Where and How in the mTOR Pathway Inhibitors Fight Aging: Rapamycin, Resveratrol, and Metformin

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Abstract

The molecular mechanisms underlying the quality and quantity of life extension appear to sometimes be orthogonal. For example, while resveratrol has continued to prove beneficial in reducing obesity, it has had less efficacy in extending lifespan. On the other hand, rapamycin and the chemically similar rapalogs extend lifespan across genera of life from yeast, to nematodes, to mice. Caloric restriction (CR) and bioavailable small molecules, which mimic a fasted state, upregulate autophagy, catabolism of fats over anabolism of carbohydrates, and decrease oxidative stress and inflammation. CR mimics are currently being investigated to elucidate the best dosage, route of administration, timing in life, where best to inhibit in the mTOR pathway, and effects of long-term use on mTORC1 versus mTORC2 complexes. Comparisons between rapamycin, resveratrol, and metformin targets, downstream pathway effects, dosage, and clinical trials will be discussed.

Keywords: rapamycin, rapalogs, resveratrol, metformin, mTOR, senescence, aging, longevity, autophagy, inflammation

1. Introduction

It has been shown across the animal kingdom that caloric restriction (CR) extends lifespan. It is logistically harder to test this in longer living animals due to the length of studies needed, but there are studies in non-human primates [1] and ongoing human test groups who show fewer signs of cardiovascular aging [2]. Two trials calorically constricting macaques began in the 1980s and initially had conflicting results. A study out of University of Wisconsin found a drastic 30% increased survival in the CR group compared to control [3], while a latter study...
by the National Institute on aging (NIA) did not find a statistically relevant effect [4]. It was later found that in the NIA study, control monkeys consumed fewer calories than expected, and some in the CR groups began consuming reduced calories as juveniles, which is known to reduce lifespan. A reanalysis of all data by both groups agreed caloric restriction appears to increase macaque longevity by almost 10% (3 years in macaques which would translate to 9 years in humans) [1].

In general results have seemed positive, extending life, but to a lesser degree than in small animal models, such as mice which have seen up to a 50% increase in lifespan from CR [5]. The search for pharmaceuticals to mimic CR life extension will need to continue the long and expensive process of large human studies due to humans’ unique interaction with calories in our post-industrial world. Study designs for larger caloric restriction studies are often questioned. Particularly concerning humans, the ability to accurately track caloric consumption in people living outside a clinical setting has often relied on caloric approximations such as food diaries, pictures of food eaten [6–8], or dietary consumption habits at a national level when comparing between countries. The larger percentage life extension effects from CR has been seen across many simpler organisms with budding yeast, fruit flies and worms having their lifespan increased 2–3 fold. However, no mammals have had such large effects. Indeed no one has suggested humans could achieve such great gains with CR which would extend our current upper lifespan from ~100 years to over 200 years. However, mammals such as rats and mice have shown a 20–50% reduction in calories can result in a lifespan increase of up to 50% [9, 10].

Even with these qualifiers in mind, it seems likely that a 30–60% reduction in calories could extend human life 10–20%. This gain of ~1% of lifetime for every ~3% reduction in calories translates to a likely ~10–20 years of extra life for humans, which is similar to the 9-year human equivalent life extension seen in the recent reanalysis of primates undergoing CR [1]. This 1:3 ratio of %life extension:%caloric restriction (LE:CR) may end up being 1:4 or 1:2 in humans, but in either scenario it is most likely caloric restriction will show a statistically significant life extension in humans. However, it is not likely to be a panacea that would give us 50 extra years bringing us past 150 years-of-age, despite that relative effect in mice.

The current dilemma has been elucidating the root cause(s) responsible for life extension which are being targeted as pharmaceutical targets. The “inputs” that one might measure which lead to an increased lifespan in humans (e.g. obesity, cholesterol, cancer, bone density) are numerous and often orthogonal in nature. For example at pharmacological concentrations resveratrol does inhibit obesity but did not inhibit cellular senescence like rapamycin does [11]. While resveratrol and rapamycin were at times thought to act similarly, their mechanistic and pharmacological issues are diverging. While resveratrol had been found to extend life in studies there have been negative results with some labs failing to find life extension in all strains of yeast [12], worms, and flies [13]. Indeed rapamycin but not resveratrol has been shown to extend lifespan in mice [14]. Resveratrol may increase our quality of life while rapamycin (and rapalogs) could increase our quantity of life. In addition, one of resveratrol’s main issues is its bioavailability (it’s good we just want more), whereas rapamycin may shut down people’s immune system too much leading to cancer (it’s good but too much is bad). The mechanism of action for rapamycin, resveratrol, and metformin, as well as animal and human studies will be discussed.
The ideal “biological scale” at which aging can be targeted is also still in question (a single gene, pathway, salvaging a cell, or killing unrecoverable cells) (Figure 1). Single genes continue to be investigated with inhibition by siRNA, conditional knockouts, or reducing posttranslational modifications such as lipid anchoring [15–21], while activation could be investigated via upregulation of transcription factors or viral therapy such as CRISPR. However, due to overlapping inputs the field often addresses how entire pathways are being affected (such as increased mitochondria biogenesis by caloric restriction). In addition while in vitro studies have often looked at modifying a cells genetic profile to have more of a centenarian profile (i.e. to rescue human cells via an intervention), it has recently been shown many cells become senescent and causing those to undergo apoptosis can save other cells thereby resulting in organism longevity [22–28]. The easiest abnormal aging targets may be the overactive cancerous cells we have become use to targeting via single genetic markers (e.g. targeting estrogen receptor sensitivity in breast cancer). Pathways can be targeted via some important individual targets, for example rescuing p53 deficiency or inhibiting mdm2 over activity to cause apoptosis. However rescuing cells from becoming senescent is the hardest and most distant task, required to truly push human longevity beyond a ~125 year limit (Figure 1). An important comparison is the case of the hydra which has been pointed to in the last couple of decades as an immortal multicellular organism [29, 30]. The hydra however, has a structure in which stem cells continually differentiate and move the periphery where they fluff off. There is not a large repository of persistent differentiated cells that can never become senescent for their hydra to continue living. In this regard the hydra can be thought of amputating any problem cells which it can replace [31–33]. Many of humanity’s growing diseases involve multi-organ

Figure 1. Therapeutics for “aging” will likely having differing levels of complexity in their targets depending on if they are targeting a single gene (easiest), a pathway, cause death of senescent cells, or trying to salvage a cell from becoming senescent (most difficult). Overactive cancerous cells can be targeted simply to kill based on one receptor or gene, while salvaging neurons from death (e.g. various dementias) is a harder therapeutic task.
systems with terminally differentiated cells which cannot be easily replaced. For example, neurodegenerative diseases such as Alzheimer disease (AD) have phenotypic effects when neurons start dying in large numbers. While CR and CR mimics may increase autophagy and delay cell death, as discussed below, there is not evidence that inhibition of the mTOR pathway can perpetually shift humans as an organism to a hydra like state of immortality. Since 1932 the correlation between mass and metabolic rate for mammals has been investigated as a foundation for humans’ upper lifespan limit [34, 35]. It could be that the lower molecular activity from CR will shift humans to a longer lifespan following the three-quarters power law (or Kleiber’s Law), although more recent studies seem to be elucidating cellular and molecular minutia in a more fine-grained manner than Kleiber’s course mass does [36–38].

2. mTOR pathways: rapamycin, resveratrol, and metformin

A wealth of studies has confirmed that rapamycin and rapalogs directly inhibit mTOR, whereas resveratrol’s targets are more numerous. Initially resveratrol was thought to act primarily through activation of sirtuins, with sirtuin-1 (SIRT1) known to help reduce obesity [39]. It is now known resveratrol also activates adenylyl cyclase and AMP-activated protein kinase (AMPK), while inhibiting a slew of proteins including lipoxygenase, protein kinase C (PKC), p53, mitogen-activated protein kinase 3 (MAPK3), proto-oncogene tyrosine-protein kinase (Src), signal transducer and activator of transcription 3 (STAT3), and IκB alpha kinase (IKK) [40]. One of the main targets is now AMPK activation which itself activates SIRT1 leading to mTOR inhibition.

2.1. Anabolic vs. catabolic energy production

AMPK is one of the primary metabolic detectors conserved across genera being activated by conditions that cause a low ATP:ADP ratio such as hypoglycemia and hypoxia. Phosphorylation of likely over 1000 targets by AMPK [41] shuts off anabolic pathways (energy-using) and turns on catabolic pathways (energy-generating). One of AMPKs targets for phosphorylation is peroxisome proliferators-activated receptor gamma coactivator-1 alpha (PGC-1α) which becomes active resulting in increased mitochondria biogenesis, membrane potential, and fatty acid oxidation [42], a recurring feature found during caloric restriction [43, 44]. AMPK also activates forkhead transcription factors of the O class (FOXO) which leads to increased autophagy and antioxidants, both leading to increased oxidative metabolism, like PGC-1α does [44].

In the case of life extending interventions dosing becomes very important. Too much of a good thing, can definitely be bad (i.e. cancer), and the molecular mechanism effecting longevity are being elucidated. For example, in Caenorhabditis elegans, metformin is found to delay development under well-fed conditions and even reduces life span during starvation [45–47]. The improved mitochondrial function, decreases oxygen consumption needed, which causes a beneficial decrease in reactive oxygen species (ROS) [48, 49]. Mitochondrial biogenesis is controlled differently depending on tissue and disease state. For example, mTOR signaling has been found to increase expression of mitochondrial genes involved in oxidative metabolism,
through PGC-1α and Ying-Yang 1 (YY1). This increased mitochondrial biogenesis in the muscle of healthy individuals, but not in obese individuals perhaps due to decreased insulin sensitivity [50]. Not only is mTORC1 activity cell specific, but it is also concentration dependent being induced and inhibited by low and high levels of ROS respectively [51]. This concentration sensitivity of mTOR is beneficial since it acts as a hub for interdependent pathways, such as mTORs ability to modify both mitochondrial biogenesis and increase autophagy (which helps degrade damaged mitochondria and other organelles). Two models of aging have been established in yeast (Saccharomyces cerevisiae): replicative lifespan (RLS) and the chronological lifespan (CLS). RLS measures the number of asymmetric mitotic divisions a cell can undergo before cell cycle arrest and is a valuable model for fibroblasts, lymphocytes, or stem cells in humans [4, 52–54]. CLS in contrast measures how long stationary (G0) cultures remain viable and is a model for postmitotic cells like neurons or muscle cells [52, 54, 55]. Organ specific analysis of human in vivo studies, while difficult, would help elucidate CR mimics at and upstream of mTOR.

Metformin is a third life extending compound worth contrasting to rapamycin and resveratrol because it inhibits the mitochondrial respiratory chain complex I, leading to decreased ATP:ADP, which activates AMPK [56]. In addition metformin has lots of human data since it is a common oral anti-diabetic drug used for overweight people with type 2 diabetes mellitus (T2DM). Metformin inhibits hepatic glucose production, reduces insulin resistance, and has recently been investigated as anti-aging therapeutic. Metformin is currently being investigated for use in various cancers [57, 58]; however, metformin has also been linked to the development of some solid tumors in humans, namely colorectal, breast, and pancreas cancer [59]. Mitochondrial complex I is clearly inhibited by metformin leading to the AMPK dependent activation of TSC2 which inhibits mTOR. AMPK can also directly inactivate mTORC1 complex via phosphorylation of its subunit Raptor. However, it has also been shown that metformin can act in an AMPK independent manner, though that mechanism is less clear but could involve nuclear pore complex (NPC) or late endosome interactions which have been documented [46]. The NPC interaction was found when C. elegans ortholog of acyl-CoA dehydrogenase family member 10 (CeACAD10) knockdown was found to have a 3-fold resistance to metformin. CeACAD10 expression was more than doubled by 50 mM metformin, and an unbiased, forward genetic screen found the nuclear pore complex is required for metformin to induce CeACAD10 [47]. That molecular pathway is currently unique to metformin, compared to resveratrol or rapamycin, and while multiple targets have been found for metformin, some pathways overlapping between these three molecules allow a more robust understanding of calorific restrictions possible of life extension mechanisms. Not only mTORC1, but even upstream AMPK, has been shown to be required for the positive effects of all three molecules rapamycin [60], resveratrol [61, 62], and metformin [63, 64]. Metformin’s molecular pathway has also been elucidated upstream of AMPK. Metformin interacts with organelle Na1/H1 exchangers (eNHE) and the V-type-ATPase (V-ATPase) which supports the idea of the late endosome/lysosome, which is required by both the AMPK and mTOR pathway, acting as a signaling hub for metabolism [45].

While gross metrics such as weight are often reported in studies and useful to follow, they are not sufficient to investigate the aging phenomenon. For example, mice administered
resveratrol have been found to not lose weight [65, 66]. The degree to which resveratrol mimics caloric restriction (CR) has been shown at a molecular level in mice with changes in gene expression overlapping in the adipose tissue, skeletal muscle, heart, liver, and neocortex. Interestingly, both resveratrol and CR slowed age-related decline in organ function, showing the benefit from resveratrol was not dependent on weight loss [65, 66]. The other side of the caloric coin which is frequently investigated independently of CR is exercise induced caloric deficit. For modern humans it is clear it is extremely difficult to exercise one’s way into the same caloric deficit that can be attained through CR. In short, it is harder to run off a fast food meal than to not have the meal in the first place. It has been shown in rodents that increased activity to achieve a 30% relative energy deficit did not extend maximal lifespan but did increase average lifespan [9, 67]. The ability of resveratrol to increase lifespan has varied significantly between studies, but been roughly 40% for yeast, 15% for worms, 30% for fish, and 10% for mice [68].

There are two mTOR multisubunit protein complexes which have been shown to be differentially regulated. mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) share the protein components DEP domain containing mTOR-interacting protein (DEPTOR), mammalian lethal with sec-13 protein 8 (mLST8, also known as GβL), telomere maintenance 2 (telO2), and telO2-Interacting Protein 1 (tti1) (shown as light blue in Figure 2). mTORC1 has three core components: mTOR, regulatory-associated protein of mTOR (Raptor), and mammalian lethal with sec-13 protein 8 (mLST8). Whereas mTOR complex 2 (mTORC2) core components share mTOR, mLST8, but also include rapamycin-insensitive companion of TOR (Rictor), and mammalian stress-activated map kinase-interacting protein 1 (mSIN1) (Figure 2) [69]. mTORC1 is activated by nutrients and growth factors while being inhibited in low energy cellular states. A known complexity with the mTOR pathway is the difference in response to inhibitors, not only by mTORC1 and mTORC2, but also by tissue. mTORC1 is universally inhibited by rapamycin, whereas mTORC2 needs long term exposure to be inhibited by rapamycin which continues to be investigated. While DEPTOR is known to partially inhibit mTORC1 it may not decrease lipogenesis or inflammation alone, however in conjunction with AKT Serine/Threonine Kinase 1 (AKT) inhibitors can result in both decreases in lipogenesis and inflammation [70]. Combination therapy may be necessary in targeting mTORC1 to attained desired effects.

While multiple targets upstream of mTOR continue to be investigated, well described downstream actions of mTOR help in analysis of in vivo, in vitro, and clinical studies. While the major downstream effect of mTOR activation is anabolic energy production (with inhibitors shifting to catabolic energy production from fat), another significant downstream effect of mTOR activation is increased inflammation. In general people living in the western world live in a state of excess inflammation. Time restricted feeding (TRF) was found to help immune response, reducing systemic low-grade inflammation and age-related chronic diseases linked to immunosenescence, without compromising muscle performance [71]. The reduced inflammation seen in calorically restricted individuals is partially due to an increase in autophagy from CR (see below). The mTOR pathway has been shown to trigger the development of T cells, B cells, and antigen-presenting cells (APC). Indeed resveratrol (found in plants such as
grapes, red wine, mulberries, and peanuts) has been described as a broad spectrum of action anti-inflammatory which attenuates microglial cell overactivation through mTOR inhibition [72]. Resveratrol inhibition of NF-κB causes an increase in superoxide dismutase (SOD) and results in decreased proinflammatory cytokines IL-1β, IL-6, and TNF-α [73–76].
Genome wide analysis will likely be needed to elucidate the beneficial molecular level causes of caloric restriction. For example, Dato et al. recently analyzed pathway-based SNP-SNP interactions of 3 pathways: the insulin/insulin-like growth factor signaling (IIS), DNA repair, and pro/antioxidants. Synergistic effects on longevity were found in the combination of growth hormone secretagogue receptor (GHSR) and double strand break repair nuclease MRE11 homolog (MRE11A) genes which are involved in IIS signaling. TP53 also had synergistic effects with either ERCC Excision Repair 2 (ERCC2) or thioredoxin reductase 1 (TXNRD1). Those results highlighted the central role of TP53 in activating DNA repair and pro-antioxidant pathways [77].

2.2. Autophagy

One pathway difference between rapamycin and resveratrol is the large magnitude with which rapamycin increases autophagy over apoptosis, which helps in regards to life extension but could prove problematic in cancer use. Pharmacological levels of resveratrol on the other hand prevent upregulation of Akt activation and autophagy thereby causing apoptosis. Resveratrol does inhibit obesity at pharmacological concentrations, prevent heightened hyperinsulinemia, or inhibit mTOR in vitro and therefore did not inhibit cellular senescence like rapamycin does [11]. Large levels of resveratrol have recently been shown to induce autophagy when inhibiting mTOR directly through ATP competition [78]. Combination therapy of rapamycin and resveratrol has proven synergistic in treatment of breast cancer cells [79, 80].

Caloric restriction has been shown to increase autophagy through inhibition of mTOR and delay molecular events associated with dementia. The rise in neurodegenerative diseases, which are exacerbated by low autophagy levels, heightens interest in mTOR inhibitors. Caloric restriction achieves mTOR inhibition through two pathways: decreased PI3K activity and increased AMPK activity (Figure 2). Cells in low energy states (calorically restricted) have low PI3K activity, lowering Akt activity, which then lowers mTORC1 via inhibition by Tsc1/2 (Figure 3). Rapamycin directly inhibits mTOR but metformin and resveratrol inhibit mTOR through upstream pathways, inhibiting the mitochondrial complex I activity and increasing AMPK respectively. In the well fed state mTORC1 inhibits autophagy via inhibition of SIRT1, Unc-51 like autophagy activating kinase (ULK1), transcription factor EB/E3 (TFEB/TFE3). Active mTOR also stimulates eukaryotic translation though phosphorylation and inhibition of 4E-BP1 which in turn releases the bound cap-binding eukaryotic translation initiation factor 4E (eif-4E). When eif-4E is released it can participate in forming the eIF4F complex required for initiation of cap-dependent translation. Ribosomal proteins S6 and S6K are also stimulated by mTOR which leads to increased protein synthesis and lipogenesis. In the fasted state ULK1 starts autophagosome maturation and TFEB/TFE3 increases lysosomal biogenesis and autophagy. Ras-related GTPases (Rags) actually tether mTORC1 to the lysosomal surface and that connection is controlled through amino acid sensing of the vacuolar H⁺-adenosine triphosphatase ATPase (v-ATPase) as well as the proton-assisted amino acid transporter 1 (PAT1) (Figure 3). SIRT1 is also activated in the fasted state, and by CR mimetics, which increases SOD, p53, and activates FOXO leading to increases in cellular autophagy and mitochondrial biogenesis (Figure 2).
Therapeutics to help extend human lifespan far past the ~100 year limit will likely need to increase autophagy to avoid dementias, a later life disease state. With various dementias (PD, ALS, HD, and AD) having mitochondrial dysfunction [81–84], and mTOR activation known to increase oxidative stress, antioxidant therapies are being investigated. It has been found conjugating a cation compound to the antioxidant increases uptake into the mitochondria 80-fold and potency up to 800-fold [85] due to its 165 mV negative potential [86]. Low levels of autophagy also result in necrosis instead of apoptosis, with the resulting ramped up immune system increasing inflammation. Intracellular stress acts through Bcl-2 to open the mitochondrial permeability transition pore (mPTP) leading to caspase dependent intrinsic apoptosis [69, 87, 88]. The mPTP is known to exist in 3 states: closed, transiently open in low conductance, and permanently open in high conductance [89–91], the latter resulting in mitochondrial depolarization, loss of ATP production, and caspase independent necrosis since the...
controlled apoptotic pathway requires energy [92]. Multiple types of cancer show increased mTOR pathway signaling which is what the first mTOR inhibitors were FDA approved for: sirolimus, everolimus (Afinitor), temsirolimus (Torisel), and ridaforolimus, with sirolimus and everolimus also finding use as immunosuppressants after organ transplants [93].

While caloric mimics will not be the panacea pushing human life past 200 years it should be pointed out the large effect it could have in humans compared to other currently measurable lifestyle interventions. The effect was recently quantified for the top five frequent lifestyle interventions: smoking cessation, physical activity, healthy diet, healthy BMI, and low alcohol consumption (Table 1) [94]. Starting at age 50 women and men were found to be able to add on average 14 and 12 years respectively, if all 5 healthy lifestyles were adopted. Never smoking was the strongest healthy habit of the five, with a close second being engaging in physical activity over 30 min a day (which included brisk walking or anything more strenuous). The healthy diet and BMI (18–25 kg/m²) can both clearly be linked to a CR lifestyle. It will be interesting to compare the magnitude of CR life extension to the years gained by aspects of a “healthy diet” which is usually cataloged by many more variables than just caloric count (e.g. vitamin/antioxidants, omega-3 vs. omega-6 vs. saturated fat content). In summary, CR alone seems likely to have as big, or slightly larger, of an effect than the 5 healthy lifestyles in concert. If rapamycin, resveratrol, metformin, or a combination thereof, prove capable of reproducing even half the years of life extension that CR extension can, it would be a multi-billion dollar market (USD) and could be among the best therapeutics measured on the Quality Adjusted Life Years (QALY) scale.

3. Preclinical and clinical studies

Table 1. Five healthy lifestyles that extend lifespan more than 10 years.

| Lifestyle                                                                 | ♀     | ♂     |
|--------------------------------------------------------------------------|-------|-------|
| Physical activity (≥30 min/day)                                          | 8     | 7     |
| Not smoking                                                              | 9     | 12    |
| Healthy diet                                                             | 5     | 4     |
| Low alcohol (15♀, 30♂ g/d = 2♀, 4♂ drinks/d)                            | 3     | 2     |
| BMI (18–25 kg/m²)                                                        | 4     | 5     |
| Extra years if all 5                                                     | 14    | 12    |

Five healthy lifestyles (exercise, healthy diet, ideal BMI, low alcohol, and not smoking) were found to add 12–14 years of life starting at age 50 when compared to people that did not follow any of the five lifestyles. The healthiest and worst habits within each lifestyle had very different life expectancies as well, with nonsmokers and excessive smokers having the largest lifespan gap (9–12 years). The second greatest gain in lifespan (7–8 years) came from getting more than 30 min of exercise a day compared to never exercising. Data from [94].
chemically distinct. Both resveratrol and metformin are hydrogen-donor rich, having hydroxyls and amides, respectively. Rapamycin is a much larger macrocycle molecule (MW = 914) with both hydrogen donor and acceptor moieties compared to the smaller resveratrol (MW = 228) and rapamycin (MW = 129) (Figure 4). Future docking, crystallography, and NMR studies would be interesting to determine if other molecules could mimic ATP, directly binding to the ATP pocket on mTOR as it has been suggested resveratrol does [78]. A structural mimic of ATP acting as an antagonist can seem conceptually attractive and likely have broad effects on multiple energy sensing proteins, but would also likely have lower than desired specificity.

All three compounds resveratrol, rapamycin, and metformin have had numerous human clinical trials. Metformin is unique among the three in that it is currently an approved and recommended therapy for a massive population, specifically obese individuals with type II diabetes, and therefore has a much larger dataset of patients to pull safety and efficacy information from. Resveratrol and rapamycin are both natural compounds with a plethora of academic papers in animal models, but rapamycin studies have the added nuance/diversity of involving a host of rapalogs with modified activity. A search of clinical trials including the keywords resveratrol, metformin and rapamycin and grouped by topic is shown in Table 2 (as of April 11th 2018). Resveratrol has the lowest number of ongoing clinical trials (137), metformin has over 2.5 fold as many (359), and rapamycin has almost fivefold ongoing clinical trials (646). Resveratrol and metformin have largely overlapping pathway targets in clinical trials with the most common being endocrine system diseases, diabetes mellitus, obesity, and insulin resistance. The main topics for rapamycin are neoplasms by histological type, vascular

Figure 4. Structures of metabolism modifiers. The structures of compounds discussed: resveratrol, metformin, rapamycin, and ATP. Some have had suggested competitive binding pockets, such as resveratrol and ATP. However on a small molecule scale metformin, resveratrol, and rapamycin have very different complexity and differ by orders of magnitude in molecular weight. All compounds do have significant number of polar groups for hydrogen binding to protein surfaces and pockets.
disease, and myocardial ischemia. Metformin and rapamycin have some overlap, e.g. metformin has 45 current trials listed under neoplasms by histological type, and rapamycin has 42 trials listed under endocrine system diseases.

4. Conclusions

The use of therapeutics that mimic caloric restriction (CR) is likely to increase and add incremental quality-adjusted life years (QALYs). Natural CR compounds, and analogs based off of them, are fairly cheap with low side effects. Controlled animal studies will likely continue to be the avenue which exposes the degree to which molecular pathways are responsible for the increased quality and quantity of life. Resveratrol and metformin seem robust at increasing molecular pathways linked to quality of health and are useful to combat obesity and type II diabetes; while their ability to increase maximum lifespan remains in question. Data suggests rapamycin and the follow on rapalogs could add years to a human lifespan, although the magnitude of the effect could be enhanced or completely ablated based on accompanying lifestyle choices (diet, exercise, sleep).

Research shedding light on the optimum dosing of caloric mimics should be interesting to follow. Caloric restriction studies in humans fall into three categories: continual modest decrease in calories consumed (~1500 kcal/day), temporary drastic reduction in energy intake (~500 kcal/day), or intermittent fasting (0 kcal/day) in which only water is consumed for 1–3 days. Intermittent fasting has actually slightly outperformed all other methods of dieting methods (atkins, zone, weight watchers, ornish/vegan) in reducing weight in humans, which is partially due to increased compliance [95, 96]. The degree to which molecular pathway changes from intermittent fasting are responsible for reduced weight, such as increased autophagy, remains to be determined. The number of calories that can be consumed above complete fasting, while still increasing autophagy and decreasing inflammation, needs further investigation [1, 10, 44, 97–108].

| Compound term search at clinicaltrials.gov | # CT ongoing | Insulin resistance | Diabetes mellitus | Endocrine system diseases | Obesity | Neoplasms by histologic type | Vascular diseases | Myocardial ischemia |
|-------------------------------------------|--------------|-------------------|------------------|--------------------------|---------|----------------------------|----------------|-------------------|
| Resveratrol                               | 137          | 28                | 24               | 23                       | 19      | 5                          | 6              | 1                 |
| Metformin                                 | 359          | 34                | 178              | 195                      | 34      | 45                         | 14             | 5                 |
| Rapamycin                                 | 646          | 1                 | 12               | 42                       | 2       | 218                        | 172            | 143               |

Clinical trials that are ongoing, as of April 11th 2018, were searched for the keywords resveratrol, metformin, and rapamycin. Resveratrol and metformin had overlapping metabolic clinical targets listed, while rapamycin had more numerous trials, which were focused on vascular diseases and cancer.

Table 2. Ongoing clinical trials for resveratrol, rapamycin, or metformin (April 11th 2018 search of clinicaltrials.gov).
5. Recommendations

It would be very useful for clinicians and patients to have a curve in which the x-axis showed calories consumed per day and the y-axis showed %change in these important life extension pathways (e.g. autophagy, inflammation, lipogenesis, lysosomal biogenesis, and protein synthesis). These studies/curves would ideally be done separately for various groups (e.g. males, females, diabetics, elderly predementia, and elderly with early dementia). The interaction of therapeutics that mimic CR in combination with a changing intake of calories from fluctuating diet will require significant large studies and clear simplifications for clinicians and patients to utilize that information and make actionable in daily life. The actionable timeline for CR mimics is still being investigated, but if studies from intermittent fasting apply then administration for months could be useful but lifetime use will be needed to maximize benefits.

Abbreviations

4E-BP1 eukaryotic translation initiation factor 4E-binding protein 1
AD Alzheimer disease
ALS amyotrophic lateral sclerosis
AKT AKT Serine/Threonine Kinase 1
AMPK AMP-activated protein kinase
APC antigen-presenting cells
CeACAD10 C. elegans ortholog of acyl-CoA dehydrogenase family member 10
CLS chronological life span
CR caloric restriction
DEPTOR DEP domain containing mTOR-interacting protein
eif-4E eukaryotic translation initiation factor 4E
eNHE Na1/H1 exchangers
ERCC2 ERCC Excision Repair 2 (ERCC2)
FOXO forkhead transcription factors of the O class
GHSR growth hormone secretagogue receptor
HD Huntington disease
IIS insulin/insulin-like growth factor signaling
IKK  IκB alpha kinase
MAPK3  mitogen-activated protein kinase 3
mLST8  mammalian lethal with sec-13 protein 8
mPTP  mitochondrial permeability transition pore
MRE11A  double strand break repair nuclease MRE11
mSIN1  mammalian stress-activated map kinase-interacting protein 1
mTORC1  mTOR complex 1
mTORC2  mTOR complex 2
NIA  National Institute on aging
NPC  nuclear pore complex
PAT1  proton-assisted amino acid transporter 1
PD  Parkinson disease
PGC-1α  proliferators-activated receptor gamma coactivator-1 alpha
PKC  protein kinase C
QALY  Quality Adjusted Life Years
Rags  Ras-related GTPases
Raptor  regulatory-associated protein of mTOR
Rictor  rapamycin-insensitive companion of TOR
RLS  replicative lifespan
ROS  reactive oxygen species
SIRT1  sirtuin-1
SOD  superoxide dismutase
Src  proto-oncogene tyrosine-protein kinase
STAT3  signal transducer and activator of transcription 3
T2DM  type 2 diabetes mellitus
telO2  telomere maintenance 2
TFEB/TFE3  transcription factor EB/E3
tti1  telO2-interacting protein 1
TXNRD1  thioredoxin reductase
ULK1: Unc-51 like autophagy activating kinase
v-ATPase: vacuolar H+‑adenosine triphosphatase ATPase
V-ATPase: vacuolar (H+)-ATPase
YY1: Ying-Yang 1

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References

[1] Mattison JA, Colman RJ, Beasley TM, Allison DB, Kemnitz JW, Roth GS, et al. Caloric restriction improves health and survival of rhesus monkeys. Nature Communications. 2017;8:14063

[2] Holloszy JO, Fontana L. Caloric restriction in humans. Experimental Gerontology. 2007;42:709-712

[3] Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, et al. Caloric restriction delays disease onset and mortality in rhesus monkeys. Science. 2009;325:201-204

[4] Mattison JA, Roth GS, Beasley TM, Tilmont EM, Handy AM, Herbert RL, et al. Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. Nature. 2012;489:318-321

[5] Weindruch R, Walford RL, Fligiel S, Guthrie D. The retardation of aging in mice by dietary restriction: Longevity, cancer, immunity and lifetime energy intake. The Journal of Nutrition. 1986;116:641-654

[6] Blechert J, Meule A, Busch NA, Ohla K. Food-pics: An image database for experimental research on eating and appetite. Frontiers in Psychology. 2014;5:617

[7] Charbonnier L, van Meer F, van der Laan LN, Viergever MA, Smeets PAM. Standardized food images: A photographing protocol and image database. Appetite. 2016;96:166-173

[8] Yue Y, Jia W, Sun M. Measurement of food volume based on single 2-D image without conventional camera calibration. Conference Proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society. 2012;2012:2166-2169
[9] Heilbronn LK, Ravussin E. Calorie restriction and aging: Review of the literature and implications for studies in humans. The American Journal of Clinical Nutrition. 2003;78:361-369

[10] Most J, Tosti V, Redman LM, Fontana L. Calorie restriction in humans: An update. Ageing Research Reviews. 2017;39:36-45

[11] Leontieva OV, Paszkiewicz G, Demidenko ZN, Blagosklonny MV. Resveratrol potentiates rapamycin to prevent hyperinsulinemia and obesity in male mice on high fat diet. Cell Death & Disease. 2013;4:e472

[12] Kaeberlein M, McDonagh T, Heltweg B, Hixon J, Westman EA, Caldwell SD, et al. Substrate-specific activation of sirtuins by resveratrol. The Journal of Biological Chemistry. 2005;280:17038-17045

[13] Bass TM, Weinkove D, Houthoofd K, Gems D, Partridge L. Effects of resveratrol on lifespan in Drosophila melanogaster and Caenorhabditis elegans. Mechanisms of Ageing and Development. 2007;128:546-552

[14] Miller RA, Harrison DE, Astle CM, Baur JA, Boyd AR, de Cabo R, et al. Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences. 2011;66A:191-201

[15] Asati V, Mahapatra DK, Bharti SK. PI3K/Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways inhibitors as anticancer agents: Structural and pharmacological perspectives. European Journal of Medicinal Chemistry. 2016;109:314-341

[16] Chuang H-C, Huang P-H, Kulp SK, Chen C-S. Pharmacological strategies to target oncogenic KRAS signaling in pancreatic cancer. Pharmacological Research. 2017;117:370-376

[17] Dang CV, Reddy EP, Shokat KM, Soucek L. Drugging the “undruggable” cancer targets. Nature Reviews Cancer. 2017;17:502-508

[18] Keeton AB, Salter EA, Piazza GA. The RAS-effector interaction as a drug target. Cancer Research. 2017;77:221-226

[19] Pan H, Finkel T. Key proteins and pathways that regulate lifespan. The Journal of Biological Chemistry. 2017;292:6452-6460

[20] Slack C. Ras signaling in aging and metabolic regulation. The Journal of Nutrition Health and Aging. 2017;4:195-205

[21] Michael JV, Goldfinger LE. Concepts and advances in cancer therapeutic vulnerabilities in RAS membrane targeting. Seminars in Cancer Biology [Internet]. 2017. Available from: http://www.sciencedirect.com/science/article/pii/S1044579X17302730

[22] Kohli J, Campisi J, Demaria M. A novel suicide gene therapy for the treatment of p16Ink4a-overexpressing tumors. Oncotarget. 2017;9:7274-7281
[23] Lecot P, Alimirah F, Desprez P-Y, Campisi J, Wiley C. Context-dependent effects of cellular senescence in cancer development. British Journal of Cancer. 2016;114:1180-1184

[24] Hodes RJ, Sierra F, Austad SN, Epel E, Neigh GN, Erlandson KM, et al. Disease drivers of aging. Annals of the New York Academy of Sciences. 2016;1386:45-68

[25] Wiley CD, Campisi J. From ancient pathways to aging cells—Connecting metabolism and cellular senescence. Cell Metabolism. 2016;23:1013-1021

[26] Campisi J. Cellular senescence and lung function during aging. Yin and Yang. Annals of the American Thoracic Society. 2016;13:S402-S406

[27] Jeon OH, David N, Campisi J, Elisseeff JH. Senescent cells and osteoarthritis: A painful connection. The Journal of Clinical Investigation. 2018;128:1229-1237

[28] Pinti M, Appay V, Campisi J, Frasca D, Fülöp T, Sauce D, et al. Aging of the immune system—Focus on inflammation and vaccination. European Journal of Immunology. 2016;46:2286-2301

[29] Nebel A, Bosch TCG. Evolution of human longevity: Lessons from Hydra. Aging (Albany NY). 2012;4:730-731

[30] Petralia RS, Mattson MP, Yao PJ. Aging and longevity in the simplest animals and the quest for immortality. Ageing Research Reviews. 2014;0:66-82

[31] Boehm A-M, Khalturin K, Erxleben FA, Hemmrich G, Klostermeier UC, Lopez-Quintero JA, et al. FoxO is a critical regulator of stem cell maintenance in immortal Hydra. Proceedings of the National Academy of Sciences. 2012;109:19697-19702. Ann Neurosci. 2013;20:17

[32] Mouton S, Grudniewska M, Glazenburg L, Guryev V, Berezikov E. Resilience to aging in the regeneration-capable flatworm Macrostomum lignano. Aging Cell. 2018;17:e12739

[33] Sturm Á, Perczel A, Ivics Z, Vellai T. The Piwi-piRNA pathway: Road to immortality. Aging Cell. 2017;16:906-911

[34] Kleiber M. Body size and metabolism. Hilgardia. 1932;6:315-353

[35] Kleiber M. Body size and metabolic rate. Physiological Reviews. 1947;27:511-541

[36] Packard GC, Birchard GF. Traditional allometric analysis fails to provide a valid predictive model for mammalian metabolic rates. The Journal of Experimental Biology. 2008;211:3581-3587

[37] Shestopaloff YK. Metabolic allometric scaling model: Combining cellular transportation and heat dissipation constraints. The Journal of Experimental Biology. 2016;219:2481-2489

[38] Donahoo WT, Levine JA, Melanson EL. Variability in energy expenditure and its components. Current Opinion in Clinical Nutrition and Metabolic Care. 2004;7:599-605
[39] Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ, et al. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. Nature. 2007;450:712-716

[40] Pirola L, Fröjdö S. Resveratrol: One molecule, many targets. IUBMB Life. 2008;60:323-332

[41] Marin TL, Gongol B, Martin M, King SJ, Smith L, Johnson DA, et al. Identification of AMP-activated protein kinase targets by a consensus sequence search of the proteome. BMC Systems Biology [Internet]. 2015;9:13. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4357066/

[42] Anderson RM, Barger JL, Edwards MG, Braun KH, O'Connor CE, Prolla TA, et al. Dynamic regulation of PGC-1α localization and turnover implicates mitochondrial adaptation in calorie restriction and the stress response. Aging Cell. 2008;7:101-111

[43] Handschin C. Caloric restriction and exercise “mimetics”: Ready for prime time? Pharmacological Research. 2016;103:158-166

[44] López-Lluch G, Navas P. Calorie restriction as an intervention in ageing. The Journal of Physiology. 2016;594:2043-2060

[45] Kim J, You Y. Regulation of organelle function by metformin. IUBMB Life. 2017;69:459-469

[46] Kim J, Lee H-Y, Ahn J, Hyun M, Lee I, Min K-J, et al. NHX-5, an Endosomal Na+/H+ exchanger, is associated with metformin action. The Journal of Biological Chemistry. 2016;291:18591-18599

[47] Wu L, Zhou B, Oshiro-Rapley N, Li M, Paulo JA, Webster CM, et al. An ancient, unified mechanism for metformin growth inhibition in C. elegans and cancer. Cell. 2016;167:1705-1718.e13

[48] Civitarese AE, Carling S, Heilbronn LK, Hulver MH, Ukropcova B, Deutsch WA, et al. Calorie restriction increases muscle mitochondrial biogenesis in healthy humans. PLOS Medicine [Internet]. 2007;4:e76. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1808482/

[49] Redman LM, Smith SR, Burton JH, Martin CK, Il'yasova D, Ravussin E. Metabolic slowing and reduced oxidative damage with sustained caloric restriction support the rate of living and oxidative damage theories of aging. Cell Metabolism [Internet]. 2018. Available from: http://www.sciencedirect.com/science/article/pii/S155041311830130X

[50] Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell. 2012;149:274-293

[51] Groenewoud MJ, FJT Z. Rheb and mammalian target of rapamycin in mitochondrial homoeostasis. Open Biology [Internet]. 2013;3:130185. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3877845/

[52] Ruetenik A, Barrientos A. Dietary restriction, mitochondrial function and aging: From yeast to humans. Biochimica et Biophysica Acta. 2015;1847:1434-1447
[53] Schmidt M, Kennedy BK. Aging: One thing leads to another. Current Biology. 2012;22:R1048-R1051

[54] Longo VD, Shadel GS, Kaeberlein M, Kennedy B. Replicative and chronological aging in Saccharomyces cerevisiae. Cell Metabolism. 2012;16:18-31

[55] Goldberg AA, Bourque SD, Kyryakov P, Gregg C, Boukh-Viner T, Beach A, et al. Effect of calorie restriction on the metabolic history of chronologically aging yeast. Experimental Gerontology. 2009;44:555-571

[56] Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017;60:1577-1585

[57] Mallik R, Chowdhury TA. Metformin in cancer. Diabetes Research and Clinical Practice [Internet]. 26 May 2018. pii: S0168-8227(17)31410-9. DOI: 10.1016/j.diabres.2018.05.023. Available from: https://www.sciencedirect.com/science/article/pii/S0168822717314109

[58] Xin W, Fang L, Fang Q, Zheng X, Huang P. Effects of metformin on survival outcomes of pancreatic cancer patients with diabetes: A meta-analysis. Molecular and Clinical Oncology. 2018;8:483-488

[59] Adak T, Samadi A, Ünal AZ, Sabuncuoğlu S. A reappraisal on metformin. Regulatory Toxicology and Pharmacology. 2018;92:324-332

[60] Campos T, Ziehe J, Fuentes-Villalobos F, Riquelme O, Peña D, Troncoso R, et al. Rapamycin requires AMPK activity and p27 expression for promoting autophagy-dependent Tsc2-null cell survival. Biochimica et Biophysica Acta (BBA)—Molecular Cell Research. 2016;1863:1200-1207

[61] Gu X, Cai Z, Cai M, Liu K, Liu D, Zhang Q, et al. AMPK/SIRT1/p38 MAPK signaling pathway regulates alcohol-induced neurodegeneration by resveratrol. Molecular Medicine Reports. 2018;17:5402-5408

[62] Alayev A, Sun Y, Snyder RB, Berger SM, Yu JJ, Holz MK. Resveratrol prevents rapamycin-induced upregulation of autophagy and selectively induces apoptosis in TSC2-deficient cells. Cell Cycle. 2014;13:371-382

[63] Auger C, Sivayoganathan T, Abdullahi A, Parousis A, Pang BW, Jeschke MG. Metformin adapts its cellular effects to bioenergetic status in a model of metabolic dysfunction. Scientific Reports [Internet]. 2018;8:5646. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5884829/

[64] Brynildsen JK, Lee BG, Perron IJ, Jin S, Kim SF, Blendy JA. Activation of AMPK by metformin improves withdrawal signs precipitated by nicotine withdrawal. Proceedings of the National Academy of Sciences of the United States of America. 2 April, 2018. 201707047. Published ahead of print 2 April, 2018. https://doi.org/10.1073/pnas.1707047115

[65] Barger JL, Kayo T, Vann JM, Arias EB, Wang J, Hacker TA, et al. A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. PLoS One [Internet]. 2008;3:e2264. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2386967/
[66] Pearson KJ, Baur JA, Lewis KN, Peshkin L, Price NL, Labinskyy N, et al. Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending lifespan. Cell Metabolism. 2008;8:157-168

[67] Holloszy JO, Smith EK, Vining M, Adams S. Effect of voluntary exercise on longevity of rats. Journal of Applied Physiology. 1985;59:826-831

[68] Agarwal B, Baur JA. Resveratrol and life extension. Annals of the New York Academy of Sciences. 2011;1215:138-143

[69] Arbor S. Targeting amyloid precursor protein shuttling and processing—Long before amyloid beta formation. Neural Regeneration Research. 2017;12:207-209

[70] Xie Q, Liang Y, Yang M, Yang Y, Cen X, Yin G. DEPTOR-mTOR signaling is critical for lipid metabolism and inflammation homeostasis of lymphocytes in human PBMC culture [internet]. Journal of Immunology Research. 2017. Available from: https://www.hindawi.com/journals/jir/2017/5252840/

[71] Gasmii M, Sellami M, Denham J, Padulo J, Kuvacic G, Selmi W, et al. Time-restricted feeding influences immune responses without compromising muscle performance in older men. Nutrition. 2018;51-52:29-37

[72] Lamouille S, Derynck R. Cell size and invasion in TGF-β–induced epithelial to mesenchymal transition is regulated by activation of the mTOR pathway. The Journal of Cell Biology. 2007;178:437-451

[73] Chong ZZ, Shang YC, Wang S, Maiiese K. Shedding new light on neurodegenerative diseases through the mammalian target of rapamycin. Progress in Neurobiology. 2012;99:128-148

[74] Soufi FG, Vardyani M, Sheervalilou R, Mohammadi M, Somi MH. Long-term treatment with resveratrol attenuates oxidative stress pro-inflammatory mediators and apoptosis in streptozotocin-nicotinamide-induced diabetic rats. General Physiology and Biophysics. 2012;31:431-438

[75] Palsamy P, Subramanian S. Ameliorative potential of resveratrol on proinflammatory cytokines, hyperglycemia mediated oxidative stress, and pancreatic beta-cell dysfunction in streptozotocin-nicotinamide-induced diabetic rats. Journal of Cellular Physiology. 2010;224:423-432

[76] Maiiese K, editor. Molecules to medicine with mTOR. In: Molecules to Medicine with mTOR [Internet]. Boston: Academic Press; 2016. pp. i-iii. Available from: https://www.sciencedirect.com/science/article/pii/B9780128027332000281

[77] Serena D, Mette S, Francesco DR, Giuseppina R, Kaare C, Lene C, et al. The genetic component of human longevity: New insights from the analysis of pathway-based SNP-SNP interactions. Aging Cell. 2018;0:e12755

[78] Park HS, Lim JH, Kim MY, Kim Y, Hong YA, Choi SR, et al. Erratum to: Resveratrol increases AdipoR1 and AdipoR2 expression in type 2 diabetic nephropathy. Journal of
Alayev A, Berger SM, Holz MK. Resveratrol as a novel treatment for diseases with mTOR pathway hyperactivation. Annals of the New York Academy of Sciences. 2015;1348:116-123

Alayev A, Berger SM, Kramer MY, Schwartz NS, Holz MK. The combination of rapamycin and resveratrol blocks autophagy and induces apoptosis in breast cancer cells. Journal of Cellular Biochemistry. 2015;116:450-457

Narendra DP, Youle RJ. Targeting mitochondrial dysfunction: Role for PINK1 and Parkin in mitochondrial quality control. Antioxidants & Redox Signaling. 2011;14:1929-1938

Manfredi G, Xu Z. Mitochondrial dysfunction and its role in motor neuron degeneration in ALS. Mitochondrion. 2005;5:77-87

Song W, Chen J, Pettrilli A, Liot G, Klinglmayr E, Zhou Y, et al. Mutant Huntingtin binds the mitochondrial fission Gtpase Drp1 and increases its enzymatic activity. Nature Medicine. 2011;17:377-382

Yao J, Irwin RW, Zhao L, Nilsen J, Hamilton RT, Brinton RD. Mitochondrial bioenergetic deficit precedes Alzheimer’s pathology in female mouse model of Alzheimer’s disease. Proceedings of the National Academy of Sciences of the United States of America. 2009;106:14670-14675

Jauslin ML, Meier T, Smith RAJ, Murphy MP. Mitochondria-targeted antioxidants protect Friedreich Ataxia fibroblasts from endogenous oxidative stress more effectively than untargeted antioxidants. The FASEB Journal. 2003;17:1972-1974

Murphy MP, Smith RA. Drug delivery to mitochondria: The key to mitochondrial medicine. Advanced Drug Delivery Reviews. 2000;41:235-250

Beau I, Mehrpour M, Codogno P. Autophagosomes and human diseases. The International Journal of Biochemistry & Cell Biology. 2011;43:460-464

Rao VK, Carlson EA, Yan SS. Mitochondrial permeability transition pore is a potential drug target for neurodegeneration. Biochimica et Biophysica Acta. 2014;1842:1267-1272

Bernardi P, Di Lisa F. The mitochondrial permeability transition pore: Molecular nature and role as a target in cardioprotection. Journal of Molecular and Cellular Cardiology. 2015;78:100-106

Oster AM, Thomas B, Terman D, Fall CP. The low conductance mitochondrial permeability transition pore confers excitability and CICR wave propagation in a computational model. Journal of Theoretical Biology. 2011;273:216-231

Wacquier B, Combettes L, Van Nhieu GT, Dupont G. Interplay between intracellular Ca\textsuperscript{2+} oscillations and Ca\textsuperscript{2+}-stimulated mitochondrial metabolism. Scientific Reports [Internet]. 2016;6:19316. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4725975/
[92] Wang K. Autophagy and apoptosis in liver injury. Cell Cycle. 2015;14:1631-1642

[93] Zanardi E, Verzoni E, Grassi P, Necchi A, Giannatempo P, Raggi D, et al. Clinical experience with temsirolimus in the treatment of advanced renal cell carcinoma. Therapeutic Advances in Urology. Jun 2015;7(3):152-161. DOI: 10.1177/1756287215574457. PMCID: PMC4485412. PMID: 26161146

[94] Li Y, Pan A, Wang DD, Liu X, Dhana K, Franco OH, et al. Impact of healthy lifestyle factors on life expectancies in the US population. Circulation. 2018 CIRCULATION.117.032047

[95] Dansinger ML, Gleason J, Griffith JL, Selker HP, Schaefer EJ. Comparison of the atkins, ornish, weight watchers, and zone diets for weight loss and heart disease risk reduction: A randomized trial. Journal of the American Medical Association. 2005;293:43-53

[96] Alhamdan BA, Garcia-Alvarez A, Alzahrnai AH, Karanxha J, Stretchberry DR, Contrera KJ, et al. Alternate-day versus daily energy restriction diets: Which is more effective for weight loss? A systematic review and meta-analysis. Obesity Science & Practice. 2016;2:293-302

[97] Betts JA, Richardson JD, Chowdhury EA, Holman GD, Tsintzas K, Thompson D. The causal role of breakfast in energy balance and health: A randomized controlled trial in lean adults. The American Journal of Clinical Nutrition. Aug 2014;100(2):539-547. DOI: 10.3945/ajcn.114.083402. Epub 2014 Jun 4

[98] Biliński T, Paszkiewicz T, Zadrag-Tecza R. Energy excess is the main cause of accelerated aging of mammals. Oncotarget. 2015;6:12909-12919

[99] Campos SE, Avelar-Rivas JA, Garay E, Juárez-Reyes A, DeLuna A. Genomewide mechanisms of chronological longevity by dietary restriction in budding yeast. Aging Cell. 2018:e12749

[100] Cava E, Fontana L. Will calorie restriction work in humans? Aging (Albany NY). 2013;5:507-514

[101] Colman RJ, Beasley TM, Kemnitz JW, Johnson SC, Weindruch R, Anderson RM. Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. Nature Communications. 2014;5:3557

[102] Fontana L, Mitchell SE, Wang B, Tosti V, van Vliet T, Veronese N, et al. The effects of graded caloric restriction: XII. Comparison of mouse to human impact on cellular senescence in the colon. Aging Cell. 2018:e12746

[103] Gillespie ZE, Pickering J, Eskiw CH. Better living through chemistry: Caloric restriction (CR) and CR mimetics alter genome function to promote increased health and lifespan. Frontiers in Genetics [Internet]. 2016;7:142. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4988992/
[104] Godar RJ, Ma X, Liu H, Murphy JT, Weinheimer CJ, Kovacs A, et al. Repetitive stimulation of autophagy-lysosome machinery by intermittent fasting preconditions the myocardium to ischemia-reperfusion injury. Autophagy. 2015;11:1537-1560

[105] Longo VD, Antebi A, Bartke A, Barzilai N, Brown-Borg HM, Caruso C, et al. Interventions to slow aging in humans: Are we ready? Aging Cell. 2015;14:497-510

[106] Nogueira LM, Lavigne JA, Chandramouli GVR, Lui H, Barrett JC, Hursting SD. Dose-dependent effects of calorie restriction on gene expression, metabolism, and tumor progression are partially mediated by insulin-like growth factor-1. Cancer Medicine. 2012;1:275-288

[107] Solon-Biet SM, Mitchell SJ, Coogan SCP, Cogger VC, Gokarn R, McMahon AC, et al. Dietary protein to carbohydrate ratio and caloric restriction: Comparing metabolic outcomes in mice. Cell Reports. 2015;11:1529-1534

[108] Gokarn R, Solon-Biet S, Youngson NA, Wahl D, Cogger VC, McMahon AC, et al. The relationship between dietary macronutrients and hepatic telomere length in aging mice. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences. 2018;73:446-449
