged level of HSP-70. If you fast an animal for a long time, the level of chaperone proteins tends to fall. So calorie restriction may have a subtle effect on some chaperones that we haven't picked up with these studies, where we looked at animals that had been fasted for 24 or 48 hours, because we missed those spikes that determined the averaged level of the protein over the long-term. So, these are very subtle and complex effects at work.

L.E.: It's a point we don't usually think about, but a lot of the things you might be measuring depend upon what time of day it is, how long it's been since you last ate, that sort of thing. The genome is very dynamic and if you look at different times, you'll get different results.

S.S.: That's so true.

L.E.: At least you're developing the tools to deal with the complexities.

S.S.: That's right.

L.E.: Another problem with using gene expression profiles to characterize aging and interventions could be that aging is a kind of mosaic. Different organs seem to age at different rates, and you die from whatever the weakest link is. So you could look wonderful based on many different markers and drop dead the next day because of one weak link that perhaps wasn't even measured.

S.S.: That's right. It's not clear to me whether we'll be able to get gene expression biomarkers from humans, for instance, from the cerebral cortex! But it is a promising technology.

L.E.: Have you found any contradictory effects of calorie restriction and aging, such as maybe having both an increase and a decrease in cell proliferation factors or both an increase and a decrease in the tendency for apoptosis or for inflammation? Or is everything consistent?

S.S.: We're not really looking at physiology. We're inferring changes in physiology from changes in gene expression. There is a lot more work to be done to see whether these changes in physiology actually occur as a result of changes in gene expression. Really, we're at the beginning of a very exciting era, and I think we need to use proteomics ultimately. We're going to need to know more about what's happening to the proteins in the cell.

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COVER STORY

L.E.: Your paper was based on the liver. What is the importance of the liver in human aging?

S.S.: The liver is an interesting organ. It is one that ages well. There are people who, of course, die from liver disease. That is a killer of the elderly. We were attracted to studies on the liver because it has such a powerful influence on physiology. One major problem that develops with aging is metabolism of drugs. They're not metabolized as well, partially because of loss of the enzymes that are involved in metabolism and partially because of a decrease in blood flow to the liver and in the volume of the liver with age. Most of the proteins that are circulating through your body in the blood, that aren't in blood cells, come from the liver, and the composition of those proteins has a major impact on wound healing, on clotting, on atherosclerosis, etc. The composition of your blood as far as glucose and insulin levels go is partially due to the responsiveness of the liver to insulin, and has a major impact on vision and on your circulatory system. So the liver sits at a very central spot. Also, it's partially mitotic. So far, gerontologists have been attracted very often to organs whose cells don't divide very much. There is a good rationale for that interest, because these organs can't create new cells when old cells get damaged or die. But most of the tissues in our body are able to renew themselves, and many of those tissues that can renew themselves have major problems with age. They have problems with being able to renew themselves properly. So I think that they also are very important to look at.

L.E.: Did the changes you observed actually reflect changes in liver function that normally occur with aging?

S.S.: We found quite a number of changes that correlate well with the known changes in liver function. We and others have shown that there is a decline in the expression of enzymes that are involved in drug metabolism in the liver, and that fits very well with the loss of those differentiated (liver-specific) functions. Other specialized (differentiated) functions of tissues begin to decline with age, and certainly there was a decline in genes that are important for cell division and an increase in genes that tend to block cell division. That, for instance, fits very well with the loss of the capacity of the liver with age to repair itself and to produce new cells that replace damaged cells.

L.E.: So, some changes that seem to be related to liver aging per se consist of the loss of liver-specific functions.

S.S.: Yes.

L.E.: Why do you think the liver does so well with aging? You've mentioned a lot of change that takes place, but in reality, compared to other tissues, not much aging seems to be happening in the liver.

S.S.: It's a difficult question. There is a standard bias that the liver ages well. This is based on physiological measurements and endpoints. People compare how many people die of or are afflicted with liver disease versus brain disease or muscle disease. I think there were certainly differences between the liver and tissues without the potential to divide. We saw new things that we think occurred because the liver does have the potential to divide. I don't really know the reason for all of the tissue-specific differences. We're going to need more data and more time to think about the data we do have before it becomes clear what's going on.

L.E.: How do the changes you do see with aging in the liver compare to changes seen in the non-dividing tissues?

S.S.: We found, as had Weindruch and Prolla in their studies of gene expression in brain and muscle, that the older the animals got, the more there was expression of genes that seemed to indicate that the animal was undergoing inflammatory stress and other kinds of stresses. Stress gene expression and inflammatory gene expression rose with age, and in the current study the majority of those changes were reversed by short-term and long-term calorie restriction, which means that even late in life you can improve your physiology and reduce the level of physiological stress rather dramatically by under-eating.

On the other hand, we saw a lot of action in the pro- and anti-apoptotic genes that you do not see in the brain and the muscle. The brain and muscle cells have to make very different kinds of decisions about whether to commit suicide or not.

L.E.: Very interesting point.

S.S.: Yes. And we also saw genes that were either pro cell division or anti cell division that were affected by aging and calorie restriction. Again, these genes would not be genes that you would expect to find changing in non-dividing tissues. Although, interestingly, they did find a few genes that are involved in cell division changing in the brain.

Dr. Spindler identifies where regulatory proteins bind to a specific gene.

L.E.: Could that be due to the presence of glial (non-nerve) cells or vascular cells in the brain?

S.S.: Yes, it could be, you're right. We know too that neuronal stem cells are capable of dividing and migrating to different regions of the brain.

L.E.: What about genes related to energy metabolism?

S.S.: We've looked in great detail at the genes that are involved in energy metabolism in the liver and in muscle. We find that calorie restriction has major effects on those. And we've looked in liver and muscle on the effects of feeding on gene expression related to energy metabolism. The results can be summed up by saying that a calorically restricted animal, even one that has just eaten, is rapidly turning over (renewing) its peripheral tissues. It's an effect that you'd expect, in fact, because insulin is a very powerful anabolic (tissue-building) hormone. Insulin levels fall in calorie-restricted animals, but you get a spike (rapid increase) in insulin levels after they eat, an intense spike, and they're very insulin-sensitive (able to respond to insulin). What I expect happens after they eat is an intense wave of protein biosynthesis under the influence of insulin. As soon as the insulin level falls, they start to break down their proteins again and put the products out into the blood for energy generation. This keeps calorie-restricted animals constantly recycling their proteins so they don't accumulate damaged, oxidized, old, defective and toxic proteins.

L.E.: Has this kind of recycling been seen in liver and muscle?

S.S.: Yes. Our data in combination with the work of others supports our general conclusion that calorie restriction causes and maintains enhanced turnover of peripheral tissue protein into old age, whereas turnover is lower and declines further with age in non-restricted animals.

L.E.: Might this explain why there is an increased inflammatory response with aging and why calorie restriction tends to reverse that?

S.S.: I don't know what that inflammatory response is due to, it's very intriguing. Is it a physiological inflammation, or is it inappropriate changes in the regulators of those inflammatory genes that are not really related to true inflammation?

L.E.: In other words, perhaps the body thinks that there is an enemy that doesn't really exist.

S.S.: Or is there real inflammation that they're responding to, and if so, what's the inducer? It's a very interesting and complex question, and something that's going to be exciting as more people begin to work on it and try to deduce really what's going on.

L.E.: Well, we certainly know from the proponents of the glycation hypothesis that macrophages (inflammatory cells) will attack glycated myelin and so forth because of the molecular change involved in glycation. There could also be other, analogous changes that would accumulate simply by failure of turnover of the proteins involved.

S.S.: We actually made sections and had them examined by the Pathology Laboratory at U.C.L.A. We didn't see any signs of increased macrophage activity in the calorie-restricted livers, or in the old livers versus the younger livers. But those studies were just preliminary and were just quick looks. I think there's a lot more work to be done to investigate that phenomenon.

L.E.: In comparing changes that you have seen and other people have seen in gene expression from tissue to tissue, can you draw any conclusions about the nature of aging in general?

S.S.: Well, so far, based on aging in brain and muscle and liver, a generalization is that aging seems to involve an increase in inflammatory gene expression and an increase in what's broadly categorized as stress gene expression. Caloric restriction seems to reverse most of that. I think that's our major consistency right now.

L.E.: The stress proteins, meaning certain proteins that stabilize other proteins against environmental stresses, were one of the major protein classes that were suggested to rise with aging in the first Weindruch and Prolla gene profiling experiment. Much was made of that observation.

S.S.: Well before the Weindruch and Prolla papers came out, we were publishing papers that showed that these stress proteins, or "chaperones," go down with caloric restriction.

L.E.: Quite right, and that they go up with aging.

S.S.: Yes. Heat shock proteins are chaperones. Chaperones respond to stress. You have to understand the purpose of chaperones to understand why a calorie restriction mediated decrease in chaperone levels may be extremely important.

Normal proteins have to have a certain shape in order to work, and stress may cause "unfolding" of proteins so that they lose their proper shape. Chaperones are required at a certain level to assist with or correct folding of proteins. They also go around and rescue proteins that have become improperly folded and refold them properly. If they can't be refolded, the chaperones tag them with ubiquitin so they can be degraded and eliminated from the cell.

When you have a stress, like, for instance, exposure to radiation, the cells that are damaged have to make a decision. Are they going to repair themselves and, if they do, has the repair been successful? Or has the damage been too severe, such that the cell, when it repairs itself, is still damaged and may become a cancer cell? Or might the cell secrete destructive local hormones to the cells around it? In such cases, the cell needs to make the appropriate decision to commit suicide and be eliminated from the body and replaced by a healthy cell.

These kinds of molecular decisions are being made all the time, not just after a dose of radiation. As part of the normal process of living, for example, there is damage to our DNA that has to be repaired, and molecular decisions have to be made about how to institute and evaluate the repair process.

Chaperones are also involved in the decision about whether a cell will commit suicide. Chaperones are a part of the machinery for regulating gene expression in some key instances. For instance, high chaperone levels inhibit apoptosis.

L.E.: Apoptosis being cell suicide or the elimination of defective cells such as pre-cancerous cells.

S.S.: That's right. Some chaperones prevent apoptosis, and if you lower the levels of those chaperones, then you encourage more apoptosis.
So, if you have high chaperone levels with age, that tends to make cells less apt to commit suicide and more apt to continue to exist, even when they're severely damaged and may be secreting harmful cytokines to the tissues around them, or possibly converting to a cancer cell. So, when calorie restriction lowers those chaperone levels, it releases some of those factors slightly more, so the decision is slightly more likely to be made to commit suicide and kill those damaged cells that might otherwise survive.

Therefore, calorie restriction is anti-cancer. That's well established. Calorie restriction is also pro-apoptotic. It promotes cell suicide of damaged cells and we think the reason it's pro-apoptotic is because it lowers the chaperone levels.

L.E.: I suppose one might imagine that over-expressing chaperones could be positive, say in a post-mitotic tissue, in which dead cells can't be replaced. But then again, you have to balance cell loss against the probability of dying very rapidly from cancer.

S.S.: That's right, and those are the kinds of complex and critical decisions that cells are making in our body all the time.

L.E.: The inflammatory proteins that you saw going up are kind of a motley crew, are they not? And a lot of inflammatory proteins that might be most informative about what's going on don't seem to be there.

S.S.: That may be partly a function of which genes were on the chip and partly a function of the subtleties of how proteins work together. If the level of one of the components of a group of proteins changes, whether that has an effect on overall activity and whether that will influence other specific proteins may not be obvious. I'll just give you an example. One of the chaperones, HSP-90, does a lot of things. It binds to some steroid hormone receptors and keeps them out of the nucleus and inactive. When the hormone that interacts with the receptor shows up, it disassociates the receptor from the chaperone and the receptor now becomes active. That same HSP-90 also binds to two pro-apoptosis factors.

L.E.: So there can be multiple effects of a given interaction.

S.S.: Yes. HSP-90 also is only one of a group of proteins with a relationship to steroid hormone receptors or with the pro-apoptotic factors. So, there's a great deal left to be found out about how all of these specific gene changes affect physiology.

L.E.: Yes, it's very complicated.

S.S.: I hope the leads that we're getting will attract people to investigate these kinds of questions.

L.E.: Yes. Basically, you're trying to understand the changes that take place over time in a system that nobody even understands to start with.

S.S.: It's an immensely complicated system, but not infinitely complicated. I'm confident that we'll be able to understand it. It's just going to take time and work.

L.E.: And in fact you've already identified a lot of pathways that have implications for the control of disease, so you're certainly making rapid progress. But progress can sometimes bring confusion, too. For example, your data and a lot of other observations indicate that calorie restriction reduces growth hormone function, thyroid hormone function and cell proliferation, and yet we need these functions in order to live. Can you make anything out of that, yet, at this point in the game?

S.S.: There's more complexity, but the functions that we need to procreate our species are not always the same as the functions we need to survive a long time. There are hormonal systems at work that want to speed us to fertility and fecundity and ensure that we'll reproduce. There are also imperatives to ensure that we'll produce offspring that will be aggressive for men, or that will be nurturing for women. Those are a different set of priorities than living a long time. In fact, calorie restricted animals are not very good at procreating, so it may be that some of these effects that we're seeing seem to be counter-intuitive because we're not keeping in mind what it is they're trying to accomplish.

L.E.: It seems that several genes whose expression changed the most with aging were not affected by either short-term or long-term calorie restriction.

S.S.: Right, and that's not to say that those changes with aging are not important, because of course calorie restriction does not stop aging. It just slows it or reverses it to an extent.

L.E.: Indeed. Do you have any plans to go beyond calorie restriction and CR mimetics and to attack those aspects of aging that persist in the face of calorie restriction?

S.S.: Not anytime soon. The challenge of finding out what causes calorie restriction to extend life span is challenging enough for now.

L.E.: Dr. Spindler, thank you very much for your interview.

S.S.: Thank you.

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