

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, Sep. 4th, the Proceedings of the National Academy of Sciences (PNAS) web site features 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?

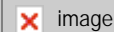
Stephen Spindler: I think the conclusion you can reach from the paper is that even in very old animals, caloric restriction will very rapidly produce most of the gene expression effects that you see in long-term calorie-restricted animals. That means, I think, that even in the short-term, older people 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. Richard 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 70 or 80 years old or older - I'm just guessing at the human equivalent age. We took a group of them and said okay guys, 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 (7 month-old) control group and a young long-term calorie restricted group (also 7 months old) so



Stephen R. Spindler, Ph.D. of the Dept. of Biochemistry at the University of California at Riverside (UCR) directs the genechip studies conducted by LifeSpan Genetics.

we could look at calorie restriction independently of aging.

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 8 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. That's as good as it gets in studies like this. 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 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.

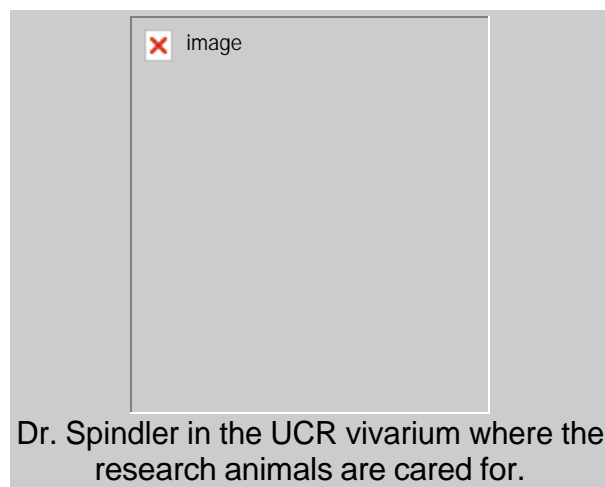
L.E.: Has anyone else ever suggested that calorie restriction could reverse aging, not just slow it? Or is your finding truly unique?

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 of gene expression, affecting genes that we can be pretty confident are going to improve the physiology of the animal.

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 much later in life will have a lifespan-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?

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 5 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 gene 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. So I think it's a very high probability that short-term reproduces the majority of the long-term effects.

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. Because our funding has been limited, we haven't been able to include the numbers of animals in each group that we would like to have to do traditional statistics. 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. The tact most users of this technology have taken is that you validate a cross section of the results that you get using another technology that's been proven to be highly reliable. 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. Whether a 2½ fold change is the same or less than a 4 fold change is difficult to say, but the fact that both changes are in the vicinity of two to four fold is highly likely to be correct.

L.E.: In the tables in your paper, if you report, for example, a two-fold decrease in expression of a given gene due to aging and a two-fold increase in expression of that same gene in old animals after long-term calorie restriction, does that mean that there was no change with age in the calorie restriction group compared to a young control mouse?

S.S.: Though it may not have been clear in the way the data were expressed, if it went down by two-fold with age and calorie restriction increased it by two-fold, we have returned it to near the youthful baseline level.

L.E.: The reason for the question is that in some cases, you might see a two-fold drop with age and a four-fold increase with calorie restriction!

S.S.: Again, I don't know if it really is four-fold greater in that particular case. You have to look at these gene chip studies as being screening studies. Probably two-fold and four-fold changes are different, but until you test each change with another technique, using large numbers of animals and get statistics that are highly reliable, it's a little difficult to say. What you can say is they both changed in opposite directions to about the same fold and you can take that to the bank.

L.E.: Okay.

S.S.: 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. The expression levels change up or change down and they change in the approximate percentage that we found. But that doesn't mean that more detailed studies aren't warranted.

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