Webinar Q&A Report:
Targeting Biological Aging: A New Paradigm for 21st Century Medicine

1. **Why does our (Western) culture value drug therapy over behavioral changes?**

   M. Kaeberlein: I agree that lifestyle plays an important role in healthy aging, but even people who practice an optimal lifestyle still undergo biological aging and develop chronic disease and disability. I don't think it's an either-or situation. I would also encourage you to recognize that lifestyle interventions have side effects very much like drugs do. Consider exercise for example. Anyone who exercises regularly at a moderate or higher level will experience side effects. Those may include muscle soreness, tendonitis, lethargy/fatigue, sprains and broken bones, even death. I don't think it's useful to think of pharmacological interventions as either better or worse than other lifestyle interventions. They are just one strategy in our arsenal.

2. **Are rapamycin and intermittent fasting similar in their biological influences on aging?**

   M. Kaeberlein: This is a bit complicated, because nearly all of the intermittent fasting studies in mice are actually caloric restriction. There isn’t much good data showing benefits from isocaloric intermittent fasting in mice and the benefits appear to be much smaller than you get from rapamycin treatment or caloric restriction. At a molecular level, yes, I'd say there are similarities - overlapping but distinct is how I think about it. Among the shared changes are reduced organ size, increased ketogenesis, enhanced autophagy, reduced growth hormone signaling, and - of course - reduced mTOR signaling.

3. **Do you have to know your dog's precise age to participate? Our dogs are rescues with estimated ages.**

   M. Kaeberlein: Any dog can be in the Pack. In order to be eligible for the Foundation, Precision, or TRIAD cohorts we need to know within about a year or so of the dog's actual age. You will be asked to provide an estimate of the dog's age and your degree of certainty as part of the Health and Life Experiences (HLES) survey.

4. **Do you take, or do you plan to take low-dose rapamycin yourself? And why?**

   M. Kaeberlein: I have taken low dose rapamycin cyclically in the past and likely will again. I don't make any recommendations on what others should do. I have not experienced any side effects associated with rapamycin personally. The only benefit I attribute to rapamycin was a striking and rapid improvement in adhesive capsulitis or frozen shoulder, which was resolved within a few weeks of starting an 8 week low-dose rapamycin regimen.

5. **Has rapamycin been used in humans with progeria?**

   M. Kaeberlein: There is preclinical data in both mice and human cells that rapamycin can improve progeria, and I believe there was a clinical trial in combination with farnesyltransferase inhibitors in progeria patients. I don't know the results of that study.

6. **Would replacing the thymus every few decades help ameliorate immune decline?**

   M. Kaeberlein: Perhaps. I'm aware of one small study aimed at thymic rejuvenation in humans which has been published. The results look encouraging but the study has obvious limitations and needs to be expanded. https://onlinelibrary.wiley.com/doi/full/10.1111/acel.13028

7. **Rapamycin promotes nitric oxide. Are these beneficial effects of Rapamycin on aging attributable to an increase in NO biology? Protecting cardiovascular function, for instance?**

   M. Kaeberlein: That's one of the many downstream effects of rapamycin that likely contribute to the various benefits in terms of healthy aging, at least in mice. Teasing out the various mechanisms downstream of mTOR and rapamycin is one of the real challenges in this field right now.

8. **Why did organisms who produce rapamycin evolve to produce it? What is its function?**

   M. Kaeberlein: The current thinking is that rapamycin is produced by certain bacteria to inhibit growth rate of competing eukaryotic cells in their niche. Bacteria do not have an mTOR protein and do not appear to be sensitive to rapamycin.

9. **Which animal disease models has rapamycin been tested in? What is the consensus opinion?**

   M. Kaeberlein: There are really too many to list here and they cover a wide range of types of diseases and disorders. In the context of aging, there is good evidence that rapamycin improves age-related declines/pathology in heart, brain, liver, kidney, muscle, reproductive, immune, and oral tissues among others.

10. **Why were there no small dogs in RCT? Do they age so much slower that you wouldn't have statistical power? What could you be missing by doing this?**

    M. Kaeberlein: In order to have statistical power to detect a chance in the 3-year time frame, we needed to have larger dogs. You are correct that it's possible we will miss something, but that is unfortunately the nature of clinical trials and limited resources. You make the best educated guesses you can when it comes to design.

11. **Has there been research on osteopenia and osteoporosis?**

    M. Kaeberlein: Yes, although this hasn't been studied as much as other age-related changes. Here is one study in rats: https://pubmed.ncbi.nlm.nih.gov/26395886/

12. Will rapamycin affect a failing heart?

M. Kaeberlein: This is a tough question to answer. My guess is that for certain types of heart failure, particularly those that involve hypertrophy, there's a good change it would. What seems to be clear in mice and perhaps in dogs is that the declines in heart function which go along with aging can be reversed by rapamycin treatment. Once it becomes true heart failure, it's unclear whether the same mechanisms are at play. I know of planned studies in dogs to look specifically at the effects of rapamycin on dilated cardiomyopathy and valvular degeneration, but no data that I'm aware of yet. I think DCM in particular is a good bet.

13. Is it known if intermittent fasting can produce any of the same effects as rapamycin?

M. Kaeberlein: Intermittent fasting with caloric restriction (nearly all mouse studies are this) has many of the same benefits as rapamycin - I think of them as partially overlapping interventions in terms of molecular mechanisms and phenotypes. Isocaloric IF has not really been studied in any detail in mice, but the existing studies suggest that IF has minimal if any real benefits for lifespan. So isocaloric IF does not recapitulate the effects of rapamycin, at least in mice.

14. Do you think the response to this medication might be increased at younger ages vs older ages?

M. Kaeberlein: Rapamycin reverses periodontal disease when started at 20 months of age in mice, so these are pretty old animals. Roughly equivalent to 60–65-year-old people. We haven't looked in older mice yet, but it's a good question.

15. Did you investigate the influence of taking Rapamycin while doing exercise on health aging?

M. Kaeberlein: As far as I know, this hasn't been carefully examined. Rats on rapamycin show preservation of muscle with aging, but I don't think the combination has been carefully studied.

16. What is Rapamycin's impact on muscle mass in older mice and/or humans?

M. Kaeberlein: There is data in both mice and rats that rapamycin preserves muscle function and mass with aging and prevents sarcopenia. I'm not aware of any human data yet.

17. Is Metformin less potent compared to Rapamycin?

M. Kaeberlein: In mice, yes. Metformin appears to have little to no effect on lifespan in mice in longer-lived strain backgrounds, although it does improve some measures of metabolic health during aging. In humans, we don't know yet, but the metformin data is fairly compelling and more extensive than rapamycin, simply because more people have been taking metformin for many years.

18. We study age-related disorders in feeding/swallowing in our lab. What is a source for rapamycin if we would like to look at how this drug may affect our behavioral outcomes?

M. Kaeberlein: We get our rapamycin from LC Labs for our yeast, worm, and mouse work: https://lclabs.com/products/r-5000-rapamycin. For human or dog studies, we use the generic Rapamune or sirolimus available from any pharmacy.

19. **Since mTORC1 is involved in regulation of skeletal muscle mass, what are the effects of rapamycin on the maintenance of skeletal muscle mass/strength in older people? Is sarcopenia helped with rapamycin?**

    M. Kaeberlein: This hasn't been studied in people as far as I know. In mice and rats, rapamycin preserves muscle function and mass during aging.

20. **What kinds of dogs are eligible to participate in the dog aging project and how can people sign up?**

    M. Kaeberlein: All dogs are eligible and you can participate by going to www.dogagingproject.org and clicking the 'nominate my dog' button. The only restriction is that, for now, we can only accept one dog per household.

21. **Do you have any insight on how castration affects aging in dogs?**

    M. Kaeberlein: This is still a bit of a controversial question in the veterinary literature but I'd say the best evidence suggests a small increase in lifespan is associated with sterilization. What seems clear is that sterilization changes the disease risk in both directions - some diseases become more prevalent and some less prevalent. There is, as you might suspect, also a sex-dependent effect. The largest study addressing this question is here: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3629191/. I think our Longitudinal Study of Aging will be able to help resolve some of the remaining questions in field around this topic in a few years.

22. **How does rapamycin compare to other interventions, such as fasting or exercise, on markers of ageing?**

    M. Kaeberlein: It depends on whether you are talking about mice or humans. In humans, we don't know much about efficacy of rapamycin for aging. Exercise clearly "works" in the sense that people who exercise tend to live longer and be healthier. Fasting gets a lot of attention, but it's still unclear how effective that is in people. In mice, caloric restriction and rapamycin are comparable in magnitude and breadth of effects across many organs and tissues. Severe CR (50%) appears to be slightly better than rapamycin at increasing lifespan. Exercise has minimal effects on lifespan in mice but does improve health. Most fasting experiments in mice are not isocaloric, so they are really CR. It's unclear whether isocaloric fasting, intermittent fasting, or time-restricted feeding really have any significant benefits in terms of aging in mice. My guess is they probably are modest at best (again I'm talking about isocaloric nutritional interventions).

23. **What happens to the inhibition of protein synthesis (and the stimulation of autophagy) with Rapamycin treatment?**

    M. Kaeberlein: The simple answer is that global protein synthesis goes down and autophagy goes up. It's obviously more complicated than that, and it's unclear whether therapeutic doses - even at the higher organ transplant levels - have this effect generally across many tissues in mammals. One model that is gaining popularity is that the cyclical dosing of rapamycin (once per week for example) is actually better at maintaining productive autophagy than daily dosing, because you need cycles of protein synthesis (high mTOR) to rebuild the autophagic machinery periodically.

24. Would rapamycin treatment increase the incidence of sarcopenia in older adults via inhibiting protein synthesis?

M. Kaeberlein: This seems unlikely, as the doses being used probably don't significantly impair protein synthesis and seem to preserve muscle mass and function during aging in mice and rats.

25. Calorie restrictions are considered to be a longevity factor in humans. What is the role of vegan/meat diet and calorie restriction in pet's longevity especially dogs and cats?

M. Kaeberlein: There is not any good data on this yet in dogs, although people have very strong opinions. We expect that the data from our longitudinal study of aging will help provide some answers that can better guide recommendations to pet owners and also provide insights into interactions between nutrition and aging in the 'real world'.

26. How was the rapamycin dosage and dose timing selected? Has there been a dose response preliminary study done?

M. Kaeberlein: The short answer is that we evaluated what was known about dosing for efficacy and side effects in mice, dogs, and humans and came up with a dose that we were confident would be safe in companion dogs and hopefully provide efficacy for the aging endpoints we are studying. We have performed two short randomized, clinical trials testing three different doses. We have also been guided by the Canine Oncology Trials Consortium studying rapamycin in dogs with cancer as to dosing. The once weekly schedule is based largely on data from humans that once weekly dosing seems to reduce side effects while maintaining efficacy, at least for restoration of age-related immune decline.

27. In the mouse studies, were both sexes used? Has anyone developed a KO mouse for mTorCi?

M. Kaeberlein: Yes, several studies have shown that rapamycin can increase lifespan and improve health during aging in both male and female mice, although rapamycin seems to be metabolized differently in male and female mice such that the effective dose - and response - is higher in females compared to males provided with the same dose. I'm not aware of any data that something similar happens in dogs or people, with respect to sex. Several genetic models of mTORC1 depletion have been developed and found to delay aging. Veronica Galvan's lab has done some elegant work with depletion of mTORC1 in the brain showing protective effects for normative cognitive aging and in Alzheimer's disease models.

28. Since aging is multifactorial, how do you think rapamycin is working?

M. Kaeberlein: There are several downstream mechanisms that likely contribute. In mammals, I think the anti-inflammatory effects are probably underappreciated. Exactly how this works is still being uncovered, but it's clear that rapamycin can potently suppress the so-called 'senescence associated secretory phenotype' which includes many inflammatory cytokines. Increases in autophagy, improved mitochondrial function, and changes in translation and transcription have been shown to be important in invertebrate models and also likely play a role in effects of rapamycin on mammalian aging.

29. Could slowing the aging of humans cause other diseases or physiological problems?

M. Kaeberlein: I suppose, but it's not clear to me what those problems would be. I think you could make an argument that what we've done during the 19th and 20th century - increasing life expectancy without slowing aging - has done this. We have a lot more people with Alzheimer's disease today than we did a century ago, for precisely this reason.

30. Are there plans to study other compounds individually and in combination with rapamycin in the dogs?

M. Kaeberlein: We're just at the idea stage, but yes, it is my hope and expectation that we will be able to test other interventions in companion dogs in the future.

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