



Anti-aging Interventions: Caloric Restriction and Beyond

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ABSTRACT

Aging is the biggest risk factor for most of the age-related chronic diseases, which carried a heavy health-care costs burden worldwide. These age-related diseases, such as cardiovascular diseases, neurodegenerative diseases, cancers, type 2 diabetes, and osteoarthritis, shared common mechanisms that associate with aging, such as chronic sterile low grade inflammation, cellular senescence, DNA damages, and stem cells dysfunctions. In this review, we will first discussed the dietary manipulation, such as caloric restriction as the mean of delaying aging, and we will also review nutraceuticals or natural compounds, and pharmaceutical drugs as the mean for intervention of aging.

Keywords: Anti-aging, caloric restriction

ABSTRAK

Proses menua dikaitkan dengan berbagai penyakit kronis terkait-usia, yang mengakibatkan beban biaya perawatan kesehatan yang besar di seluruh dunia. Penyakit-penyakit tersebut meliputi penyakit kardiovaskuler, penyakit neurodegeneratif, kanker, diabetes tipe 2, dan osteoarthritis, yang mempunyai gambaran umum serupa yaitu inflamasi steril kronis kadar rendah, penuaan sel, kerusakan DNA, dan disfungsi sel punca. Tinjauan ini berkenaan dengan manipulasi diet berupa restriksi kalori, dan *nutraceuticals* serta senyawa alami, juga sediaan farmakologis untuk tujuan mengintervensi proses penuaan. **Khing S. Ong, Zack S.T. Lim, Boenjamin Setiawan. Intervensi Anti-aging: Restriksi Kalori dan Upaya Lain**

Kata kunci: *Anti-aging*, restriksi kalori

INTRODUCTION

In February of 2017, an international team of experts in aging had published The DrugAge database through literature searches of 324 publications, featuring 418 different drugs and compounds that extend lifespan in model organisms, including yeasts, worms, flies, and mice.¹ The results of lifespan extension changes are species specific.

Lower form organisms, such as worms and flies, have the most dramatic life extension changes when tested with these “anti-aging” drugs or compounds. Whereas mice showed only a modest extension of lifespan.¹ If size is the determining factor and we translate these data from lower organisms to human, the predicted results will not be that impressive. Another hurdle is that human average age is far longer than this lower model organism. Thus evaluation of lifespan extension would

need a long follow up period to achieve. In addition, application of any drugs for human would need a toxicological screening, clinical trials and FDA approval, before it can be marketed for public consumption.

In this review, we therefore, limit ourselves to relatively well established drugs/compounds or methods of anti-aging. Senolytics for the elimination of senescent cells will be left out, as it has been discussed in some detail elsewhere.²

As age is the primary risk factor for many age-related diseases, currently most scientists in this field are using the term “health-span” instead of lifespan. As a matter of fact, we also don't know if there is a limit or not to human lifespan. Recently, however a study analyzing global demographic data strongly suggest that the maximum lifespan of human is fixed

and subject to natural constraints.³

There is a need to validate any anti-aging drugs or compound, and any interventions that seems to work, and this is where well designed clinical trials come in. An alternate is to measure aging biomarkers. A recent study⁴ described an easy measurement of urinary 9-oxo-7, 8-Dihydroguanosine (or 8-oxoGsn) as marker of our biological age that can be used to compare against our chronological age. Based on the premises that oxygen products produced during normal metabolism can cause oxidative damage to DNA and RNA in the cells, as age advances. This damage increases and reflected by the level of 8-oxoGsn excreted in the urine. If this results are confirmed and reproducible by others labs, then it can be a convenient method of validating the effect of any anti-aging modalities.



DIETARY MANIPULATION

Caloric Restriction (CR)

Numerous studies have shown that reducing calorie intake by 30 – 40 percents, will extend the lifespan of model organisms (worms, flies, and mice) by at least one third.⁵ However, in primate (rhesus macaques), the results of CR on lifespan are contradicting each other in two recent studies. The 23-year study from NIH showed that CR does not improved life expectancy,⁶ whereas study from the Wisconsin National Primate Research Center (WNPRC) with a 30 percent CR, improved the survival of the primates.⁷ However in both studies CR does delay age-associated diseases, indicate a health-span extension.

Analysis of responses to CR in multiple tissues reveal an increase in the expression of genes for pathways of mitochondria energy metabolism, mitochondria redox metabolism, protein synthesis, and a decrease in the expression of genes involved in inflammation and immune pathways.⁸ In addition, CR links to the average species lifespan in mammal with the rate at which DNA undergoes age-related epigenetic changes. CR slows down this process of epigenetic drift and extend the lifespan.⁹ Despite of some controversies, CR is considered by most as a beneficial mean against aging. Even in light of recent finding that CR accelerates the loss of gray matter throughout much of the cerebrum in grey mouse lemur primate,¹⁰ however it is without apparent affects on cognitive function. In another study on rodent model, low-fat diet with CR resulted in reduced activation of microglia, and suggests that this might be the mechanism for the protective role of CR during aging associated decline.¹¹

CR in Human Subjects

Evidence that CR delays aging and extends lifespan in animal models, draw many researches on CR in human subjects. Most studies focused on intermittent fasting, to mimic the effects of CR, but still palatable for most people. In addition, a continuous long term CR, might also be detrimental if essential nutrients are not supplemented, and could cause muscles and bones density loss. However, a recent study from University of Southern California¹² indicates that prolonged fasting can protect patient's lymphocytes from chemotoxicity and regenerates stem cells by diminishing IGF-1 (insulin-like growth factor 1)

or PKA (protein kinase A) signaling. Researchers also designed a "fasting mimicking diet" with 5 days of fasting in a month for 3 months, and repeatable as needed, it is safe and effective in reducing risk factors for aging and age-related diseases.¹³ From evolutionary perspective, this intermittent fasting make sense, as our body's metabolism is adapted and conserved for intermittent of scarcity of food in the hunters gatherers era.

The so-called "Longevity Diet"

Okinawan diet: Okinawan residents are well known for their long lifespan and high numbers of centenarians which is thought to be due especially to their diet and may be a healthy life style. Changes in diet and life-style in the recent decade has reduced their lifespan to the average Japanese lifespan. However, their original diet is worth to be study for its lifespan extension.

This traditional diet is heavy on vegetable and fruit, but reduced in meat, refined grains, saturated fat, sugar, salt and full fat dairy products, and relatively rich in carbohydrate as compare to Mediterranean diet, because of the intake of antioxidant rich, low calorie orange-yellow root vegetable such as sweet potatoes.¹⁴

Mediterranean diet: Like Okinawan diet, Mediterranean diet also includes large amount of fruits and vegetable, bean, whole grains with moderate amount of fish, dairy and wine, and limited red meat, but lots of olive and olive oil. In a recent study of older people who followed a Mediterranean diet shows they retained more brain volume than those who do not follow the diet as closely.¹⁵

NUTRACEUTICALS

Resveratrol (RV): initially found in grapes and red wine, and later from many other sources include Melinjo (*Gnetum gnemon L.*) seeds that are rich in resveratrol dimmers.¹⁶ RV seems to mimics calorie restriction in some ways and activate sirtuins¹⁷ for its anti-aging effects. There are other synthetic sirtuin activators developed mainly against Sirt1, such as SRT1720 (18), SRT1460 with potency closer to SRT1720, and SRT2183 with potency closer to RV developed by Sirtris Pharmaceuticals. NIH researcher found that an enzyme PDE4 (phosphodiesterase type 4) in the skeletal muscles is the primary target for

the health benefit of RV.¹⁹ Another benefit of RV is increased in hippocampal neurogenesis, microvascularization and reduced microglial activation, hence improving or prevent age-related memory decline and mood dysfunction.²⁰

Sirtuins: are a group of Silent information regulator (Sir) proteins that regulate lifespan in many model organisms. Sir2 is an NAD (nicotinamide adenine dinucleotide)-dependent deacetylase that links metabolism with longevity in yeast, worms and flies.^{21,22} The homologs of yeast Sir2 in mammals are SIRT 1-7. These sirtuins are divided phylogenetically into 4 classes, Sirt 1-3 in class I, Sirt 4 in class II, Sirt 5 in class III, and Sirt 6-7 in class 4. Sirt 1, 6 and 7 are located in the nucleus, Sirt 3, 4 and 5 in the mitochondria, and Sirt 2 found predominantly in cytoplasm.^{22,23} Most studies of mammalian sirtuins support a link between sirtuins and CR phenotype of increased lifespan. Sirt 1 null mice have been shown to have shorter lifespan compare to wild type.²²

The following are some of the known functions of Sirt 1-7.^{23,24}

- Sirt 1: Main biological functions are related to cell survival and metabolisms, including gluconeogenesis, fatty acid oxidation, cholesterol scavenging and thermogenesis. It also involved in mitochondria activity and stemness maintenance.
- Sirt 2: Main functions in cell cycle, microtubule stability and promotes differentiation and stress response.
- Sirt 3: Metabolism, thrmogenesis, and stemness maintenance.
- Sirt 4: Negative regulation of insulin secretion
- Sirt 5: Positive regulation of urea cycle
- Sirt 6: Glucose homeostasis and regulate DNA repair, promote differentiation, and maintenance of stemness.
- Sirt 7: Positive regulation of stress resistance and rDNA (recombinant DNA) transcription, and maintenance of stemness.

NAD+ /NMN (Nicotinamide Mono nucleotide): is a coenzyme with intimate connection to sirtuins and provide a foundation that translates the regulation of energy metabolism into aging and longevity



control in diverse organisms. It has been shown that as we age, level of NAD⁺ decreases and without sufficient NAD⁺ Sirt 1 loses its ability to block HIF-1 alpha (hypoxia-inducible factor 1 alpha). Increasing level of HIF-1alpha in turn block the communication between nucleus and mitochondria at the cellular level and also between the hypothalamus and adipose tissue at the systemic level.²⁵ The breakdown in nuclear-mitochondria communication caused rapid decline in mitochondrial function, which lead to development of age-related diseases. Administration of NAD⁺ precursor NMN restores the communication and mitochondrial function.²⁵ The reason for depletion of NAD⁺ is increase activity of PARP1 (poly[ADP-ribose] polymerase1) for DNA damage signal as we aged. While producing poly(ADP-ribose) polymers to generate the signal, PARP1 consumes NAD⁺ and causes depletion of NAD⁺ in its cellular pool. In addition, decrease level and activity of NAMPT (nicotinamide mononucleotide phosphate transferase) in aging also lead to low level of NAD⁺.²⁶ SIRT1 inhibit PARP1 through deacetylation, and their activity on key cellular protein are opposing too, e.g. SIRT1 suppress NF-kb (nuclear factor kappa beta), but PARP1 activate NF-kb. Since decrease level of NAD⁺ contribute to the aging process and to pathogenesis of age-related chronic disease, it will be appropriate to recommend the supplement of NAD⁺ precursors such as NMN or Niagen.²⁷

Curcumin/J147:²⁸ J147 is a novel molecule found in turmeric, a modified version of curcumin molecule. J147 targeting mitochondrial alpha-F1-ATP synthase, and causes an increase in intracellular calcium, that lead to a sustained calcium/calmodulin dependent protein kinase, kinase beta (CAMKK2) dependent activation of AMPK/mTOR pathway, an accepted longevity mechanism. By modulating mitochondrial process through ATP synthase, J147 prevent age-associated drift in the hippocampal transcriptome and plasma metabolome in mice and extends lifespan in drosophila.

T-006:²⁹ T-006 is a novel molecule synthesized from tetramethylpyrazine by replacing the methoxyphenol group of J147. T006 has multifunctional neuroprotective effects at very low concentration. T006 significantly ameliorate memory impairments in APP/

PS1 transgenic mice (a mouse model of Alzheimer's disease) suggest its potential as a possible treatment for Alzheimer's disease.

Probiotic/Microbiomic: Researchers has compared fecal microbiota of frail elderly people with a similar but more robust control elders group. The frail group had lower level of short chain fatty acid made by microbes in the gut from dietary fibers.³⁰ A recent large study of healthy old and young (age from 3 to >100) in China, found no significant differences in their gut microbiota,³¹ however, such study is valuable for future comparison reference.

ALT-711/Alagebrium: ALT-711 acts by catalytically breaking the cross-links of advance glycation end products (AGEs), commonly generated in food prepared with heat process. AGEs can initiate cell inflammation, and causes cardiovascular diseases in older people.³²

Telomerase activators: TA-65 an extract from Astragalus root. A recent paper³³ has shown that T-65 moderately activate telomerase in human keratinocytes, fibroblasts, and immune cells in cultures. Another study³⁴ has found that TA-65 effects both telomerase and proliferative activity of human CD4 and CD8 T cells with a 1.3 to 3.3-fold increased of telomerase activity compared to control, they also demonstrated that TA-65 activates telomerase by an AMPK-specific pathway.

Klotho: Human klotho gene encodes the ala Klotho protein, produced in kidney and the brain, then enter the circulation as a hormone. It is a multifunctional protein that regulates the metabolism of phosphate, calcium, and vitamin D. It suppresses oxidative stress and depressed level of klotho increases expression of senescence-associated proteins, such as beta-galactosidase, p16, p21, and p53.³⁵

PHARMACEUTICAL DRUGS

Metformin

Metformin has been used for over 60 years in type 2 diabetes (T2D). A study in 2014 reported that subjects with T2D taking metformin lived longer than a control group of similar people without T2D,³⁶ suggesting that metformin might protect against basic aging process, and not just T2D. Metformin has also been shown to extend the lifespan of worms.³⁷ Metformin decreases hepatic gluconeogenesis and increase insulin sensitivity. It activates AMPK

(adenosine monophosphate activated protein kinase), an enzyme important in cellular energy balance, and in glucose and fat metabolism.³⁸

Rapamycin/Rapalogs:

Rapamycin is first discovered from isolated bacteria found in Easter Island (Rapa Nui) soil, as an immune suppressant for organ transplant. It is an inhibitor of TOR (target of rapamycin) protein kinase.³⁹

A study in 2009 with genetically heterogeneous male and female mice⁴⁰ fed at 600 days of age with rapamycin, and based on age at 90% mortality, rapamycin led to an increased of 14% for female mice and 9% for male mice, suggest rapamycin could increased lifespan at least in mice. Long term uses has been found to cause a variety of adverse effects, including impaired wound healing, anemia, pneumonitis, and other infections. Chronic inhibition of mammalian TOR (mTOR) by rapamycin caused diabetes in mice, but intermittent rapamycin feeding increase lifespan in mice.⁴¹

Rapalogs are rapamycin analogs design more specific to certain cellular situation. Analog RAD001 was develop that could affect aging in human, and reported that older people taking RAD001 improved their response to vaccine.⁴²

Spermidine:

Spermidine is a natural polyamine that when feed to yeast, worms and flies triggers autophagy, hence extend their lifespan.⁴³ Endogenous spermidine decreases with age in human except centenarians they keep higher level of this polyamine. The impact of long term effects of spermidine has not been studied. However, long term feeding of spermidine promotes increase of healthspan.⁴³

PROTEINS FROM YOUNG BLOOD/PLASMA

GDF-11 and GDF-8 (Myostatin):

Earlier studies using parabiosis technique has generated discovery of anti-aging protein GDF-11.⁴⁴ This discovery has created debates over the effect of GDF-11 on aging whether it is real or an artifact.⁴⁵ A more recent study from UCSF shown that relatively high blood level of both GDF-11 & GDF-8 were less than half as likely to die from any cause.⁴⁶ Despite some controversial, GDF11/8 does seem to be



a potential anti-aging protein.

Recent study from USC⁴⁷ has identified a protein originated from mitochondria called humanin which is a neuroprotective factor. In addition, there are 6 small humanin like peptides (SHLPs) encoded in the same region of mtDNA as humanin. SHLP2 and 3 and humanin significantly reduced apoptosis, and the generation of ROS, also improved mitochondrial metabolism in vitro. The level of circulating SHLP2 were found to decrease

with age, suggest the important role of mitochondria play in relevance to aging.

CONCLUSION

We understand the difficulty of clinical study of this anti-aging agent, due to human lifespan. However, randomized control clinical trial can be done as those for type 2 diabetes with comparison in certain period of 5 or 10 years.³⁶

Microbiomic studies not only interesting but

also could yield significant findings for anti-aging intervention. Example: a molecule in pomegranates transformed by gut microbes: Urolithin A,⁴⁸ that enable muscles to protect themselves from aging. Secondly, it would be interesting to perform fecal-transplant of gut microbes from centenarian to people at high risk or having age-related diseases to see if these new gut microbes can assist in preventing or delaying the age-related diseases.

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