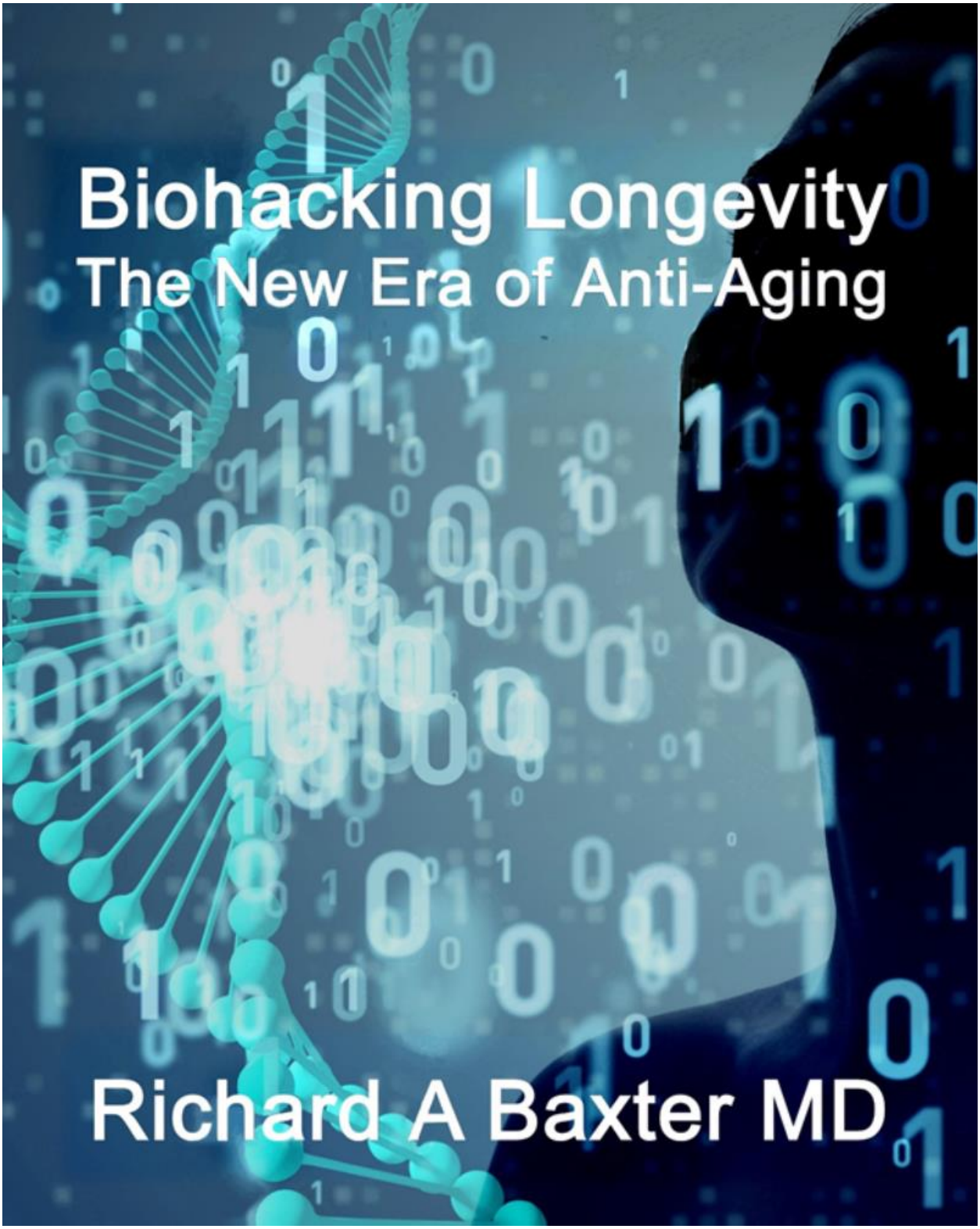

Biohacking Longevity
The New Era of Anti-Aging
Richard A Baxter MD

March 2025 edition

This book is based on emerging science as well as established clinical practices. It is not intended as specific medical advice.

Phase Plastic Surgery &
Longevity Institute

Nature, Sept 2019: "A small clinical study in California has suggested for the first time that it might be possible to reverse the body's epigenetic clock, which measures a person's biological age … The results were a surprise even to the trial organizers. 'I'd expected to see slowing down of the clock, but not a reversal,' says geneticist Steve Horvath at the University of California, Los Angeles, who conducted the epigenetic analysis. 'That felt kind of futuristic.'"

SciTechDaily, December 2020: "Harvard Medical School scientists have successfully restored vision in mice by turning back the clock on aged eye cells in the retina to recapture youthful gene function. 'Our study demonstrates that it's possible to safely reverse the age of complex tissues such as the retina and restore its youthful biological function,' said senior author David Sinclair. [Update March 2025: "Life Biosciences, the company co-founded by Sinclair, is gearing up to launch the first ever partial epigenetic reprogramming candidate… Assuming everything goes to plan … Life Bio will enter clinical trials within a year.]

Liveforever.club, August 2024: A recent study [from the Buck Institute for Research on Aging] conducted a phase 3 controlled clinical trial to investigate the effects of different TPE [Therapeutic Plasma Exchange] modalities on biological age. The study revealed that TPE has a significant impact on reducing biological age.

The researchers measured biological age using 35 different epigenetic clocks, which are tools that assess age-related changes in DNA methylation patterns. These clocks are among the most accurate methods for estimating biological age.

Table of Contents

Introduction

It is now possible to reverse aging in humans. Not just to look younger or feel younger, but to literally turn back the biological age clock. After decades of unfulfilled promises, controversies, and sometimes outright quackery, longevity medicine is gaining traction as a legitimate clinical specialty. Prospects for extension of healthy lifespan are engaging some of the brightest minds in science and medicine. This coming of age in anti-aging is happening in research labs around the world and beginning to enter clinical practice. We're biohacking longevity.

The advancements fueling this change are nothing short of mind-boggling. But misinformation about anti-aging and longevity remains ubiquitous and persistent; a lot remains to be proven. Finding the true signals amidst this noise is plenty challenging for experienced researchers, and for the consumer nearly impossible. Nostrums and nonsense are everywhere, even as concepts such as gene reprogramming, tissue regeneration, and biologic age clocks are developing swiftly. This book will be your guide to what works now, what might work, and what doesn't.

I'll reveal some powerful discoveries just now emerging from research labs and moving into clinical practice, and introduce what is coming to be known as 21st century medicine.

My personal involvement in the topic started many years ago with an interest in the health effects of wine. After the "French Paradox" was reported in the 1990's, there was a lot of research seeking to explain the association of wine with health and longevity. Wine drinkers were found to have *lower rates of all of the diseases of aging*, so there had to be some essential common denominator that wine drinkers were benefiting from. Few had any idea at the time that the seeds of a scientific revolution were just beginning to germinate. We now know that it isn't just wine, or anything in wine alone, that holds the secret.

As these revelations unfolded, my interest expanded from the healthful joys of wine into an obsession over longevity science. I began to spend absurd amounts of time looking up the latest studies and learning from the world's experts on anti-aging. I became one of the first to complete a new certificate course on Longevity Medicine. I'm convinced that we are on the threshold of one of the most profound transformations in biomedical science in history.

As I started thinking about how to incorporate this new science into my clinical practice, I recognized the need for a sort of briefing document for patients considering participation in this new version of anti-aging. This book is just that; a plain language overview, a living document, to be updated regularly.

My First Longevity Medicine Certification

Even the term "anti-aging" is itself becoming passé, in favor of "longevity medicine." The practice of longevity medicine is not intended to replace traditional medical care (yet), but I have no doubt that it will transform the way we think about health and health care. A fundamental change is underway, framed by the curiosity of science, grounded in massive data known as bioinformatics, and propelled by the lure of solving biology's biggest questions.

A brief history of anti-aging

Anti-aging hasn't always had a good reputation. Pressure to capitalize on anti-aging treatments has often resulted in shortcutting validation through clinical trials, diluting the impact of genuine breakthroughs. Stem cell therapies, hormone clinics, and a range of supplement products have all gained marketplace traction without clinical corroboration or honest reckoning of risks. Similarly, long held beliefs such as antioxidant supplementation persist even as clinical trial evidence repeatedly points to their futility. Biohacking longevity aims to move anti-aging into the 21st century by leveraging AI-driven revolutionary advances in bioscience.

A pivotal breakthrough was figuring out how lifespan extension via caloric restriction (CR) works. It had been known for years that in experimental models, restriction of caloric intake

triggers a metabolic change that prolongs healthy lifespan. It requires an impractical degree of CR however, so scientists wondered if there could be a way to replicate it without semi-starvation. What they discovered ushered in the modern era of anti-aging.

Lost in translation

The journey from the research lab to the clinic is known as translational medicine, and the process can be long, unpredictable, and expensive. What works in a test tube or cell culture may not do the same in animal studies, let alone humans. Because there are limits on what sort of studies you can do to test your idea in clinical trials, you need to determine not just if it is likely to have the desired result, but also if the doses needed are toxic or have unexpected side-effects. Lab rats make a convenient model for trying out new therapies, but they are not people. For example, at least 9 out of 10 cancer treatments that appear promising in animal studies fail in human clinical trials. The odds aren't any better with anti-aging products, though AI computing can accelerate the process and make increasingly reliable predictions.

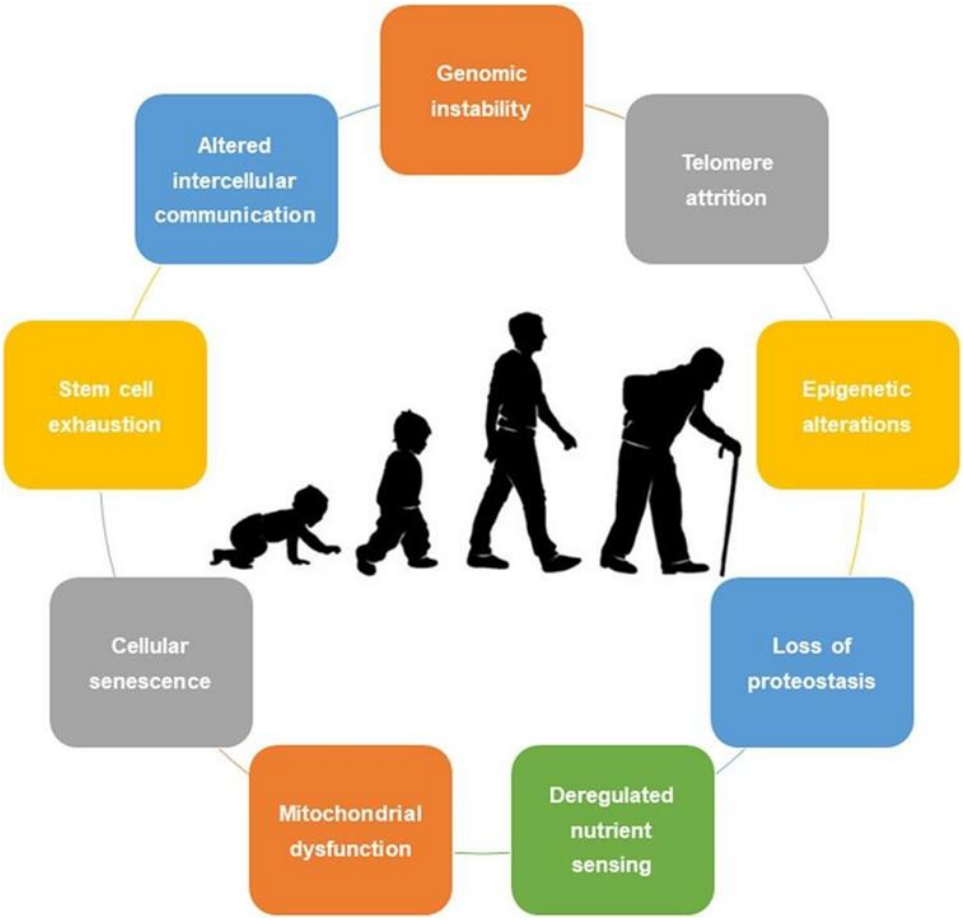
Resveratrol, a molecule concentrated in wine from grape skins, was the catalyst. For a while, it appeared that resveratrol could explain the CR effect and possibly the whole French Paradox.

A pioneering advocate of resveratrol's potential was Professor Joseph Vercauteren of Université Montpellier in France, who extracted resveratrol from the lees typically discarded after pressing wine. Few others considered resveratrol to be more than a novel antioxidant until a group at Harvard headed by geneticist David Sinclair, Ph.D. identified it as an activator of a type of gene regulator called sirtuins. Sirtuins had earlier been shown to turn on the genes responsible for the CR effect, so now we had the first true anti-aging candidate molecule.

Myself, Professor Vercauteren, and David Sinclair

The anti-aging market grew rapidly, with resveratrol supplements appearing everywhere. But while these products referenced progress in science, they often oversimplified it, and anti-aging's credibility problem persisted. That is changing, as several lines of research converge and possibilities unimaginable a generation ago appear within our grasp.

Defining aging



Hallmarks of Aging

Before we can define anti-aging, we need some detail on the aging process itself. The visible and functional aspects are easy enough to see – loss of muscle mass, joint stiffness, skin wrinkles, decline in mental sharpness, less energy – but what we really need is to understand the underlying causes. Here's how scientists see it now: At the cellular and subcellular level,

interactions among genes and environmental factors result in accumulation of genetic damage, manifested by specific and interrelated aging hallmarks.

These hallmarks form a framework for identifying targets for anti-aging therapies. Anti-aging aims to slow or reverse the aging process at several levels, with a goal of physiologic improvements, reduction in disease, and potential lifespan extension. We'll touch on a few of these that are becoming important opportunities in the practice of longevity medicine.

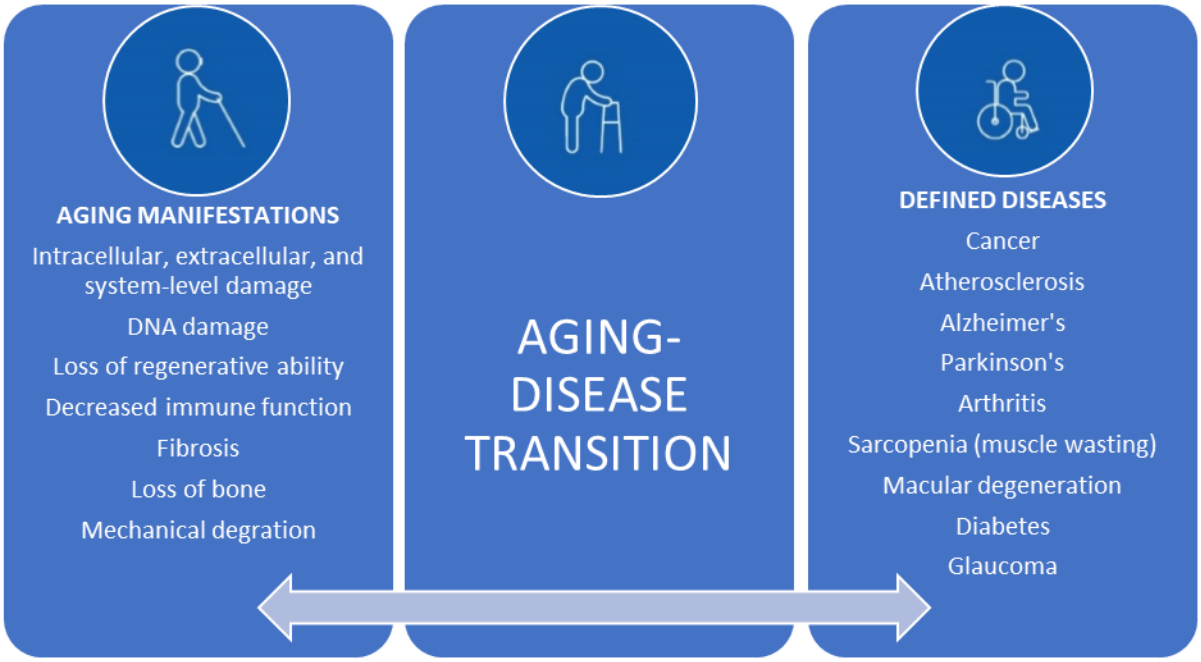
Longevity Medicine: The new era of anti-aging

This new era of exponential progress in anti-aging science is propelled by three developments:

1. *The recognition that the biological mechanisms of aging at a cellular level are largely the same as those underlying major age-related diseases;*
2. *A substantial influx of capital driving anti-aging research and development; and*
3. *The unprecedented power of applied artificial intelligence computing.*

The understanding that aging and disease are intertwined points to therapies that might reverse aging while simultaneously addressing cancer, cardiovascular disease, neurodegenerative diseases, diabetes, and many others. Beyond the implications for health care, this has a pragmatic benefit for development of longevity products; given the impracticality of clinical trials for anti-aging as the primary outcome within a realistic timeframe, it informs a strategy for navigation of regulatory constraints on drug and device development. If for example your anti-aging compound also happens to be effective against cancer, you now have a pathway to approvable on-label uses while you continue to test it for anti-aging effects. Once it becomes commercially available, off-label uses for longevity therapeutics can be explored.

Note: Although non-prescription supplements and treatments may not be subject to the same degree of regulation as drugs, using them without well-documented proof of safety and efficacy is a fool's errand. Let the experts do their thing.



This increasing recognition that the factors underlying degenerative diseases are the drivers of aging has led some to propose that aging itself be classified as a disease. Researcher Matt Kaeberlein, formerly at the University of Washington, went so far as to say that this will be a defining feature of 21st Century medicine. Also here in Seattle is the Institute for Systems Biology founded by Leroy Hood MD, PhD, whose book *The Age of Scientific Wellness* makes a similar case, as does Peter Attia MD in his *book Outlive: The Science and Art of Longevity.* I agree with both of them on this. Realizing the full benefits of next- generation longevity medicine will require a dramatically revamped model of health care delivery, shifting from sickness care to wellness care. Currently, because aging isn't considered a specific disease entity, your health insurance won't cover longevity medicine therapies; there aren't any diagnosis codes for aging and no billable treatments. That is the reason for the ground rules governing anti-aging and longevity medicine practice at the present time:

- Both the physician services and prescribed treatments, supplements, or drugs are not billable to a traditional third-party payer.

- Anti-aging/longevity medicine practice is not (yet) a replacement for traditional medical care.

Longevity Medicine vs. Age Management

Age management is healthcare designed to address the needs of people as they get older and has been practiced by mainstream physicians for decades. A central component of age management is hormone replacement therapy, which is not without its controversies (page 19). But because the ability to measure biological aging is a comparatively recent development, age management doctors have traditionally avoided the term "anti-aging" since aging per se was not a quantifiable outcome. Longevity medicine incorporates aspects of age management as well as interventions *specifically intended to measurably slow or reverse biological aging.*

The second driver of anti-aging research and development is a massive influx of capital. Dedicated venture capital-funds and endowed research facilities such as the Buck Institute and Google-backed Calico Labs are leveraging the most up-to-date technology to identify and develop anti-aging products. The Switzerland-based Longevity Science Foundation announced plans in October 2021 to devote $1 billion toward the goal of extending human lifespan. In 2022 the Saudi Arabian-based nonprofit Hevolution Foundation announced plans for that amount every year for support of longevity research globally. While the sector remains a high- risk investment category littered with some spectacular failures, many see longevity as "the next trillion-dollar opportunity." In 2023 a $101 million Xprize competition was announced to be awarded to the first team that can restore at least 10 years' worth of muscle, brain, and immune function in older adults. There are now dozens of companies working exclusively on anti-aging therapeutics and more than a hundred others with anti-aging products in their pipeline. Investment in longevity companies is in the $billions and is increasing.

A legitimate concern is whether the profit motive will have a corrupting influence and reanimate the credibility question that has plagued anti-aging practice. The counterargument points to increasingly open sharing of data, and the fact that it costs a lot of money to do this kind of research properly. The work these labs are doing is being followed with intense interest and scrutiny. If one of these longevity moonshots pans out, we should all benefit.

The third pillar of the anti-aging imperative is the exponential power of applied artificial intelligence.* This not the ChatGPT version of AI but uses programs with the ability to discern patterns in massive amounts of data, make predictions, and validate them. Analysis of these immense sets of biological data is called "omics," (e.g., economics or genomics) and for all practical purposes was not possible at scale until relatively recently. AI reveals detailed insights into central biochemical processes of aging at every level, from the whole body to the cellular, subcellular, and on down to the molecular level. Hidden patterns and connections are being revealed on complex "metro maps" of cellular metabolism and expression of anti-aging genes.

There's a hugely practical application of AI in anti-aging: AI-based analysis is how your biological age, vs. chronological age, can be determined. More on that later, but knowing your biological age means that you can objectively measure the results of anti-aging interventions. Biological age tests are now readily available, reliable, and increasingly affordable.

A valuable AI resource is the advent of online databases of anonymized health information accessible to researchers around the world. Examples include the UK Biobank, which houses genetic and health information from more than a half million subjects; the website Geroprotectors.org, a catalog of compounds identified as having anti-aging properties; the Genotype Tissue Expression project (GTEx), an atlas of human gene expression; the U.S. National Genomics Data Center, with database resources for support of research in both academia and industry; and my favorite acronym, the "BIG" (Beijing Institute of Genomics) Data Center at the Chinese Academy of Sciences, also available worldwide for researchers.

Collectively these "big medical data" warehouses are used for AI-assisted data mining for precision medicine and anti-aging research.

AI is also helping to discover new anti-aging compounds. A particularly useful feature of AI is the ability to create virtual 3-D models of biological molecules, called *in silico* modeling. The specific ways that molecules interact can be understood and predicted, and in silico screening of large

* For engaging explanations of AI in anti-aging, there are 3 essential books: *Deep Medicine* by Dr. Eric Topol; Alex Zhavaronkov's *The Ageless Generation: How Advances in Biomedicine Will Transform the Global Economy*; and *Live Longer with AI* by Tina Woods.

numbers of potential therapeutics can now be done rapidly. But identifying candidates is only the beginning, due to a nagging feature of biomolecules: They tend to be “promiscuous,” meaning that they have a lot of relationships, metabolically speaking. These so-called off-target interactions may result in unpredicted side effects. However, in silico modeling can imagine and design modified versions of candidate drugs, creating thousands of possible tweaks to the structure of the candidate molecule and predict the results. In silico molecular engineering can potentially improve the effectiveness of new drugs while simultaneously making them safer.

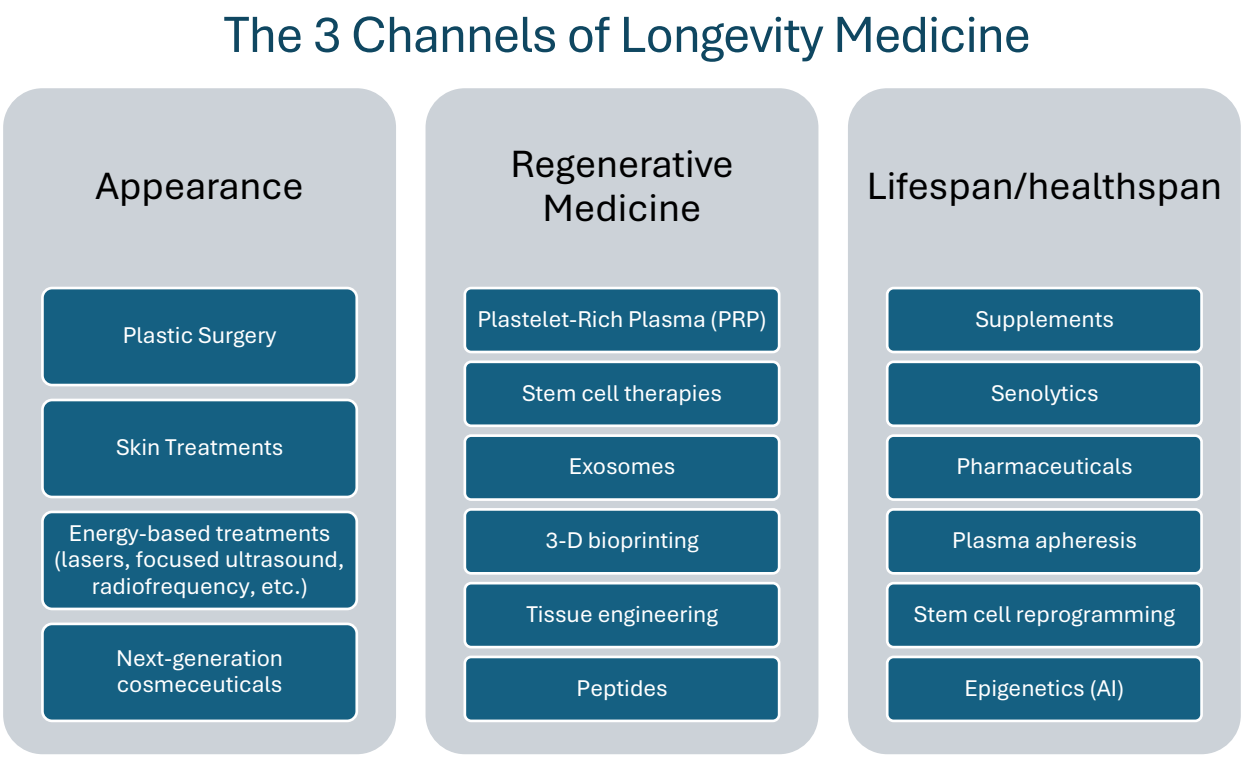
Longevity without restrictions

So we know that caloric restriction is a sure-fire if utterly impractical lifespan extension strategy. Cut your calories back by 25% or more, and add a similar percentage to your lifespan. It’s been proven in everything from yeast to worms to mice. Almost certainly in primates* too. Although a controlled experiment in humans with length of life as the endpoint is unlikely to happen, there is evidence however that caloric restriction can slow the pace of aging. A clinical study released in February 2023, funded by the U.S. National Institute on Aging, included 220 healthy adults randomized to either a 25% calorie restriction diet or a normal diet for two years. The pace of aging was measured with a DNA test called DunedinPACE (more on that below). The CR group saw a 2–3% reduction in the pace of aging over the control group. That may not sound like much, but it equates to a 10–15% reduction in mortality risk, comparable to quitting smoking.

A true CR diet is unsustainable, so research has shifted to learning how to activate the CR effect pharmacologically or with more realistic diet strategies such as intermittent fasting. There are simple supplements that add to the effect as well. But CR is just one of many anti-aging channels being fleshed out; leveraged by the three pillars of the aging↔disease linkage, record investment, and AI, the fruits of this research are beginning to move into clinical practice.

* A study on rhesus monkeys, whose average life span is 40 years and whose aging patterns are similar to humans, found that the monkeys on a CR diet lived longer. However the results have been questioned because the control group monkeys were apparently given a less healthy diet than they would normally consume.

I see this manifest by three facets of longevity medicine: first, aesthetic treatments and surgery targeting appearances of aging; a second category of regenerative medicine aimed at restoring physiologic function at a macro level; and a third category intending to slow or reverse aging at a cellular and genomic level. We shift from disease-specific "whack-a-mole" treatments to a focus on healthy longevity, or healthspan. While longevity medicine integrates AI-based genomics and personalized medicine, a complete approach to anti-aging includes multiple points of attack. For many, this starts with strategies to look as young as you feel.



1. If you look good, you feel good – and live longer?

While plastic surgeons and others practicing aesthetic medicine know about the positive impact of their craft, some would debate whether they can accurately be called "anti-aging" since they do not affect biological aging per se. Or do they? Cosmetic surgery does have documented value in terms of quality-of-life measures, which may translate into biological changes. One review of the psychology of facelift patients found that more than 95% experienced positive changes in their life, increased self-confidence and self-esteem, and overall improvement in quality of life. And a positive outlook has been shown to be associated with longevity; a study from the Mayo

Clinic some years ago suggested that women who have facelifts live up to 10 years longer than women who don't. The authors attributed the lifespan benefit to a boost in self-image and the resulting optimism. There are obvious confounders with studies of this type, but it does support the concept of youthful appearance contributing to longevity.

This connection is supported by a concept known as the “Socioemotional Selectivity Theory” or SST, developed by Stanford psychologist Laura Carstensen (now Director of the Stanford Center on Longevity). SST holds that subjective age predicts late life health outcomes. The longer a person expects to live - time horizon view - the younger their self-perception of age. SST evinces that time horizons are pliable and modifiable with behavioral changes. *Subjective* age can predict *objective* health and lifespan, thereby explaining the potential longevity benefit from aesthetic plastic surgery. Whether the decision to undergo restorative surgery marks the onset of a personal anti-aging effort, or simply resets one’s time horizon view, either way it validates the role of plastic surgery and cosmetic treatments in longevity. Cosmetics giant Estée Lauder is throwing their support behind this idea, announcing in 2023 the formation of a “longevity expert collective” and underwriting research on longevity, appearance, and well-being.

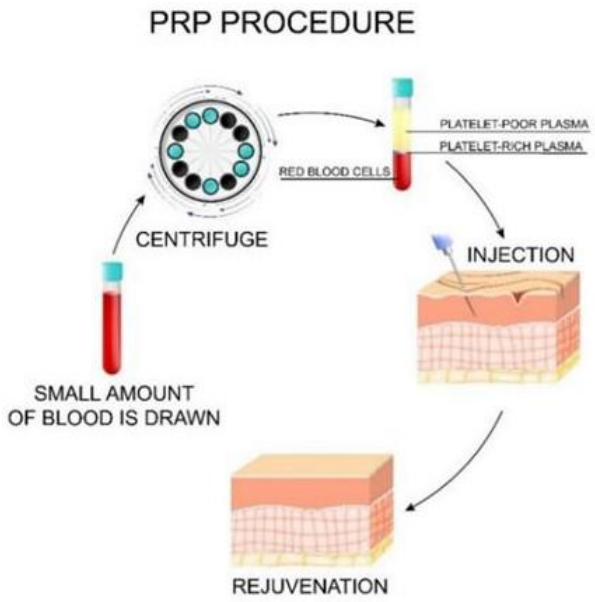
2. Regenerative medicine: form and function

I think of regenerative medicine as integrating the visual and functional aspects of aging. Healthy skin is beautiful skin, heathy muscles form an athletic and attractive physique, a healthy central nervous system retains mental sharpness, and healthy cells give youthful energy. Regenerative medicine considers aging as it relates to coordinated system-wide signaling, (e.g., hormones) as well as restoration of individual body parts and organs.

Platelet Rich Plasma

Platelets are tiny cells in the bloodstream associated with clotting, and platelet-rich plasma (PRP) is defined as the plasma fraction of blood with a high platelet concentration. PRP is an abundant source of growth factors. Much of the early use of PRP was in orthopedics, and it remains widely used in sports medicine. It is now used for an array of clinical applications from oral surgery to gynecology. Yet despite an abundant medical literature on PRP in

regenerative medicine, there are few high-quality studies. One reason for this is the large number of available systems for processing PRP and lack of standardization of preparations and treatment protocols, which makes it challenging to compare effectiveness.



How PRP works: A small blood sample is drawn. This is then placed in a centrifuge, which separates the blood into its components, and the plasma layer is drawn off. Processing takes less than an hour, and the PRP is then ready for use. For skin rejuvenation, it is either injected or applied topically after microneedling. Several sessions a few weeks apart are required for maximum effect.

In the United States, products such as PRP are regulated by the FDA, which deems products such as PRP to be exempt from oversight, up to a point; while the product itself may be exempt, the devices for their preparation are not. No devices are specifically approved for regenerative or aesthetic medicine, so their use in this setting is considered "off label."

Stem cells

Stem cell therapies are increasingly viewed as being synonymous with regenerative medicine. As it happens, harvesting stem cells is surprisingly easy: Adipose tissue (fat) is an abundant source. A minimally invasive liposuction procedure, a few processing steps, and you've got a vial of stem cells in concentrations 500-fold greater than bone marrow. What's more, adipose-derived stem cells (ASCs) have distinct advantages over other sources of stem cells, not the least of which is their ubiquity in tissue that is often present in excess.

ASCs are multipotent, meaning that they have the potential to differentiate into tissues of several types including bone, cartilage, muscle, and nerve. ASCs are being studied for a range of applications including aberrant wound healing, organ repair, and cartilage regeneration.

In aesthetic surgery, much of the early focus on ASCs was on soft tissue augmentation. Because depletion of facial fat is an important aspect of aging, fat grafting is often done in conjunction with a facelift. Because volume retention of fat grafts is variable however, a concept called cell-assisted lipotransfer (CAL) aims to improve this by adding extra ASCs to the fat. For this procedure, the fat from liposuction is divided, the ASCs are isolated from one portion, then added back to the fat to be used in the graft. The enhanced content of ASCs is believed to contribute to blood vessel growth into the grafted fat and cell renewal.

Despite technical progress, there are still several controversies with the use of ASCs. The so-called “stem cell facelift” (really just cell-assisted fat grafting) has been earnestly promoted, and just as vigorously condemned as a misleading exaggeration. As stem cell procedures became popular, regulatory agencies began to take a critical look in the U.S. and elsewhere. A well-publicized closure of stem cell clinics after 3 cases of blindness from stem cell injections for macular degeneration furthered these efforts. Stricter FDA guidelines went into effect in 2021.

Nevertheless, the regenerative potential of ASCs has become progressively more recognized. In order to leverage these benefits with less reliance on costly and time-consuming devices, simpler processing methods have been developed. Using low-tech filtration techniques, specific types of grafts can be prepared, tailored to intended effect and placement site. These are classified as millifat, microfat, and nanofat, the latter largely consisting of stromal vascular fraction. Nanofat is used as a superficial subdermal injection for skin rejuvenation rather than volumization, while millifat and microfat are placed deeper.

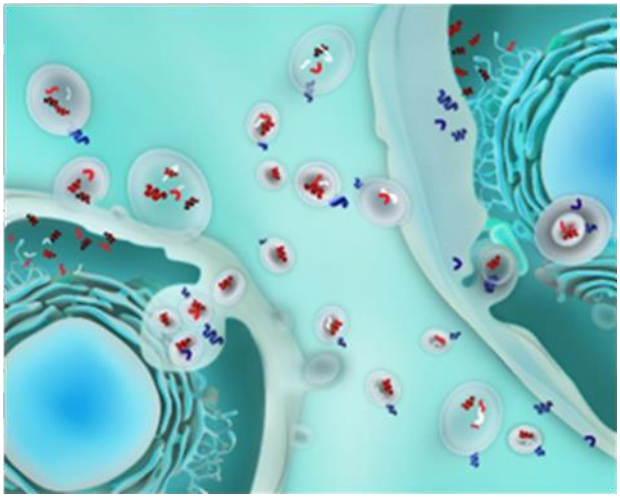
Realizing the full potential of ASCs likely requires more elaborate processing however. A study by plastic surgeons in Brazil evaluated the effects of skin injection of concentrated ASCs prepared by using an enzyme to separate the stem cells and then culturing them in a lab, a procedure requiring 3 weeks. They then placed injections into facial skin of 20 patients who were scheduled for facelift, so that the skin could be evaluated in the sections that were removed 3-4 months later. Impressively, they found full regeneration of damage from aging and the effects of lifetime sun exposure. The elastin fiber network (the type of collagen that gives

youthful skin its elasticity) was restored, and the deeper layers of the skin were reconstituted after a single albeit elaborate adipose-derived stem cell treatment.

I am excited about the potential for stem cell treatments, but do not want to get ahead of what regulations allow or clinical science proves. Stem cell treatments remain experimental. In 2024 the FDA successfully sued two clinics offering stem cell therapies. Fortunately, there is a less risky way to leverage the benefits of stem cells, by understanding how they communicate with neighboring cells. They do this with little bubbles of biomolecules called *exosomes.*

Exosomes

The local tissue effects of stem cell therapies are mediated largely by what are called "paracrine" actions – effects on other cells in the immediate area – rather than by differentiating into cells of a particular type. This phenomenon is termed the *paracrine hypothesis*, and it is important because it suggests a sort of shortcut. Paracrine communication between neighbor cells occurs by exchange of tiny packets containing cargoes of proteins, peptides, RNA, and other biomolecules. These are categorized by size, the smallest of which are called *exosomes*. Stem cell-derived exosomes could possibly be used as a surrogate for stem cell therapies, skipping the step of extracting and injecting the stem cells. Exosome treatments have a further advantage in that they can be purchased as an off-the-shelf solution, though regulations limit their use to topical applications as with PRP. These commercially produced exosomes are derived from cell cultures, and the effects are related to the specific type of parent cell. I expect to hear a lot more about exosomes in the next few years, as a number of clinical trials are underway for a wide range of applications. For now, their primary clinical use is skin rejuvenation, typically combined with microneedling to enhance penetration. Think of exosomes as supercharged PRP or stem cells.



Artists rendition of exosomes

One workaround to the restriction for topical use is to extract exosomes from your own plasma, which a company called ZeoScientifix© has developed. These are called autologous exosomes and can be injected. We are exploring possibilities for autologous exosomes for a variety of regenerative purposes, but this is still new. Stay tuned.

Hormone replacement therapies

Declining hormone levels are a hallmark of aging, and hormone replacement improves quality of life for millions. Yet evidence for positive effects on the aging process is often contradictory; in many cases, it may do the opposite. There are questions as to whether making the body perform as it does in youth makes it biologically younger, now that this can be measured. All hormones operate with elaborate feedback loops, so tinkering with them may cause unanticipated side-effects if not managed expertly. For these reasons, hormone replacement therapy (HRT) has long been considered as age management medicine rather than anti-aging. That may change as approaches to HRT are updated and re-evaluated.

Testosterone supplementation is a controversial case in point. Studies of testosterone replacement in older men are comparatively few and generally have been of short duration, with small numbers of participants and frequently lacking adequate controls. What studies there are show modest anti-aging effects. Though longevity is a common theme in marketing testosterone, convincing evidence of a healthspan benefit is lacking.

Human growth hormone (GH) has been widely marketed for antiaging, promising to deliver weight loss, improved energy and mood, and better sleep. As with testosterone, support for these claims is mixed. One study in elderly men reported increased muscle mass, reduced body fat, and improved bone density with GH supplementation. Animal studies however suggest that declining levels of GH are associated with greater longevity, are protective against cancer, and that GH supplementation actually *accelerates* aging. This fits with human epidemiologic studies, which generally associate lower levels of GH with longevity.

GH use is further confounded by its effects on glucose metabolism. A primary action of GH is to elevate blood sugar levels, producing a diabetes-like effect. This leads to a compensatory increase in a hormone called IGF-1 (Insulin-like Growth Factor 1), which acts to lower blood

sugar. This relationship of growth hormone and IGF-1 is called the GH/IGF-1 axis, and is an extremely important anti-aging intervention target. The study reporting age reversal that I cited in the box at the beginning of this book also used the diabetes drug metformin to counteract the adverse effects of GH on blood sugar.

Because of these and other issues, access to GH is heavily restricted. Alternative approaches using peptides to stimulate natural production of GH may offer a safer option (see below).

DHEA (Dehydroepiandrosterone) is a precursor hormone produced in the adrenal gland. DHEA helps produce other hormones such as testosterone and estrogen. Natural DHEA levels peak in early adulthood and then decline with age. Studies on DHEA supplementation are mixed in terms of anti-aging effects, and there is concern that it may promote growth of hormone-sensitive tumors such as breast cancers.

Estrogen replacement has a controversial past but is increasingly considered an indispensable part of a comprehensive anti-aging strategy for postmenopausal women. Thanks in large part to work done at the Buck Institute's Center for Female Reproductive Longevity and Equality, menopause is now known to be associated with accelerated aging. Seen in this context, the use of estrogen replacement becomes more than a quality of life and sexual health issue; it may literally be life-saving.

Peptides

Peptides are ubiquitous molecules that serve a variety of functions. Insulin and semaglutide (Ozempic) for example are peptides, and more than 7000 peptides have been identified in the body. Around 60 peptides have been FDA-approved as drugs, with many more being explored in clinical trials. Peptides play critical roles in senescence, immunity, and overall aging.

The high guru of peptides is orthopedic surgeon William Seeds MD, who has unbridled enthusiasm and many years of experience with them. I've taken his training course and I'm using several peptides in my clinical practice.

GHRH: Growth-Hormone-Releasing Hormone, as its name implies, is a peptide that signals the body to generate and release growth hormone in its own natural cycle. GHRH is naturally

produced in the brain, and is thought of as a safer alternative to GH. Other peptides that stimulate production of GH are known as GHRH mimetics.

CJC-1295 (also known as Mod-GRF) is a widely preferred GHRH mimetic for anti-aging, because of its favorable side-effect profile and potency.

Sermorelin is a well-known GHRH mimetic originally FDA-approved to elevate growth hormone levels in children of short stature.

Tesamorelin (brand name Egrifta) is a 44 amino acid peptide first FDA-approved to treat a condition known as lipodystrophy that occurs with HIV. Tesamorelin may improve muscle mass, blood lipid profiles, cardiovascular disease risk, and reduce visceral fat.

Ipamorelin headlines the category of what are known as Growth Hormone Releasing Peptides or GHRPs. While GHRH mimetics promote production of natural growth hormone, release of the hormone into the blood stream is under additional feedback control involving the cell receptor known as ghrelin, which responds to conditions of hunger and satiety. GHRPs act on the ghrelin receptor to promote release of GH. Ipamorelin is considered a third-generation GHRP and is preferred because of its high potency and minimal side-effects, and has a range of potential anti-aging properties. Ipamorelin should be used in cycles, as continuous use may lead to receptor desensitization. Ipamorelin is typically compounded with CJC1295, so they can be given together in a single injection.

All of these are given by subcutaneous injection, usually once a day before bedtime, 5 days a week. Because natural growth hormone release is pulsed and highly linked to a stage of deep sleep, the benefits of GHRH/GHRP treatments are amplified by syncing to this natural cycle.

Ibutamoren or MK-0677 is the last in our list of GHRP mimetics and worth mentioning because it can be taken orally. Technically it isn't a true peptide because of modifications needed to prevent it from being broken down by digestive enzymes, but it has a similar safety profile. There is a risk of irreversible growth hormone receptor involution if taken continuously, so it is used in 3-month cycles alternating with a different GHRP.

Epithalon: A synthetic version of the peptide epithalamin, an endocrine bioregulator naturally produced in the pineal gland (a tiny structure in the mid-brain.) There is evidence that epithalon decelerates aging, suppresses tumor development, enhances antioxidant defenses, and moderates stress response.

BPC-157: Body Protection Compound 157 is a medium-sized cell repair peptide working on the brain-gut axis. BPC-157 promotes wound healing, reduces neuroinflammation, and promotes regeneration. It is also available in pill form but not absorbed enough for systemic effects.

Thymosins: The thymus is an organ that sits just in front of the heart, and is so named because its two lobes resemble leaves of the thyme plant. T-cells, an important factor in adaptive immunity, are produced in the thymus. After puberty the thymus undergoes gradual shrinkage, senescent cells accumulate, and peptides produced by the thymus (thymosins) diminish. Two thymosins are important clinically: alpha-1 (TA1) and beta-4 (TB4).

TA1 (Zadaxin) is a multifunctional peptide that helps T-cells mature and restores immune system homeostasis. It is used to treat autoimmune disease, viral infections including hepatitis, Lyme disease, and other inflammatory conditions.

Thymosin Beta 4 is also involved in immune system functioning and is important in tissue repair and regeneration. It is a potent anti-inflammatory and has been studied for a variety of conditions from traumatic brain injury to dry eye.

GHK-Cu is a multifunctional copper-containing peptide used to stimulate synthesis of collagen and elastin in the skin, accelerate healing, reduce inflammation, and promote tissue regeneration. It is used as a topical ingredient or given by subcutaneous injection.

In October 2023 the US FDA issued a “guidance statement” that all but prohibits access to many peptides, citing lack of safety data. There were few specific adverse effects identified however, and it seems clear that the move was intended to crack down on the compounding pharmacies that source these products rather than the practitioners who prescribe them. Paradoxically this has resulted in some peptide users turning to alternative sources whose safety standards and purity are questionable. Some of these restrictions were later lifted.

Plasma pheresis

Here's a simple idea, and it appears to work: Infuse plasma from a young donor into an older individual, and it measurably turns back the clock on many markers of age. The origin of this remarkable phenomenon is one of science's weirder stories: Parabiosis, an experiment in which two animals (mice for example) have their circulatory systems surgically joined. The goal was to determine whether factors in the blood of one "parabiont" have physiological effects on its partner. Uniting an older animal with a younger one is called heterochronic parabiosis, and it produces dramatic rejuvenating effects on the older animal.

Harold Katcher, a former Professor at University of Maryland who lectures on the biology of aging, has isolated a plasma fraction called E5 that he believes is responsible for the effect. In 2020 he published the results of an experiment in which 2-year-old rats (elderly for a rat) were given E5 from younger rats; the old rats reportedly had an average epigenetic age reduction of 54%, more youthful levels of over 20 biomarkers, and improved physical strength and cognitive ability. The implication of this, as Katcher argues in his book *The Illusion of Knowledge: The Paradigm Shift in Aging Research that Shows the Way to Human Rejuvenation*, is that it is the organism that determines the age of the cells, not the other way around. In other words, aging is *centrally controlled,* not driven by random deterioration at the cellular level.

But did the E5 rats live longer? In February 2023, the final results were released showing that the treated rats indeed outlived their untreated counterparts, with the longest survivor going to the equivalent of 120 human years and 5% longer than the previously known longest living rat (on caloric restriction.) A few months later, we learned what plasma fraction E5 is: **Exosomes!** Katcher has formed a company to commercialize E5 as *Elixir*, but will likely be a while before we see human trials in the U.S., especially in light of the FDA's dubious view of exosome injections.

Another company, California-based Alkahest, may be making better progress toward a clinically viable product. Alkahest is working to isolate aging-related proteins called chronokines in the constellation of circulating proteins in blood plasma. Seemingly, identifying them is just a question of connecting the dots, but there are a lot of dots – around 8,000 different proteins and peptides to screen. But the three trends seem to align: age-related diseases to target in

addition to anti-aging per se, financial backing, and AI. Alkahest have identified a few plasma fractions and completed stage 2 clinical trials for Alzheimer's and Parkinson's, and other applications like hastening recovery after surgery. In February 2025 they reported results using a fraction from young donors on knee replacement surgery patients, showing less pain and inflammation. In 2020 Alkahest was acquired by the Spanish pharma firm Grifols for $147 million, and Katcher's company is reportedly attracting investment interest as well.

A cautionary note on this approach comes from the experience of another company called AmbrosiaPlasma. They were charging several thousand dollars a pop for plasma infusions from young donors, but were shut down by the FDA in 2019 for lack of clinical proof even though their model was a pay to play clinical trial, ostensibly to determine whether it works.

In the meantime, there is mounting evidence that a much simpler and widely available procedure called Therapeutic Plasma Exchange (TPE) may work as well. In this procedure, the patient's plasma is replaced with ordinary IV solutions rather than with young donor plasma. It is presumed to work by clearing toxic or damaged proteins, in particular the main circulating plasma protein albumin. Results on an impressive clinical study were released in pre-print in 2024, in which different TPE protocols were compared and the results measured with a battery of known epigenetic age clocks (see below). When combined with fresh albumin and immune globulin, *a series of treatments produced impressive age reduction and at the present time is the only procedure known to do so*. The lead researcher on the study, Dr. Dobri Kiprov, has been working on TPE for many years, and I have spoken with him about it. This most recent study was underwritten by a company called Circulate, which has plans to bring the treatment to market in longevity clinics. I am currently looking in to having it in my office.

How TPE works: The procedure is similar to plasma donation. You sit in a comfortable chair and have 2 IV lines placed; one of these removes blood, which then goes into a centrifuge device to separate blood cells from plasma. These blood cells are then added to albumin solution and infused back through the other line. The whole process takes only about 3 hours and is painless. The main hurdle is cost, typically upwards of $6000 per session. And since these types of studies are costly and Dr. Kiprov has a patent on the protocol, there aren't any proven alternatives.

3. Epigenetics: The new era

As our cells age and go through multiple replicative cycles, our DNA degrades, like a photocopy of a photocopy. Traditionally, aging models based on this cumulative DNA damage have been classified into two broad categories: The *error hypothesis* and the *programmed hypothesis.* The error hypothesis attributes aging to the accumulation of mutations in genes. In this model, the primary culprit is oxidative damage, mediated by reactive molecules called free radicals. While it is known that oxidation leads to DNA mutations, clinical studies consistently find no correlations to longer lifespan or healthspan with antioxidant vitamins or supplements despite decades of study. The fact that antioxidants are ineffective for anti-aging is still not widely appreciated, but the evidence is comprehensive and conclusive. There's clearly more going on here.

What could explain this apparent contradiction? In terms of cellular metabolism, some level of free radicals is actually beneficial. Here's why:

- Free radicals are key information mediators in cellular response to stress
- Most antioxidants are easily transformed into pro-oxidants
- Antioxidants can actually increase tumor cell viability in some cases

So paradoxically our bodies need free radicals, and even under the most optimistic circumstances antioxidants can turn against you.

The *programmed hypothesis* of aging holds that it is caused by evolved biological mechanisms. In this paradigm, a predetermined genetic program tamps down hormone levels as we age, dials down the immune system, and expends fewer resources on DNA repair as the organism (or person) gets older. Genes known to be associated with aging are either activated or suppressed depending upon their function at various stages of life.

After decades of research, neither hypothesis has produced proven longevity therapies. The *information hypothesis* of aging, developed by David Sinclair, unifies these concepts and points to new strategies. It is based on *epigenetics*, the processes that regulate how genes are turned on (expression) or turned off (silencing). It is how each of the 30 trillion cells in the human body can become for example a skin cell or a brain cell though they all have the exact same DNA.

Epigenetics also regulates the day-to-day activity of cells. If genes are the pages in a reference manual, epigenetics is the bookmarks, dogeared corners, and highlighter markups. This brings us back to sirtuins, which are epigenetic regulators of genes involved in aging. Basically, sirtuins flip certain genetic "switches" on and off. These switches are found on proteins called histones, which organize DNA. Each time a gene activation switch is accessed, it is altered with a "tag" called methylation. Because epigenetic changes are inherited through cell replication cycles, these tags leave a permanent record of gene expression and/or silencing. This is where AI comes in: lifetime accumulation of methylations can be counted with AI-derived algorithms. Biological age is determined by tallying methylations on genes associated with aging, and this can now be done with a high degree of accuracy. These epigenetic clocks (also called methylation clocks) have been validated and continue to be refined. Like they say, it isn't the years, it's the mileage; where aging is concerned, methylation clocks are the odometer.

To take the analogy a step further, if the genome is our biological hardware, the epigenome is software; aging can then be seen as a software problem that could be restored by rebooting from a backup copy! The possibility of epigenetic reprogramming was the subject of a 2012 Nobel Prize to Japanese scientist Shinya Yamanaka, whom I had the privilege of seeing at a conference in Kyoto in 2016. His work showed that mature cells could be induced to revert all the way back to primal stem cells, which could then become a completely different type of cell. This involved exposure to four small molecules now known as the "Yamanaka factors."

In February 2023, Sinclair released results of a study using Yamanaka factors in which he demonstrated how to make cells younger but stopping before going too far, which would result in loss of their indentity. He first caused the mice to age faster by exposing them to a chemical that caused breaks in their DNA. This activated DNA repair processes but resulted in loss of epigenetic information. It was these epigenetic changes that caused the mice to age faster.

Using Yamanaka factors, the integrity of the epigenome was restored and organs and tissues returned to a youthful state; the experiment was *driving aging "forward and backward at will."* This "epigenetic reboot" led to improved biomarkers of aging in multiple tissues.

Epigenetics is where aging and disease intersect. For example, epigenetic methylation signatures are increasingly used for estimation of disease susceptibility. As these tests become more sophisticated, they can be used to point to specific and individualized interventions that form the basis of a growing specialty called precision medicine. For longevity medicine, epigenetic clocks provide an objective standard for measuring efficacy of anti-aging therapies.

The first generation of methylation clocks attempted to predict chronological age. But what we really want to do is measure the aging process itself, to learn why some people are more fit or frail, or appear younger or older than their actual age. These features of aging are known as the *aging phenotype.* Second-generation clocks began to appear around 2017, trained to predict disease and lifespan. For example, it has been shown that for a middle-aged individual, each one-year increase in epigenetic age over chronological there is a 6% increased risk of developing cancer within 3 years and a 17% increased risk of dying of cancer within 5 years.

The aging phenotype, which reflects the visible manifestations of aging, can also be combined with epigenetic markers to form a "phenotype age clock." The link between the appearance of youthfulness and system-wide epigenetic changes is powerful, as shown in this illustration:

Facial aging phenotypes. Each of these images were generated by digitally combining actual photos of 10 individuals, with the left column the slowest agers, middle is average agers, right the fastest agers, based on DunedinPACE. All of the subjects are the same chronological age! Source: TruDiagnostic

Third-generation clocks are designed to measure *rate of aging*. In order to do this, the data set used to train the AI algorithm requires measurements taken over a long period of time. There is really only one today, called DunedinPACE, which derived from a decades-long study in Dunedin, New Zealand. Based on analysis of banked samples from childhood through adulthood, the DunedinPACE test yields a rate of aging prediction as well as biological age.

The most comprehensive age clocks combine epigenetic data with measurements of phenotype markers, metabolic parameters, composition of proteins, and gene transcription, respectively called phenomics, metabolomics, proteomics, transcriptomics, epigenomics, and genomics. Together these create the most accurate prediction of biological age, known as the OMICm Age. The algorithm identifies numerous "proxy" markers based on specific methylation sites, revealing a very comprehensive portrait of how you are aging. This is the basis for the promise of 21st century medicine: Extremely precise and highly personalized. (See an example in the addendum.)

Because longevity medicine practice centers on sophisticated genomics analysis, selecting the right test is important. My minimum criteria are:

1. The clock algorithm has been published and shares data on its relationship to disease outcomes. As of March 2023, companies like Elysium, Tally Health (founded by Sinclair), DoNotAge, Mudho, and EpiAge have no published data on their algorithms and whether they predict disease. TruDiagnostic with DunedinPACE is the only one.

2. The test should be comprehensive and produce actionable data, supported by clinical studies. TruDiagnostic produces by far the most extensive amount of data and is involved with multiple clinical studies.

3. The lab should be independent and not tied to supplement marketing or lifestyle apps. Be skeptical of a company that is selling you more than the test result and recommendations.

4. They must have strong policies around data privacy. Your DNA methylation profile is highly personal information. TruDiagnostic does their testing in house.

Comparison of Epigenetic Age Clocks

	TruDiagnostic	MyDNAge	Elysium	DoNotAge	EpiAge	Tally
Published Algorithms	✓	X	X	X	X	X
# of Age Outputs	17	1	1	1	1	1
ICC Values*	>98%	Not Published	Not Published	Not Published	Not Published	Not Published
Data Test Size**	~950,000 CpGs	2,000 CpGs	~350,000 CpGs	-	300 CpGs	5,000 CpGs
Generation Clock	2nd & 3rd	1st Gen	1st Gen	1st Gen	1st Gen	1st Gen
Sample Type***	Blood	Blood + Saliva	Saliva	Saliva	Saliva	Saliva

*ICC value is a measurement of the reliability and reproducibility of a test. 98% is extremely good.

**CpGs are the methylation sites on DNA which reflect the activity of specific genes.

***A study published in January 2025 found that saliva-based tests often overestimate biological age and yield sometimes misleading results. Blood tests are better.

Mitochondria

Cumulative epigenetic alterations of aging are mediated by activation or silencing of specific genes in response to circumstances such as caloric restriction. Much of this occurs in structures within cells called mitochondria. These are the energy processors of the cell, and a lot of aging and anti-aging action involves mitochondria. They're sort of a cell within the cell, having their own DNA and their own sirtuins. Mitochondrial sirtuins reflect the metabolic state of the cell, positioning them as stress sensors (nutritional stress, oxidative stress, etc.) If you are interested in healthy longevity, you need to be mindful of your mitochondria.

Caloric restriction mimetics

Because CR-mediated longevity occurs with such consistency across species, whatever drives it is biologically fundamental. A primary aim of longevity medicine therefore is to replicate the CR

effect without semi-starvation. CR triggers a sirtuin-mediated metabolic change which likely evolved as an adaptation to disruptions in food supply, which activates the genes responsible for these metabolic changes. The result can be experimentally reproduced without nutrient restriction by sirtuin activators such as resveratrol. Many were eager (as was I) to credit resveratrol for the French Paradox and longevity. But there are problems with this hypothesis, and some high-level re-examinations of the original studies have cast doubt about the results. The discovery did however provide an opening to probe cellular aging at a molecular level.

Substances like resveratrol that activate sirtuins are called caloric restriction mimetics (CRMs). With its numerous potential clinical actions, resveratrol was the flagship CRM. A wide range of potential health and anti-aging benefits have been investigated, including cancer prevention and treatment; diabetes; viral, bacterial, and fungal infections; cardiovascular health; senile dementia/Alzheimer's Disease; osteoporosis; arthritis; immune dysfunction; hormone imbalance; and others. These are all mediated at least in part by sirtuin activation, highlighting the commonality between the underlying drivers of disease and aging.

Despite the proliferation of resveratrol-based supplement formulations, validation from clinical trials has been elusive, with only a handful of studies showing any measurable benefits. Reasons for this include low bioavailability, first pass metabolism (things absorbed from the gastro-intestinal tract are processed through the liver before circulating), and hormesis, a phenomenon characterized by differential and sometimes opposite effects at lower vs higher levels of exposure. Bioavailability is limited by resveratrol's poor aqueous solubility and variable absorption. For these reasons, we can't assume that in vitro resveratrol studies extrapolate to in vivo clinical effects. We need to look for other options.

But what if we could find already available compounds and drugs that can be repurposed for anti-aging? Repurposing existing medications would bypass the expense and time required for premarket approval, and make them available for off-label use. This idea is being exploited with some notable successes using in silico screening, and has resulted in identification of several candidate anti-aging molecules. This process hopes to find more drugs like the cheap and well-tolerated anti-diabetic drug metformin, a CR mimetic that has been available for years.

Metformin

Originally derived from the French lilac plant in 1922, Metformin was developed as an antidiabetic treatment in the 1950's and remains the most widely prescribed medication for type 2 diabetes. As with resveratrol, metformin mimics aspects of CR by activation of SIRT1 in mitochondria.

In addition to optimizing mitochondrial metabolism, metformin has several other beneficial effects. It inhibits expression of cytokines associated with diseases related to cell senescence, immunity, and inflammation. Interestingly, other evidence suggests that metformin also restructures the gut microbiome, promoting the growth of beneficial bacterial species. It's an all-around good team player, especially where anti-aging is concerned.

Epidemiologic evidence strongly suggests that metformin users have a lower incidence of cancer and better overall survival rates, despite having diabetes. Metformin's potential as an anti-aging drug came to light in a large study using the UK Biobank (page 12) which compared type 2 diabetics taking metformin or another type of diabetes drug called sulphonylurea with matched non- diabetic controls. One would expect diabetics to have higher rates of mortality, but the opposite was found for those on metformin. This implies that metformin has anti-aging properties beyond its anti-diabetes effects, and powerful enough to overcome its damaging effects. (The poorest outcomes were in the sulfonylurea group.)

Proving this in a prospective trial is another matter. A leading advocate for this is Nir Barzilai, MD, founding director of The Institute for Aging Research at the Albert Einstein College of Medicine. (Check out his book *Age Later: Health Span, Life Span, and the New Science of Longevity.)* In 2015 he conducted a small clinical trial called Metformin in Longevity Study (MILES), a placebo-controlled randomized trial in 15 subjects of average age 70. All subjects had metabolic improvements, and gene expression analysis in muscle and fat tissue from biopsies demonstrated significant shift to youthful patterns. This set the foundation for a much larger trial dubbed TAME (Targeting Aging with Metformin), with the explicitly stated goal of demonstrating that aging can be targeted in a FDA-sanctioned clinical trial. Success of TAME would also show the feasibility of clinical trials for aging as a target for intervention.

Funding issues stalled the launch of TAME, likely to due lack of sponsorship for an inexpensive, off-patent drug. Word is that TAME is finally underway now. It's an important study because it's not clear that metformin is entirely without adverse effects. One concern is that it may suppress mitochondrial respiration in response to exercise, negating the anti-aging benefits of exercise and paradoxically antagonizing exercise-induced improvements in cardiorespiratory fitness.

An oft-cited study on adults in their early 60's looked at changes in insulin sensitivity and cardiac fitness after aerobic exercise training (AET), and its effects on mitochondrial respiration and protein synthesis in muscle. AET decreased fat mass and improved blood sugar control in both groups, but metformin attenuated the increases in overall insulin sensitivity and exercise capacity. Interestingly, the effect seemed to appear in only half of the subjects. It is not yet known whether this effect impacts the anti-aging properties of metformin. Notably, it was not an adverse effect per se, but a lack of the expected result of exercise. Nevertheless, the study has created some uncertainty about the wisdom of routine metformin use in nondiabetics.

Take home message: The potential benefits of metformin probably outweigh its possible adverse effects for most people. Metformin is a prescription medication. If you decide to use metformin and you are not diabetic, you shouldn't have it filled at a regular pharmacy, because the on-label indication is diabetes. You don't want your insurance company categorizing you as a diabetic, and shouldn't expect your prescriber to fudge the diagnosis. Fortunately metformin is inexpensive. (We have made arrangements to dispense metformin through our office.)

There is some evidence suggesting that *berberine,* a botanical compound in a Chinese longevity medicine, may have similar properties to metformin without the adverse effects on exercise adaptation. It hasn't been tested in clinical trials, though there is evidence that berberine increases insulin sensitivity and alleviates a condition called metabolic syndrome - a cluster of conditions including increased blood pressure, high blood sugar, excess central body fat, and elevated cholesterol – that jointly increase risk of cardiovascular disease and related problems.

While berberine has the advantage of being available without a prescription, it is known to have issues with bioavailability; only very small amounts are absorbed when taken as an oral

supplement. A derivative called **dihydro-berberine** appears to do better. As with all nonprescription supplements, sourcing from reliable providers is important.

NAD+

Mitochondrial sirtuin activity is dependent on the molecule *nicotinamide adenine dinucleotide*, usually expressed as its oxidized form NAD+, and is central to metabolism and energy processing. NAD+ has a fundamental role in nutrient sensing, linking directly with the energy-processing enzyme AMPK in mitochondria. Because NAD+ is a requisite substrate for sirtuins,

NAD+ depletion correlates with aging and age-related pathologies. For example, a 2023 study found low levels of NAD+ in people with high blood pressure, related to dysfunction of cells lining blood vessels. In mice, an experimental method whereby NAD+-generating enzymes can be “over-expressed” shows that lifespan and healthspan can be prolonged, and restoration of NAD+ levels ameliorates age-induced pathologies associated with diabetes. Caloric restriction, resveratrol, and resveralogs activate sirtuins, which need NAD+; so why not just try to boost levels of NAD+? You can, and easily.

For these reasons, NAD+ has become a hot topic anti-aging research. The NAD+ precursors nicotinamide mononucleotide (NMR) and nicotinamide riboside (NR), a form of vitamin B3, have been shown to raise levels of NAD+ when taken orally in both human and animal models. NR supplementation has been shown to extend the lifespan of mice even when administered late in life, while enhancing stem cell and mitochondrial function.

Because the NAD+ precursors NR and NMR occur in the human diet naturally (albeit in very small amounts), they are generally regarded as safe, and several clinical studies line up in support. A 2018 trial of NR plus pterostilbene – a polyphenol related to resveratrol but with higher bioavailability – assessed safety and efficacy in a population of 120 healthy adults ages 60 to 80 over 8 weeks. NAD+ blood levels showed sustained increases with this supplement, with no significant adverse effects. In December 2022 the results of a randomized, placebo-controlled clinical trial of NMN supplementation for 2 months on 80 middle-aged healthy adults evaluated physical performance (six-minute walking test), a blood biological age test (Aging.AI 3.0 calculator), NAD+ blood levels, and a standardized 36-item general health assessment. All

subjects in the NMN group showed increased NAD+ levels, improved physical performance, and better general health compared to placebo. Biological age remained unchanged with NMN but increased in the placebo group.

Many anti-aging clinics offer intravenous infusions of NAD+, claiming a range of benefits. However, there is little clinical data to support the practice, and what there is suggests that it is rapidly cleared from the body. This is one situation where oral supplements with precursors outperform injections in terms of bioavailability, which is usually the opposite.

Bottom line: Use of supplements containing the NAD+ precursors NR and/or NMR is almost certainly safe and might have longevity-promoting properties. Brand names to look for include *Basis, Chromodex,* and *Elevant.* Intravenous NAD+ infusions are probably a waste of money.

Making sense of senescence

The phenomenon of cellular senescence was first described by the renowned biologist Leonard Hayflick as a condition of cellular dysfunction occurring when cells reach their replicative limit but don't die. Senescence can be triggered by telomere shortening, oxidative stress, and accumulated DNA damage. Once they reach this stage, senescent cells have 3 options: die using an "auto-destruct" program called apoptosis, devolve into cancer, or lapse into a zombie-like state. Senescent cells are an important feature of aging because they release pro-inflammatory cytokines and other harmful molecules, a condition called the senescence-associated secretory phenotype (SASP). Like the bad apples in the barrel, SASP zombie cells adversely affect nearby cells and the tissue environment, resulting in what is called *inflammaging*: chronic low-grade inflammation driving accelerated aging.

One presumed explanation for why SASP occurs is that cellular senescence suppresses tumor genesis, because precancerous cells share many characteristics with senescent cells. The inflammation caused by SASP factors incites destruction of precancerous cells by eliciting an immune system response.

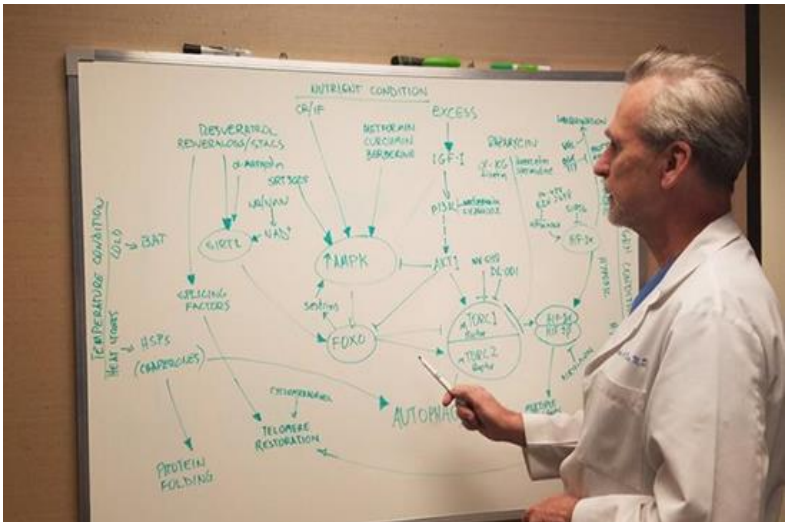
SASP is a blunt instrument though, and may actually provoke adjacent premalignant cells into malignancy, so on balance it's believed to be better to eliminate senescent cells if it can be done

selectively. Substances targeting clearance of senescent cells are termed *senolytics,* and are a major focus of research and product development.

One promising approach uses a combination of the flavonoid *quercetin* and the anti-cancer drug Dasatinib, reported in a 2019 clinical trial to be effective at clearing senescent cells. Though quercetin is available in supplement form and is nontoxic in usual doses, Dasatinib must be administered under close medical supervision. Subjects in the study were in hospital. The picture is further complicated by a 2024 study which found that the treatment unexpectedly *increased* epigenetic age. There's clearly more to learn; as it is said, don't try this at home.

Rapamycin and autophagy

Autophagy (literally "self-eating") is an essential natural method of suppressing cellular senescence. Its importance was recognized in the 2016 Nobel Prize for physiology and medicine, awarded to biologist Yoshinori Ohsumi for the discovery of how it works. Ohsumi's lab and others identified autophagy-related genes (called Atg), which code for proteins that package cellular waste. These are controlled by an enzyme complex called Target of Rapamycin (TOR).



The metabolic "metro map"

TOR got its name when it was identified as the site of action for *rapamycin*, a naturally occurring compound known for many years (like metformin.) Rapamycin was first isolated from a bacterium found in the soil of Easter Island (Rapa Nui) in the 1960's. Originally developed as an antifungal agent, it was found to have immunosuppressant properties and its on-label use now is to suppress rejection with kidney transplantation. Anti-aging researchers today feel that it can be better described as an immune *modulator* and anti-inflammatory drug, which blocks

hyper- immunity rather than bluntly suppressing immunity, or even that it “rejuvenates immunity.”

Exactly how rapamycin worked remained unclear until the 1990’s, when it was recognized as an inhibitor of the enzyme complex that would later be called TOR. We now know that TOR is a principal orchestrator of cell growth and functions as a central coordinator of metabolism in response to both environmental and hormonal signals such as caloric restriction.

Experimentally, rapamycin has been shown to extend lifespan in mice, even with treatment initiated late in life. In fact, *rapamycin is currently the only known pharmacological treatment that increases lifespan in all model organisms studied.* Conversely, aberrant mTOR signaling is linked to a variety of diseases, ranging from epilepsy to cancer. For this reason, rapamycin derivatives (called *rapalogs*) may find a path to approval as disease therapies but with potential use in anti-aging. For example, some neurodegenerative disorders are associated with impaired autophagy due to the suppressed removal of neurotoxic misfolded proteins (like origami, proteins have to be folded just right). Rapamycin provided the first proof that pharmacologically stimulating autophagy can protect brain tissue. For now, rapamycin is off-label for anti-aging.

TOR straddles a major intersection of busy metabolic pathways. Autophagy is regulated two subtypes of TOR, conveniently called TORC1 and TORC2. Inhibition of TORC1 prolongs lifespan, whereas inhibition of TORC2 does the opposite. The goal therefore is to identify TORC1 inhibitors that don’t inhibit TORC2. Rapamycin is a relatively nonspecific inhibitor of TORC1, and long-term use results in impaired glucose tolerance related to TORC2. And as with other nutrient sensing regulators, TORC pathways exhibit significant hormesis (opposite effects at different doses), and the effects of intermittent vs regular dosing differ. So, it’s complicated.

A program called The Dog Aging Project started by Matt Kaeberlein, PhD, is evaluating potential anti-aging effects of non-immunosuppressive doses of rapamycin in middle-aged pet dogs. The trial, called TRIAD (Test of Rapamycin In Aging Dogs) is based on the rationale that dogs share the human environment, have similar risk factors, receive comparable medical care, and develop many of the same age-related diseases. Plus they live longer than mice, so their aging characteristics are more similar to humans. So far, the study has found improvement in age-

related measures of heart function and no side effects in the rapamycin-treated dogs. The study has been expanded nationally to around 50,000 dogs.

The Dog Aging Project (DAP) has produced several important publications of relevance to both humans and dogs. One reported that dogs fed only once daily had better cognitive function and lower odds of having gastrointestinal, dental, orthopedic, kidney, liver, and pancreas disorders, providing evidence for the practice of intermittent fasting/time restricted eating. However, in December 2023, the National Institutes of Health pulled their funding for DAP. If you are interested in supporting the DAP or learning more about it, visit dogagingproject.org.

There aren't many human trials on rapamycin for aging though. The most often-referenced one is a 2014 study that gave a group of healthy adults age 65+ either 0.5 mg daily, 5 mg once weekly, or 20 mg weekly for 6 weeks. The low dose groups had no significant adverse reactions. All subjects then received a seasonal flu vaccination 2 weeks later, and had their responses measured by sampling antibody titers. Both low-dose groups had a 20% increase over those not taking the drug! Interestingly, the levels of antibodies against flu strains not covered in the vaccine also increased, implying a broader immune-enhancing effect. This is a meaningful result because this 20% increase is known to correlate to reduced flu illness severity and incidence.

Another study in the UK is looking at rapamycin's effects on muscle in older adults, and one more in the US called Participatory Evaluation of Aging with Rapamycin for Longevity (PEARL) is specifically intended to investigate efficacy and safety of rapamycin to promote longevity. Preliminary results after 48 weeks were released in August 2024 and are encouraging. The use of rapamycin for anti-aging is becoming increasingly accepted, and in low doses (2-5 milligrams once a week) probably safe.

But don't think more is better. Tech entrepreneur Bryan Johnson, whose influential personal longevity quest called Project Blueprint has been the subject of a Netflix documentary, announced in early 2025 that he had stopping rapamycin. Citing side-effects such as skin infections, blood lipid and glucose abnormalities, and increased heart rate, he concluded that it was also *accelerating* his biological aging. Of note however is that he reportedly was taking 13 mg biweekly, which would be immunosuppressive.

If you are considering taking rapamycin, make sure you are prescribed the brand name *sirolimus.* Compounding pharmacies often prepare rapamycin in capsule form, which has been shown to have significantly lower bioavailability.

Spermidine

Caloric restriction mimetics also trigger autophagy. Like most biological processes, autophagy can be promoted by either increasing its activators or decreasing its repressors, which is what CRMs do. One inhibitor of autophagy repressors is spermidine, a naturally occurring molecule which is involved in regulation of cell growth. (It is abundant in sperm, hence the name.) Spermidine is produced in various cell types, the gut microbiome, citrus fruits, animal proteins, and especially in fermented foods. Dietary spermidine has high bioavailability and has been found to correlate with longevity even after adjustment for lifestyle factors. Further evidence suggests favorable effects on brain aging, immune senescence, and cardiovascular health. Clinical trials of spermidine are mostly preliminary, but with generally positive results.

Alpha-Ketoglutarate

Another potentially useful mTOR inhibitor is alpha-ketoglutarate (AKG), a supplement widely used to improve athletic performance (though with debatable evidence.) AKG levels are known to change with fasting, exercise, and aging. In mice, adding AKG to the diet decreases systemic levels of inflammatory cytokines and prolongs healthspan and lifespan.

A product called *Rejuvant*, developed by Ponce de Leon Health in conjunction with the Buck Institute, is backed by some impressive science. To prove their case, the initial order comes with a DNA methylation age test, which customers are encouraged to repeat after 6 months of use. As supplements go, it's not inexpensive considering that the primary ingredient is widely available in other formulations. The manufacturer claims that combining AKG with vitamin D for women and vitamin A for men optimizes effectiveness, so there is a different formulation for each. In 2021 they published a study showing an average 8-year reduction in biological age after an average of 7 months of use, measured by the TruAge DNA methylation test (not TruDiagnostic). This test is based on an unpublished algorithm, and it isn't known how it compares to the more widely used ones. In February 2022, Ponce de Leon promised that results

of a larger, placebo- controlled trial would be out soon. The company's founder, Tom Weldon, reports that his own biological age is reversing faster than his chronological age is advancing.

C15:0 – A fatty acid for longevity?

A recently discovered saturated fatty acid called C15:0, found in whole fat dairy and some types of fish, is attracting a lot of attention from longevity researchers. It appears to do a lot of what rapamycin and metformin do, targeting inflammaging, regulation of metabolism, cancer, and mTOR. Levels of C15:0 decline with age, and higher levels are associated with longevity. Although C15:0 has not been tested clinically, it is considered to be a safe and essential nutrient at optimal doses. It is marketed under the brand name *Fatty15*.

Urolithin

An important type of autophagy targets worn out mitochondria, a process called *mitophagy.* It is important because dysfunctional mitochondria are a hallmark of aging, manifest for example by age-related loss of muscle mass. For that reason, mitophagy-activating compounds hold promise for maintaining and restoring muscle strength. In 2022, clinical trial results were reported for a mitophagy promoting compound called Urolithin A, a gut-microbiome-derived metabolite of a substance in foods such as pomegranate, berries, and walnuts. After 4 months of supplemental Urolithin A, the data revealed significant gains in muscle strength, aerobic endurance, and physical performance compared to placebo in adults 40-64 years of age. The supplement (brand name *Mitopure*) was shown to be nontoxic and is available online.

What can telomeres tell us?

Telomeres are caps that prevent unraveling on the ends of chromosomes. Telomeres shorten during each cell replication, eventually depleting, which results in senescence by disabling cell replication. Telomere length was one of the earliest hallmarks of aging to be identified, but recent studies paint a conflicting picture. *Telomerase* is an enzyme capable of re-elongating telomeres, but factors that regulate it are complex and differ within cell lines and between individuals. Activation of telomerase is a tantalizing prospect in anti- aging, but as a target for intervention it has yielded inconsistent results.

It isn't even clear that telomerase is a central mediator of aging. Consider for example the Baltimore Longitudinal Study of Aging, which prospectively measured changes in telomere length over 13 years in a large number of subjects. The study did find that average telomere length shortens with aging, but the scope of change varied considerably in different cell types and across individual subjects. Another study of older adults in Spain similarly found that baseline telomere length failed to predict what is called "frailty phenotype" or mortality.

Studies on identical twins reveal some interesting insights on the relationship between telomere length and the effects of environmental stressors. A unique opportunity to evaluate the effects of long duration space flight on telomere length and other aging biomarkers was provided by the NASA twin study, when astronaut Scott Kelly had a "ground control" twin Mark. After a year in the International Space Station, assessments on Scott identified spaceflight-specific changes, including genome instability, DNA methylation alterations in immune and oxidative stress-related genes, and unexpected telomere *elongation*. Average telomere length and global gene expression returned to near preflight levels within 6 months after returning to Earth, though increased numbers of short telomeres were observed and expression of some genes was still disrupted. This paradox remains unexplained.

Nevertheless, the conceptual simplicity of telomere restoration by activation of telomerase remains compelling. And it might not be especially difficult, if unpredictable; traditional Chinese medicines, a regularly dredged source of anti-aging remedies, has identified at least one telomerase activator in the herb *Astragalus*. There is good evidence to support the claim of telomere lengthening, and it appears to have a favorable toxicity profile. The active compound is a small molecule called cycloastragenol, patented under the name TA-65.

Undoubtedly the most controversial approach to telomere restoration involves gene therapy. A company called Libella Gene Therapeutics is pushing hard to get this accomplished. The idea is to transplant an extra copy of the telomerase gene, and it has been shown to have potential in studies on mice. This type of treatment carries significant risk however, not the least of which is activation of dormant cancers or premalignant cells. Because cancer is the prime example of cellular immortality, this illustrates a fundamental challenge in all anti-aging interventions. How

do we selectively extend the life of healthy cells but not precancerous ones? And as if the idea of a clinical trial of telomerase gene therapy at this stage of development wasn't provocative enough, Libella announced that subjects would have to pay a $1 million fee to participate, and travel to South America for the procedure. No results have been posted that I could find, though the study was announced in 2019.

Bottom line on telomerase activating treatments? Be skeptical. Nonspecific telomerase activation as an anti-aging target remains a work in progress.

Oxygen sensing

Nutrient sensing has received the most attention in aging biology, but oxygen sensing may play a vital role as well. Both hyperbaric oxygen treatments (high oxygen) and hypoxia (low oxygen) point to possible longevity-promoting interventions. Hypoxic conditions activate what is called the Hypoxia-Inducible Factor (HIF) pathway, which facilitates adaptation to low oxygen. HIF is a key driver of regeneration involving sirtuins, mTORC1, and mitochondrial activity.

HIF signaling has been identified as a target longevity pathway and an opportunity to use AI-based "omics" screening for compounds that can be repurposed for anti-aging. For example, the California-based biotech company BioAge used a multi-omics database of human aging and found that HIF activation was linked to multiple functional improvements as well as healthspan and lifespan. In 2020 they licensed a HIF-activating drug in development for kidney disease by the Japanese pharma company Taiko to commercialize it to treat diseases of aging.

Conversely, under certain conditions repeated hyperbaric exposure can induce effects which normally result from hypoxia, which is known as the hyperoxic-hypoxic paradox. It has been shown that hyperbaric oxygen treatment (HBOT) can induce HIF and sirtuin expression, and promote stem cell proliferation, mitochondrial function, and telomere elongation. A study on 35 healthy adults aged 64 or older given 60 daily HBOT sessions found telomere length increased significantly, and the number of senescent white blood cells declined after the treatment. That's a lot of time in hyperbaric chambers though, and these are the "hard" chambers of the type used for decompression for deep sea divers, which require medical supervision.

Temperature stress

Cold plunges have become a hot topic in longevity medicine, but is there any real evidence that they work? Studies of temperature stress responses have yielded meaningful insights into aging pathways, but until recently they have received less research interest. However, an understanding of cold and heat stress response is important, and there are potentially meaningful therapies worth considering.

Benefits of cold exposure relate primarily to brown adipose tissue (BAT), the function of which is thermogenesis in response to cold, and importantly also to modulate energy balance and insulin sensitivity. BAT develops in the embryonic stage, and was believed to diminish by adulthood. It's hard to detect, but a recent series of positron emission tomography (PET) scans – which create images based on metabolic activity – identified a cohort with metabolically active BAT. The subjects with BAT had lower odds of type 2 diabetes, dyslipidemia, coronary artery disease, cerebrovascular disease, congestive heart failure, and hypertension.

"Normal" fat or white adipose tissue (WAT) can be induced by cold exposure to undergo partial browning, a phenomenon termed beiging (as in turning beige). Beiging can be produced by daily application of ice packs to the thigh, with systemic effects mediated by mitochondrial respiration. Even a daily cold shower has measurable benefits, if you can do it.

Heat stress has been more thoroughly studied and may play a more central role in longevity. A family of molecules called heat shock proteins (HSPs) function to refold proteins that have acquired faulty conformations, and to prevent the aggregation of misfolded proteins. HSPs are termed "chaperones" and work on diverse proteins including enzymes, transcription factors, and hormone receptors. Because these various proteins are involved in multiple cellular signaling pathways, HSPs have been implicated in a range of diseases. The TruDiagnostic OMICs test has identified HSP70 (the number indicates the size of the molecule) as a strong correlate to Alzheimer's disease, Parkinson's, and cancer.

HSPs have a dual role, operating in concert with systems that surveille and dispose damaged proteins, as well as facilitating new protein assembly. For this reason, HSP inhibitors have been

recognized as potential anti-cancer and antiviral therapeutics. Several tumor types overexpress HSP105, which has led to its possible use in designing RNA-based anti- cancer vaccines. In contrast, the citrus-derived flavonoid nobiletin extends lifespan in laboratory models, mediated in part by promoting expression of HSPs. A clinical study on the effects of exercise and protein supplementation in healthy subjects in their 60's found that improvements in lean body mass were linked to increased expression of HSPs.

Evidence indicates that beyond their role in proteostasis – folding new proteins, refolding misconfigured ones, and clearing those too damaged to salvage - HSPs operate as central lifespan determinants. HSP activity declines with age along with increasing aggregation, a hallmark of aging. This manifests in neurological disorders, cancer, cardiovascular disease, and other degenerative diseases. Because of this promiscuity of interactions by the various HSPs, it is a challenge to find anti-aging HSP modulators without off-target effects.

Extreme athlete and cold therapy advocate Wim Hof, aka "The Iceman" has developed a program called *The Wim Hof Method*, which consists of breathing exercises and cold exposure. He has set a number of cold exposure records including standing in a container filled with ice cubes for nearly 2 hours. This is obviously a practice requiring considerable self-discipline.

Skin and anti-aging

Skin health is of obvious interest in aesthetic medicine and plastic surgery, but its role in systemic health and aging is often overlooked. The accumulation of senescent cells in response to environmental damage has implications beyond the visible manifestations of aging skin. Further, because the skin is the largest organ in the body and its continuous interface between the internal and external environment, it reflects overall biological age. Consider the phenotype images on page 27; the fastest aging individuals show it in their skin health. Skin biological age with a high degree of accuracy using AI-based algorithms based only on photographs. One AI platform called PhotoAgeClock outperformed the Horvath DNAm clock in predicting chronological age using only photos of the eye area! At the same time, because tissues age at different rates, DNA methylation age estimators trained using internal tissues are less likely to

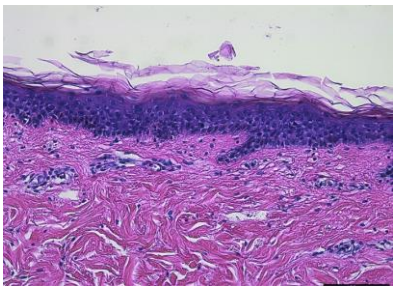
be accurate predictors of skin age. Truly anti-aging skin treatments require validated measures specific to skin to precisely quantify the effects of various treatments.

Topical senolytics are an exciting new strategy for skin rejuvenation. Rapamycin has been tested as a topical senolytic, resulting in reduction of senescence markers. A small placebo- controlled trial was conducted in subjects greater than 40 years of age with age-related photoaging (sun damage) and thinning of the skin. Subjects showed progressive reduction in levels of a cell senescence marker called p16INK4A protein, and an increase in collagen.

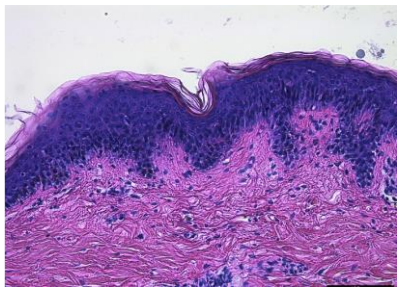
Improvement in skin appearance was noted in multiple participants as well. A California-based company called OneSkin has developed a skin care product (called OS-01) with a proprietary senolytic peptide called Pep 14. Haut.ai validated their results, and research published in 2023 demonstrated that Pep 14 reduced senescence markers and promoted DNA repair in human skin. I believe that senolytics for skin care is a major advance and I recommend it frequently. Anecdotally, we are seeing faster healing after facial peels and surgical scars with OS-01. OneSkin has products for face and body.

Retinol vs. OneSkin OS-01

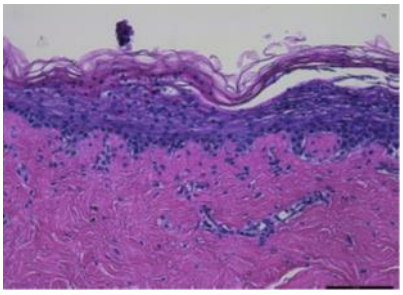
Although retinoids (tretinoin, retinol) have long been the gold standard anti-aging ingredients for their ability to improve visible signs of aging, they are also known to cause skin irritation and sensitivity, resulting in redness, peeling, and flaking. These effects may actually *impair* long-term skin health. The scientists at OneSkin compared the two in a head-to-head test on human skin samples in a lab, analyzing key biomarkers on skin at a cellular level. They found that both retinol and OS-01 FACE significantly increased a key biomarker associated with collagen production, COL1A1. However, retinol also increased a biomarker associated with skin aging, CDKN2A, while OS-01 FACE did not. Plus, OS-01 FACE increased a marker associated with cell growth, called MKi67, while retinol did not. Microscopic comparison of skin samples further revealed that retinol appeared to *worsen* cellular structure and organization!



Untreated skin



OneSkin OS-01



Retinol 1%

Conversely, treatment with OS-01 produced a significant increase in epidermal thickness and promoted a more defined general structure and cellular organization, indicating that the skin's barrier was strengthened and intracellular function was improved with OS-01.

A surprising anti-aging effect of Botox©

Botulinum toxin A (Botox© and other brands) has also been shown to have anti-aging effects via a senolytic process. One test used human skin cells in vitro, which were induced to undergo premature senescence using Ultraviolet B exposure, similar to photo-aging in living skin. The cells treated with botulinum toxin demonstrated a decrease in a senescence marker called SA-beta-gal, an increase in collagen production, and other restorative effects.

There's further clinical evidence of skin rejuvenation beyond botulinum toxin's role as a wrinkle relaxer. A technique called *microbotox,* in which tiny doses of diluted botulinum toxin are injected into the facial skin (rather than the standard practice of placement into the muscle under the surface) found improvements in skin texture, tone, and pore size.

Nanofat

The basis for using nanofat (page 17) in skin rejuvenation has also been shown to involve senescence pathways. A study on cultured human skin cells pretreated with nanofat 24 hours prior to inducing senescence by exposure to UVB demonstrated significantly increased cell proliferation, reduced production of free radicals, increased collagen, and fewer cells expressing SA-beta-gal compared to non-treated samples.

For this reason, the use of nanofat in facial rejuvenation surgery is becoming more common. In facelifts it has become common to use fat grafting for volume restoration (volume loss is a

feature of facial aging), and nanofat injected into the skin, or at least very superficially, enhances the result. This adds a biological anti-aging benefit to facial rejuvenation surgery.

Estrogen and skin

Post-menopausal estrogen depletion is a significant challenge in anti- aging skin care. Declining estrogen levels have multiple impacts on both the visible and structural qualities of aging in skin, including accelerated collagen breakdown, decreased elastin, and impaired moisture retention. These translate into thinning, loss of elastic recoil, dryness, and wrinkling. One answer to this is skin care products that selectively target the beta type of estrogen receptors (Erβ), which are abundant in facial skin. Estrogen taken orally does not get into the skin, but topical application risks absorbing too much. Something that stays in the skin without a systemic effect would be the optimal solution.

VEGF: A very good factor for skin rejuvenation?

In 2019, scientists in Israel and Germany reported that they found a way to experimentally restore aged human skin to a genetically and functionally youthful state. The protein that was identified as the mediator of this is called Vascular Endothelial Growth Factor-A, or VEGF-A. VEGF is found in PRP and exosomes, and is expressed in stem cells. If VEGF-A passes muster in clinical trials, skin rejuvenation will be only the beginning; the researchers believe that the model can be extrapolated to just about any organ or tissue. You would be correct however to surmise that a lot remains to be worked out before we start squirting VEGF into people; for example, HIF (page 40) seems to be involved, though exactly how is unknown. VEGF is available in skin care products, though the rejuvenating effects probably don't compare with injections.

Glycation: Targeting a fundamental aspect of aging

Age-related changes in skin have one thing in common with aging in tissues throughout the body: degradation of the extracellular matrix (ECM), the material between cells. This is comprised of proteins such as collagen, hydration molecules such as hyaluronic acid, and many others. A prominent feature of aging in the ECM is the result of sugar molecules attaching to proteins or lipids, a process called *glycation*. A familiar example of glycation is hemoglobin A1c, which reflects the level of blood glucose levels over time. Glycated proteins are dysfunctional

and accelerate tissue deterioration as they accumulate. In the skin this is manifest with thinning, loss of elasticity, and inability to retain moisture. In muscles and joints the result is stiffness and loss of strength. Ultimately, these glycated proteins form what are called Advanced Glycation End products, or AGEs. Buildup of AGEs is a big deal in anti-aging across the board.

Glycation is the result of a chemical process called the Maillard reaction, which is also the cause of browning in cooking that makes food so tasty. The crust on your bread, the char on your burger – Maillard reaction. Recent research from the Buck Institute shows why these foods are so hard to resist and also why they are so detrimental: certain AGEs in food activate a signaling pathway that promotes hunger and overeating, while simultaneously provoking neural tissue damage. As if all this bad-for-you goodness wasn't tempting enough.

Our friends at SkinCeuticals have been working for some time on products to restore the ECM in skin. They have developed a cream based on proxylane, a sugar-protein hybrid molecule that helps repair the ECM. The flagship product in the category is cleverly called "anti-A.G.E." Other anti-glycation compounds include resveratrol, metformin, and the peptide TB-4 (page 18).

Another bit of encouraging news is a report from Japan finding that a fish-derived collagen peptide supplement reduced AGEs in skin. The randomized prospective placebo-controlled 12-week trial also found an improvement in insulin resistance. The product, from Nitta Gelatin, has high concentrations of two specific compounds (prolyl-hydroxyproline and hydroxyprolyl-glycine), so it cannot be presumed that other collagen peptide supplements would have the same effect.

AGEs affect more than just skin; there is even an age clock based on glycation, called GlycanAge. As with other biological age clocks, GlycanAge is predictive of diseases of aging. The good news is that glycation is something that can be improved. Avoiding refined sugars makes a difference and metformin seems to help as well.

Turn.bio: Turning back the clock on aging skin with epigenetic reprogramming

One of the companies to watch in this space is called Turn.bio. They seem to have the inside track on leveraging techniques for epigenetic reprogramming, with their first clinical application likely to be for aging skin. They are one of several companies working on technologies for the use of Yamanaka factors (page 26) in reversing epigenetic age. In order to do that safely and predictably there are several challenges that need to be overcome: First, too much exposure causes cells to revert back to stem cells, which means they lose their identity. The goal is to make skin cells, for example, more youthful—not turn the clock back too far. The second challenge is how to deliver the factors into living cells. Turn.bio appear to have solved this by using mRNA, similar to how it was developed for vaccines. While there has been a fair amount of disinformation about mRNA vaccines, the use of mRNA is actually what makes the technique safe because it does not enter the cell nucleus and so does not alter DNA. What it does do is carry a signal for the cell to naturally make Yamanaka factors in specific proportions which then result in controlled epigenetic reprogramming. They have also solved the problem of delivering the mRNA by creating a sort of artificial exosome which delivers the mRNA cargo into the cell. These reprogrammed skin cells manifest all of the desired characteristics of younger cells. They make better collagen, more elastin, show fewer signs of senescence, and in every important way are functionally younger. An additional benefit is that the treatment does not need to be repeated frequently. The technology is not limited to skin, and has been demonstrated in the lab to work on a range of tissues and organ systems. I'm keeping a close eye on this company.

The aging brain

Ultimately no aspect of longevity medicine is more urgent than the aging brain. The baseball hall of famer Satchel Paige is credited with saying that "Age is a case of mind over matter; if you don't mind, it doesn't matter." I won't argue with that, but when it comes to the gray matter of the brain it's going to take more than a good attitude. Demographic projections foretell an impending crisis as age-related cognitive disorders crest over the coming decades. There is encouraging progress however, if recent findings can be validated clinically. In fact, there has never been more cause for optimism.

There are several big challenges to the development of treatments for age-related cognitive decline, whether from effects of aging or specific conditions such as Alzheimer's disease. For one, the brain is protected by the blood-brain barrier, a layer that restricts what molecules are allowed to enter the central nervous system. Many promising compounds are simply undeliverable to the target tissue. Another is the impracticality of sampling the tissue with brain biopsies for analysis of the disease process or the effect of treatments. And finally, there is zero margin for error with toxic side-effects where the brain is concerned.

Stem cell treatments are potentially able to overcome all these obstacles because they know how to deliver messenger molecules where they are needed. Adipose-derived stem cells are particularly attractive because they are your own cells, can promote regeneration of nerve tissue, and are generally safe. Numerous clinical trials of stem cell treatments are underway for a range of conditions, from Alzheimer's and Parkinson's diseases to brain injury from stroke.

I still try to keep up with research on wine and health, and this brought me to an interesting finding on brain function. It has to do with what is called the glymphatic system, which is the lymphatic system of the brain. As in the rest of the body, metabolic waste products in the central nervous system are cleared by the glymphatic system. Glymphatic channels open up during sleep, explaining a big part of why sleep is so important in anti-aging. What caught my attention was the finding that *alcohol* enhances glymphatic flow, at least up to a point. Since every major study of risk factors for Alzheimer's disease finds it lower in wine drinkers, that seemed to fit. At least a cause to raise a glass and offer a toast to the researchers.

In the big scheme of things though, the benefit doesn't go nearly far enough. Delving deeper into the biology of the aging brain, we find declining protein synthesis that in turn correlates with defects in protein folding. As misfolded proteins accumulate, they activate what is called the Integrated Stress Response (ISR), which regulates the protein synthesis required for memory formation. The response to damaged proteins thereby also impairs memory, and explains why ISR activation is seen in states of cognitive decline with age. There are limited other options to prevent toxic proteins from building up, so inhibition of ISR is a double-edged sword.

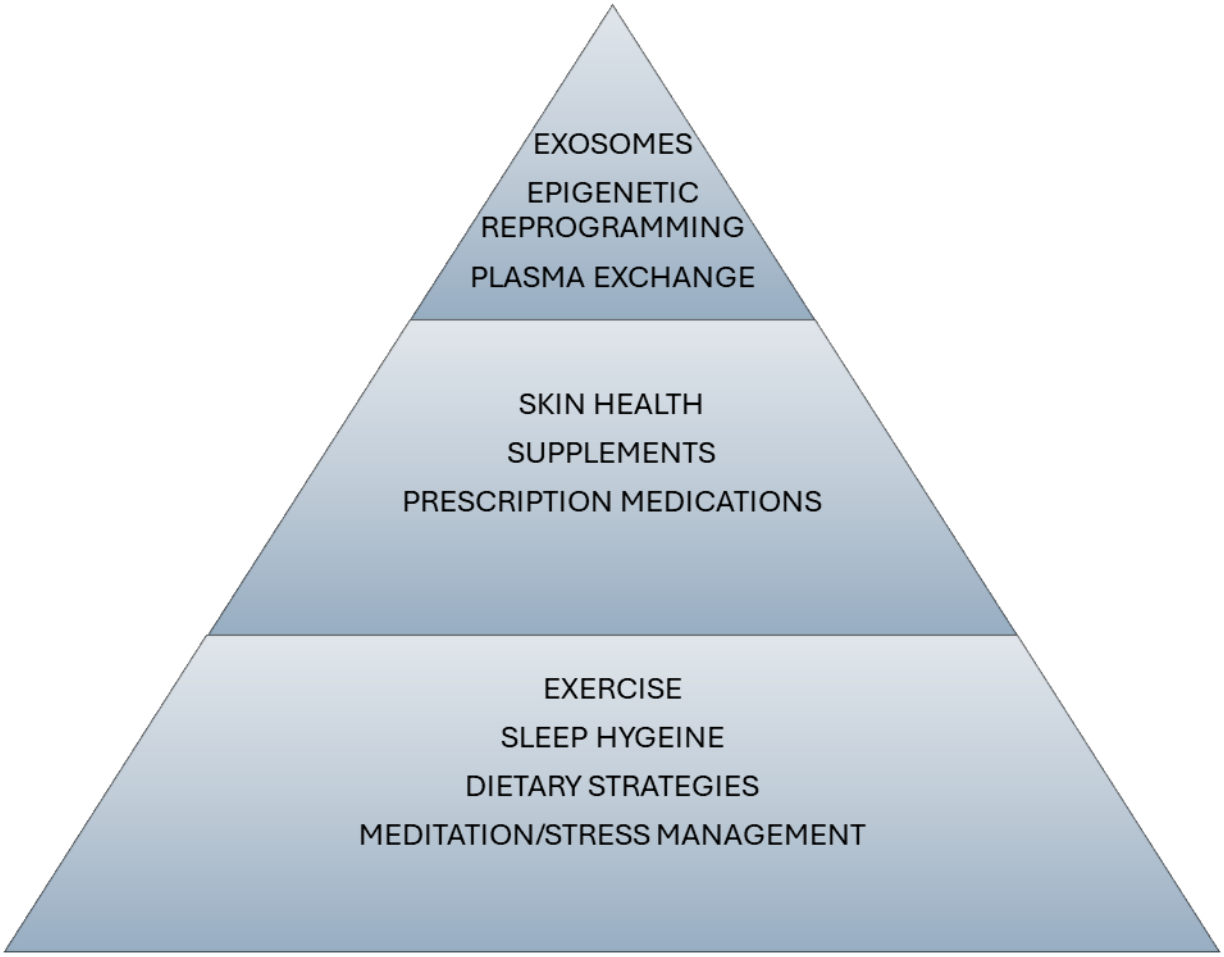
A promising candidate is the drug-like molecule ISR inhibitor ISRIB, which has been shown in studies from Calico labs to restore memory function months after traumatic brain injury and to enhance cognition in healthy animals. A subsequent study found rapid and lasting restoration of spatial learning and memory in aged mice within a week after a series of 3 injections of ISRIB. The implications of this potentially long-lasting, simple, and effective way to reverse age-related cognitive decline cannot be overestimated. ISRIB has yet to be tested in humans though.

Biohacking Longevity

Fast-forward a few years from when I first started writing this, and the idea of biohacking longevity has gone mainstream. But my take on it differs from what a lot of the gurus on social media promote. As with anti-aging, the cycle of hype outpacing science is being repeated. Longevity biohackers are seen as a fringe group of kooks experimenting on their own bodies, taking every supplement or medicine with even a hint of promise in lab studies. Or the other extreme which shuns biotech approaches in favor of rigid lifestyle regimens. Exercise in precisely the right ways, optimal nutrition and sleep, all tracked with wearables tracking your every breath and heartbeat is the way to go. Add in daily cold plunges and you're on your way.

The way I see it, a rational longevity strategy is built on a foundation of lifestyle changes, but biohacking longevity means going beyond what you can accomplish with healthy living. It seeks to understand aging at a fundamental level and leverage that knowledge to find ways to amplify the impact of things that slow or reverse aging. How can we reap the benefits of caloric restriction without starving ourselves? Are there pharmacologic ways to reproduce the effects of cold exposure for those without the self-discipline to endure it? Can we undo glycation and still have the occasional sweet treat? The real discipline required is patience while waiting for scientific validation.

Longevity Medicine Hierarchy



Putting it all together: What you can do now

Many of those sponsoring and performing research in longevity go by the mantra "Don't do anything bad for your health now, so that you will be here to take advantage of the breakthroughs that are coming." That doesn't mean that there aren't things that work now.

Despite the momentum propelling the longevity medicine field, completed prospective clinical studies are relatively few in number but revealing. Now that biological age can be measured, there are many studies in progress and we can expect results from these studies to validate anti-aging in ways not previously possible. Here are a few highlights of what we know now:

Time-restricted eating: Also called intermittent fasting, the idea here is to mimic the effects of caloric restriction by eating only during a limited time frame each day rather than reducing calories. The evidence is good: One crossover study (each group did both parts) compared gene expression patterns for a 6-hour eating schedule (8:00 am to 2:00 pm) to a 12-hour (8:00 am to 8:00 pm) on overweight adults. The time-restricted subjects showed stabilized glucose levels, increased expression of sirtuins, the autophagy gene LC3A, and mTOR. I try to practice time-restricted eating (with an 8 hour eating interval) and have not found it too difficult.

TRIIM trial: The "Thymus Regeneration, Immunorestoration, and Insulin Mitigation" trial investigated the use of human growth hormone to prevent or reverse signs of immune senescence in middle-aged healthy men. Growth hormone was used based on prior evidence that it has thymus and immune reconstituting effects in animals, but because of the undesirable diabetes- like effects, it was combined with metformin and DHEA. After one year of treatment, the mean epigenetic age approximately decreased to 1.5 years less than baseline, a −2.5-year change compared to no treatment at the end of the study. The decrease in epigenetic vs. chronological age persisted after discontinuing treatment. In my practice we use growth hormone-releasing peptides (sermorelin, CJC-1295) instead of GH.

A follow-up study called TRIIM-X is seeking to confirm the results with a larger group of subjects which includes women, and is ongoing. This phase of the study is sponsored by a company called Intervene Immune, and there is a cost of up to $18,000 to participate.

Note: Because use of growth hormone is highly restricted and has certain adverse effects, I prefer to use growth hormone-releasing hormones and peptides such as sermorelin, CJC-1295, and ipamorelin. These also have the advantages of cycling with the body's circadian rhythm, which releases growth hormone in pulses during sleep. I also prescribe metformin and DHEA.

Diet & Lifestyle study: This randomized controlled clinical trial on 43 healthy adult men aged 50- 72 tested a plant-centered, low carb diet plus a special fruit & vegetable powder, a specific probiotic, at least 7 hours/night sleep, exercise 30 minutes/day 5 days/week and twice daily relaxation exercises. DNA methylation analysis found a more than 3 years decrease in DNAmAge compared with controls.

Rejuvant® supplement: Retrospective study of -ketoglutarate supplement (AKG) showing an 8-year reversal in biological age as measured by their proprietary TruAge DNA methylation (not TruDiagnostic) analysis, after an average of 7 months of use. (Rejuvant changed their formula to include B vitamins, so it is important to check your other vitamin-containing supplements to make sure that you aren't taking too much. It is also possible to buy AKG as a stand-alone powder.)

CALERIE™ (Comprehensive Assessment of Long term Effects of Reducing Intake of Energy): Designed to determine the biological effects of two years of caloric restriction in humans, this study has produced several findings of interest. Reduction of caloric intake by only 14% produced improvements in immune function, systemic inflammation, and metabolism. The researchers subsequently identified the specific gene responsible for the effect (Pla2g7). So if you are up for long-term extreme dieting, it will work.

Therapeutic Plasma Exchange (TPE): In 2024, Dr. Dobri Kiprov and the Buck Institute reported the results of a clinical trial evaluating the biological age effects of TPE. They found that when supplemented with intravenous immunoglobulin, TPE produced robust 'omics' responses, reversed age-related immune decline, and modulated cellular senescence. A regimen of 6 monthly sessions gave the best result. *TPE is the only procedure available today documented to reverse aging.* This is not an inexpensive option however, with each session costing upwards of $6000.

TranslAGE Response Study: Also in October 2024, the results of a massive study from Yale in collaboration with TruDiagnostic came out. What they did was to evaluate 51 separate published studies of various interventions intended to impact aging biomarkers, and compare them across 16 different age clocks and 95 biomarkers. This way they were able to identify the interventions that had the strongest and most consistent effects. The data revealed that pharmacological interventions like metformin and a class of drugs called TNF-alpha blockers induced the strongest responses compared to lifestyle changes, supplements, and medical procedures.

Summary and Future directions

It is now possible to slow and even reverse aging in humans. Longevity medicine in the new era integrates basic science and clinical practice across multiple disciplines, bridges boundaries between academia and private enterprise, applies artificial intelligence, and reconfigures health care models from disease-based to healthspan-based.

Biohacking longevity is big business already, and getting bigger. Competition to capitalize on this 21st century gold rush is intense. No matter how savvy the consumer is, it is ever more challenging to navigate the many options available now. Privacy of genomic data is a vital issue in this enterprise. As longevity science advances, so too does the sophistication of its scammers.

This is where the practice of longevity medicine finds the value proposition. My goal is to provide independent and unbiased guidance. I will make recommendations according to the best information available. I won't get ahead of the science, but I don't intend to lag behind either. I want patients for the anti-aging practice to start by reading *Biohacking Longevity* because longevity medicine represents a fundamental shift from the way doctor-patient relationships have been built. I want you to be an insider and empower you to know when you are being sold a bill of goods. Our goal is to objectively track outcomes, whether it is skin rejuvenation, prescriptions such as metformin or rapamycin, peptides, or aesthetic treatments. Your anti-aging plan will be collaborative and personalized.

Glossary

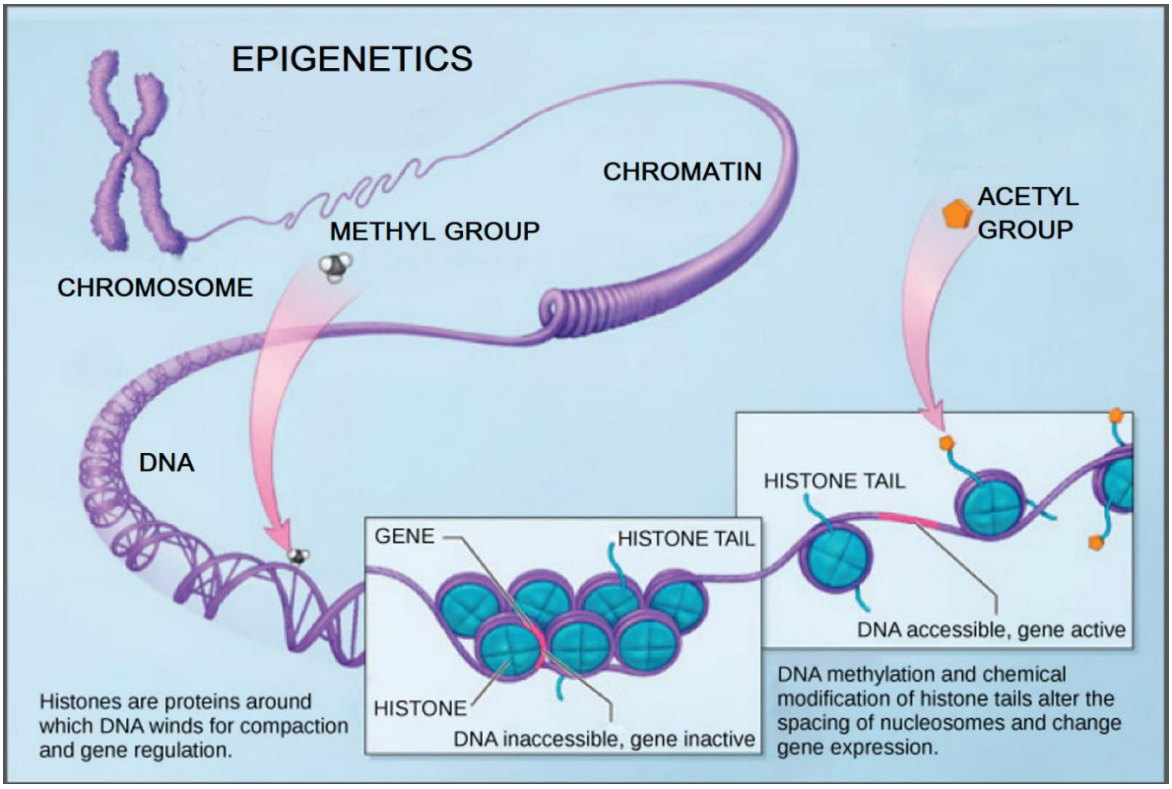
Aging biomarkers Measurable factors that reproducibly, qualitatively and quantitatively reflect the rate of human aging. Examples include DNA methylation, blood levels of proteins related to inflammation such as C-reactive protein, and glycated proteins such as hemoglobin A1c.

Artificial Intelligence (AI) The general definition of AI is a form of computing science which enables the computer to process information in the way that the human mind does. The types of AI relevant to biomedical science and anti-aging include Machine Learning – the computer's ability to learn from examples and experiences – and Deep Learning, a subset of machine learning using algorithms that enable software to train itself and process multiple layers of data.

Alpha-Ketoglutarate is an amino acid precursor that promotes protein synthesis and autophagy. There is evidence that AKG prolongs lifespan in animal studies and may reverse aging in humans.

Autophagy Literally meaning "self-devouring," autophagy is the mechanism by which cells remove damaged or dysfunctional components. Autophagy facilitates the orderly recycling of cellular debris. Although it was initially identified as a primordial response to starvation, it is now known that it also plays an important role in cell metabolism under normal conditions.

Epigenetics The molecular "software" that controls gene activity. Epigenetics is how cells transform into various types even though they all have the same DNA, and also directs the day-to-day function of mature cells. Mechanisms of epigenetic function include DNA methylation and modification of histones, leaving a permanent record of gene activation and/or silencing. These methylations leave a permanent record that can be transcribed and analyzed to determine biological age, rate of aging, and identify specific areas for individualized treatment.



Exosome A bubble formed of cell membrane containing a cargo of molecules to be delivered from one cell to another. Exosomes are a primary means of communication between cells.

Genomics The study of all of an individual's genes, how they interact with each other and the environment, and the resulting impact on physiology and health.

Glycation Sugar molecules binding to proteins, resulting in Glycation End Products or AGEs, which cause tissue damage and accelerate aging as they accumulate over time. Hemoglobin A1c is an example of glycation, reflecting average blood glucose levels over time.

Histones Protein structures forming spools around which DNA wraps, forming units called nucleosomes. Histones regulate gene expression and prevent DNA from becoming tangled.

Inflammaging Low-grade, chronic, systemic inflammation leading to more rapid aging.

In silico A term that means "done on a computer". In biology it usually refers to the computational modeling of biological processes. Examples include docking simulations, which model how biomolecules fit together and interact, and AI-based predictions of clinical effects.

In vivo In the living organism.

Longevity medicine AI-driven practice incorporating precision medicine concepts and interventions intended to prolong healthspan and lifespan.

Methylation One of the mechanisms of epigenetics. DNA consists of four bases, called cytosine, guanine, adenine, and thymine (the 4-letter "alphabet"). A chemical unit called a methyl group can be added to cytosine, resulting in methylation of that area of the DNA, suppressing activation of the gene. This leaves a record of genetic activity.

Mitochondria (singular is *mitochondrion*) Structures with the cell that are responsible for energy production. Mitochondria have their own DNA and are critical for healthy metabolism.

mRNA Messenger RNA (ribonucleic acid) is essentially a template transcribed from a gene. The mRNA strand then moves from the nucleus of the cell into the cytoplasm where it directs the assembly of proteins.

Nanofat A type of fat graft in which the fat cells are removed by filtration, leaving the platelets, stem cells, and other factors to promote regeneration. It is typically used in conjunction with a facelift where it is injected at a superficial layer under the skin.

Omics The study of collective sets of data within biological systems, and how they translate into structure and function. Examples include genomics, transcriptomics, proteomics, and metabolomics. The ending "-ome" is used to address the respective categories of study, such as the genome, proteome, etc. The objective of omics is to identify, characterize, and quantify biological molecules that are involved in the dynamics of a cell, tissue, or organism.

Peptides Mini-proteins that often act as hormones or signaling molecules, such as insulin. Peptides are involved in every aspect of aging, immunology, metabolism, and disease. Peptide levels decline with age.

Phenotype A term used in genetics for the observable characteristics or traits of an organism, including physical form and structure and its physiological properties. Phenotype results from the expression of its genetic code, (genotype) and the influence of environmental factors.

Precision medicine (PM) Also called personalized medicine, PM is a model that customizes healthcare decisions, treatments, practices, or products based on the individual's genome. PM has been widely applied in cancer therapeutics, and increasingly relevant in longevity medicine.

Protein folding The process of forming strands of amino acids into 3-dimensional shapes to make functional proteins. Misfolded proteins are associated with several specific diseases and manifestations of aging. Enzymes that assist in protein folding are called chaperones.

Senescence In the context of longevity medicine, this refers to cellular senescence, a zombie-like state cells can enter into when they reach the end of their replicative cycle but don't die. Senescent cells typically have accumulated DNA damage and secrete inflammatory molecules which contribute to both localized and systemic degradation (*inflammaging*.)

Senolytic Senolytics are drugs or other substances that selectively clear senescent cells. These include the drug Dasatinib and the flavonoids quercetin and fisetin. In contrast, *senomorphics* are compounds that restore senescent cells.

Sirtuins A class of signaling proteins that modulate the activity of genes involved in cellular metabolism, stress response, and aging.

Telomere Telomeres are bits of non-coding DNA and protein that cap the ends of chromosomes to prevent unraveling. Typically, they shorten with each cell replication cycle. Short telomeres are associated with aging. The enzyme that restores telomeres is called telomerase.

Therapeutic Plasma Exchange (TPE) A procedure in which blood plasma is removed and replaced with a solution of albumin and immune globulin protein. TPE has impressive age-reversal potential but is expensive.

Transcription factor (TF) A protein that controls the transcription of genetic information from DNA to messenger RNA, by binding to a specific DNA sequence. TFs regulate genes to ensure that they are expressed in the right cell at the right time and in the right amount.

Yamanaka factors are a group of transcription factors that together direct epigenetic reprogramming of mature cells to a more youthful state.

Resources

1. Subscribe to the Buck Institute's newsletter: BuckInstitute.org
2. Fight Aging! Newsletter: FightAging.org
3. FirstLongevity.com
4. Aging Biotech List of Therapeutics: This frequently updated website is a list of available therapies thought to slow or reverse aging. agingbiotech.info/therapeutics/
5. Forever Health Foundation: This non-profit organization based in Germany has a mission to "enable people to vastly extend their healthy lifespan through science." lifespan.io/organizations/forever-healthy-foundation/