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# Intermittent Fasting (IF) Promotes Longevity through Alterations of the Mammalian Target of Rapamycin (mTOR) and the Epigenome

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*By* Tayt Boeckholt

**Table of Contents**

**Abstract**

Many studies with a range of subjects from *Nematodes* to *Homo sapiens* have found intermittent fasting (IF) to significantly improve the cardiometabolic health of individuals, but how IF promotes longevity through epigenetic modulations remains a sparse understanding throughout the literature. The process of aging may be characterized by a loss of cellular identity sprouted from a disrupted epigenome rich with information for a cell, while also losing the ability to recycle ineffective cellular components. The nutrient-sensing kinase Mammalian Target of Rapamycin (mTOR) is disrupted during bouts of fasting which allows for the recycling of cellular components through increased autophagy. Furthermore, the Sirtuin (SIRT) family of $NAD^+$-dependent deacetylases is a prominent transcriptional repressor via histone deacetylation and increased DNA-methyltransferase (DNMT) activity upon deacetylation of the enzyme. Although longitudinal studies spanning many years will be needed to provide definitive evidence for the long-term effects of IF. To date, the most profound pro-longevity evidence for IF is a significant reduction in the rate of biological aging determined from global genomic DNA methylation, of which is a more accurate measure of age in comparison to chronological age. Aside from specific cases in which IF may be detrimental to health, the practice of IF may add years onto an individual's life, and more importantly, healthy years contributing to a better quality of life.

## Introduction

The proper practice of intermittent fasting (IF) has the potential to influence our body through physiological and genomic modifications. Until recent years, research and discussions regarding IF have been lacking. With the increase in studies underlying the scientific basis to support the practice of IF, the scientific communities' support for the practice has grown as well. Most importantly, IF can slow the process of aging and therefore extend lifespan (Belsky, Huffman, Pieper, Shalev, & Kraus, 2017; Catterson et al., 2018; Liang et al., 2018; Maegawa et al., 2017; Petkovich et al., 2017; Thompson et al., 2018; Wang et al., 2017; Weir et al., 2017). But it is important to first understand the pre-historic relationship between IF and humans.

Throughout the history of time, our ancestors survived on relatively small amounts of food, or periodic bouts of eating large portions with gaps of time in between each bout ranging from hours to days (Eaton & Konner, 1985; Mattson et al., 2014; Strohle, Hahn, & Sebastian, 2010). Between long periods of no food consumption, which we now refer to as fasting, our ancestors had to function and thrive in order to find the energy to hunt, forage for food and collect resources. As humans have evolved through thousands of generations, our bodies now function at optimal capacity for performance when we are fasting, as we then achieve maximal cognitive functioning and peak awareness to perform better in vital situations (Mattson, 2015). If a human could not perform well cognitively and physically when enduring periods of food deprivation, such as a 12 to 24-hour our period of fasting, *homo sapiens* may have been eliminated through natural selection such as the Neanderthals were (Banks et al., 2008; Hortolà & Martínez-Navarro, 2013). Of course, the periods of food deprivation may have ranged from a few hours to even days at a time for our ancestors, depending on the circumstances faced. Fortunate as humans are today with unlimited resources and a consistent access to food, we may

capture the opportunity to reap the benefits associated with fasting through consuming proper nutrition in periods of eating and adhering to some type of fasting schedule.

To understand how IF mechanistically slows the aging process to promote a longer and healthier life, it is important to know why and how we age. From a broad scope of factors that drive aging, this paper will focus on the environment and what we can control in respect to how we age. On a side note, purely genetic influence on aging is less significant considering studies on twins found that at the most, 27% of the variation in life span among individuals is due to genetics (McGue, Vaupel, Holm, & Harvald, 1993 1993; Skytthe et al., 2003 2012). At the molecular level, the factors of focus for this paper can be summed together in an overarching idea: the loss of information. The information lost is responsible for telling our cells how to maintain a younger and healthier state of homeostasis, and without this information, our cells no longer function as well as when they were younger, eventually leading to cell death. Common factors throughout the literature that underlie aging are mutations to our DNA, small non-coding RNAs, shortening of our telomeres, and epigenetic alterations (Bayersdorf & Schumacher, 2019; Christensen et al., 2009; Kane & Sinclair, 2019).

This paper will discuss the outcomes associated attributed to fasting and in relation to IF. While consuming only water or coffee with zero calories, IF can include any of the following patters: fasting for 16 to 20 hours of a 24-hour day for 1-3 days a week, fasting for 24-hours two times a week, or alternate-day fasting (ADF) in which an individual restricts energy intake by 75%, every other day (Arnason, Bowen, & Mansell, 2016; Overland et al., 2018; K. A. Varady, 2011; Krista A. Varady & Hellerstein, 2007). There are many other subcategories of IF, and the understanding as to which type of IF is necessarily the best is yet to be determined. No specific IF intervention will be of focus, but it is important to know the most common characteristic of all

types of IF is decreased energy intake and bouts of no caloric intake at all, which allows the body to utilize other mechanisms to provide energy for us to survive. While many studies utilize caloric restriction (20-50% reduction in daily energy intake) as the mechanism for energy intake reduction, intermittent fasting has shown to provide equally effective outcomes for health measures such as weight loss and cardiovascular protection (K. A. Varady, 2011; Krista A. Varady & Hellerstein, 2007).

Although telomeres and aging often pair together as a correlation, an in-depth discussion of telomere length and aging will be excluded. There is evidence supporting telomere length and aging, on the contrary, there exist inexplicable findings associated between telomere length and aging. For example, a study of 1000 70-year-olds found males to have significantly longer telomeres than females and concluded telomere length to be of "little evidence" as a biomarker of normal aging (Harris, Martin-Ruiz, von Zglinicki, Starr, & Deary, 2012). Another study found greater mitochondrial DNA in a cell was found to be positively correlated with longer telomeres, except for individuals of the 90-100-year-old cohort, who had more mitochondrial DNA but shorter telomeres than the 60-89-year-old cohort (Zole, Zadinane, Pliss, & Ranka, 2018). Lastly, an analysis of telomere length and aging-related outcomes (n=261,000) found that at most "telomere lengthening may offer little gain" with respect to an individual's health as they age (Kuo, Pilling, Kuchel, Ferrucci, & Melzer, 2019).

To narrow in on information from the broad range of data pertaining to aging, two aspects of aging will be of focus. In respect to molecular pathways relating to metabolism, Mammalian Target of Rapamycin (mTOR) serves as a well understood kinase that responds to or is turned off, in response to plethora of nutrients or fasting, respectively (Hay & Sonenberg, 2004; D.-H. Kim et al., 2002; Lipton & Sahin, 2014). The second piece of information pertains

to the epigenome. The biological age or "epigenetic age" of an individual has in recent years come to the forefront of aging. The biological age of an individual is found by determining DNA methylation values at known CpG dinucleotides, of which undergo aberrant patterns of hypomethylation and hypermethylation as we age (Horvath, 2013). Even with the correction for factors contributing to increased fatality of an older individual, the biological age can predict all-cause mortality (Chen et al., 2016; Marioni et al., 2015). It is fair to say the biological age of an individual is a more predictable and accurate representation of one's "true" age in comparison to the chronological age of an individual. Lastly, an analysis of how the Sirtuin (SIRT) family of histone deacetylases contributes a vital role in the regulation of the epigenome will be reviewed.

## Intermittent Fasting Pro-Longevity Effects on Molecular Pathways and the Epigenome

### 1. Mammalian Target of Rapamycin (mTOR)

The mTOR protein kinase, encoded by a single mTOR gene, is constituted of two separate complexes termed mTORC1 and mTORC2. The similar molecular components of mTORC1 and mTORC2 include mLST8 (mammalian lethal with SEC13 protein 8) and DEP (DEP domain-containing mTOR-interacting protein). The two complexes differ in that mTORC1 contain regulatory-associated protein of mTOR (RAPTOR) and proline-rich AKT1 substrate 1 (PRAS40) while mTORC2 contains RICTOR (rapamycin-insensitive companion of mTOR), mSIN1 (mammalian stress-activated protein kinase interacting protein 1), and Protor-1/2 (Saxton & Sabatini, 2017). Overall, mTOR functions as a serine/threonine kinase in response to insulin-like growth factors, glucose, and amino acids, to function in an anabolic role through regulating different processes (Hay & Sonenberg, 2004; D.-H. Kim et al., 2002; Lipton & Sahin, 2014). The

usefulness of the anabolism promoting capabilities of mTOR is essential in development and growth, especially at a young age. But as we age, the growth-promoting capabilities of mTOR become less essential and eventually lead to negative outcomes when prolonged or frequent activation occurs. The confounding role of mTOR is described as antagonistic pleiotropic, with the specific genes activating early in life being beneficial to an organism and becoming detrimental later in life when hyperactive (Schmeisser & Parker, 2019). Aging associated cellular processes regulated by mTOR include cell proliferation, autophagy leading to disrupted proteostasis, protein synthesis, mitochondrial dysfunction and other metabolic growth-related pathways (Gonskikh & Polacek, 2017; Koga, Kaushik, & Cuervo, 2011; Papadopoli et al., 2019)(figure 2).

There are three general mechanisms the activation of mTOR, which may occur independently of one another or simultaneously to one another. In response to nutrient uptake, the liver will secrete insulin-like growth factors into the blood, which activates insulin-like growth factor-1 receptor when bound with insulin-like growth factor-1 (IFG-1) (Yin et al., 2016 2016). Ligand-bound IGF-1 receptor allows the tyrosine kinase activity of the receptor to initiate a signaling cascade characterized by protein kinase B (Akt) phosphorylating tuberous sclerosis complex 2 (TSC2). Phosphorylated TSC2 breaks from TSC1, which inhibits the GTPase-activating function of the TSC1/2 complex, and the Ras homologue enriched in brain (RHEB) protein is therefore never inhibited, allowing continual mTORC1 activity (Kwiatkowski & Manning, 2005). Since TSC1/2 complex is one of the central components that regulate mTORC1 activity, other pathways converge onto the complex in addition to the IGF-1 receptor signaling. Depending on the ratio of ADP: AMP, a signature of a cell's energy status, 5' AMP-activated Protein Kinase (AMPK) will increase in activity as the concentration of AMP increases.

Activated AMPK phosphorylates TSC2 but activates the GAP activity of the complex rather than inhibit. Active TSC2 hydrolyzes the RHEB-GTP into GDP, inactivating RHEB, which inhibits mTORC1 (Mihaylova & Shaw, 2011). Also, an increase of intracellular amino acid levels activates a Ras GTPase that will go onto localize mTORC1 to the lysosomal membrane to initiate protein synthesis (Bar-Peled, Schweitzer, Zoncu, & Sabatini, 2012).

Upon activation via GTP-bound RHEB, mTORC1 kinase activity phosphorylates the protein 4E-BP1, a translation inhibitor, and p70-S6 Kinase 1 (S6K1) (Figure 1), which will begin the steps to start protein synthesis and therefore upregulate protein translation (Roux & Topisirovic, 2018). Further facilitation of pro-protein synthesis is favored by the mTORC1 induced inhibition of autophagy. mTORC1 phosphorylates autophagy-related protein 13 (Atg 13), preventing the protein from complexing with other Atg related proteins, to contribute to the Unc-51-like autophagy-activating kinase (ULK1), effectively inhibiting autophagy (Alers, Loffler, Wesselborg, & Stork, 2012; J. Kim, Kundu, Viollet, & Guan, 2011 2011). This combination of factors creating a pro-protein synthesis state within the cell leaves minuscule opportunities for the cell to perform functions like the unfolded protein response (UPR) and impedes on cellular restoration functions

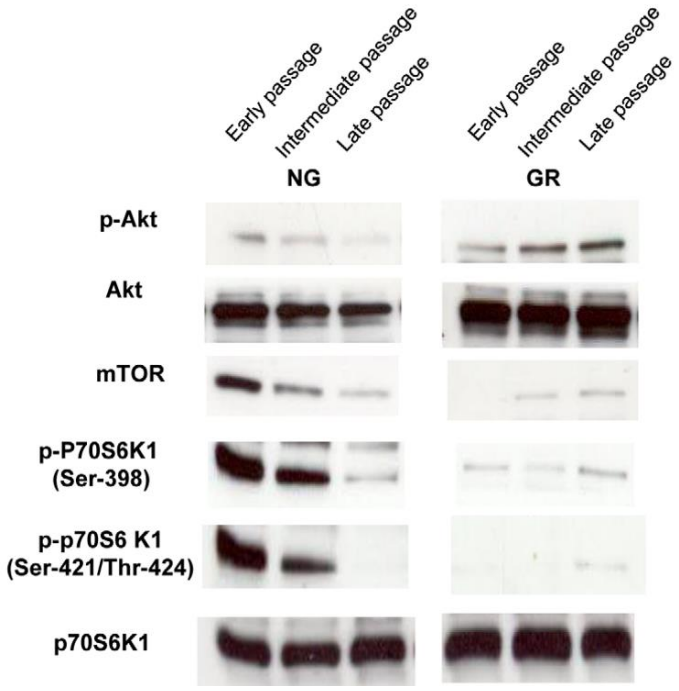


**Figure 1.** Protein extracts taken at various intervals of proliferation from normal Human Fibroblasts (WI-38) fed either normal glucose (NG) or glucose restricted (GR) medium. Each protein was probed with the corresponding antibody on a nitrocellulose membrane. (Li & Tollefsbol, 2011)

like tagging denatured proteins with ubiquitin for degradation. Johnson, S. C., et al. 2013, demonstrated in *C. elegans*, *Drosophila*, yeast, and mice, that when rates of protein translation decrease, life span inversely increases and vice versa. This is reasonable because when autophagy is not inhibited, the cell will be able to recycle misfolded or dysfunctional proteins, leading to a clearer and improved signal cascades within the cell and a simple mechanism of which replaces worn-out components of the cell.

The background knowledge, literature and molecular components involved with mTOR signaling support intermittent fasting, which is often accompanied by a decrease of caloric intake, as a means of decreasing mTOR activity to improve longevity (Figure 1). Longevity has shown to be greatly improved when practicing dietary restriction (DR) and done so by promoting the regulation that occurs between TORC1, AMPK activation, insulin and insulin-like growth factor signaling axis (Hou et al., 2016). While mTOR functions in metabolism, inhibition of autophagy, and growth, it also regulates other cellular processes. In mice, the inhibition of mTORC1 activation promoted the expression of DNA repair proteins, N-myc downstream-regulated gene 1 (NDRG1) and O-6-methylguanine-DNA methyltransferase (MGMT). Both proteins directly work to undo DNA damage. Although the mechanism of NDRG1 is not clear, MGMT is needed to maintain a stable genome for DNA replication and transcription by removing erroneous methyl groups from guanines in the genome (Dominick, Bowman, Li, Miller, & Garcia, 2017).

As we age, our cells slowly erode at their ability to keep levels of ATP high, high levels of oxidized nicotinamide adenine dinucleotide ($NAD^+$) activate sirtuins, preventing the accumulation of reactive oxygen species (ROS) that leak into the other compartments of the cell. These deficiencies are resultants of deteriorating mitochondrial function such as the decreased

ability to induce mitophagy and mitochondrial biogenesis. Both ATP and $NAD^+$ are key molecules in informing the cell of nutrient or energy status, via interactions with AMPK, and sirtuin proteins. mTOR activity directly regulates mitophagy and therefore regulates mitochondrial biogenesis as well (Bartolome et al., 2017; Palacios et al., 2009). As will be discussed later, the other nutrient-sensing pathway proteins like AMPK and sirtuins can work to halt mTOR inhibition of renewing our mitochondria to a higher level of function.

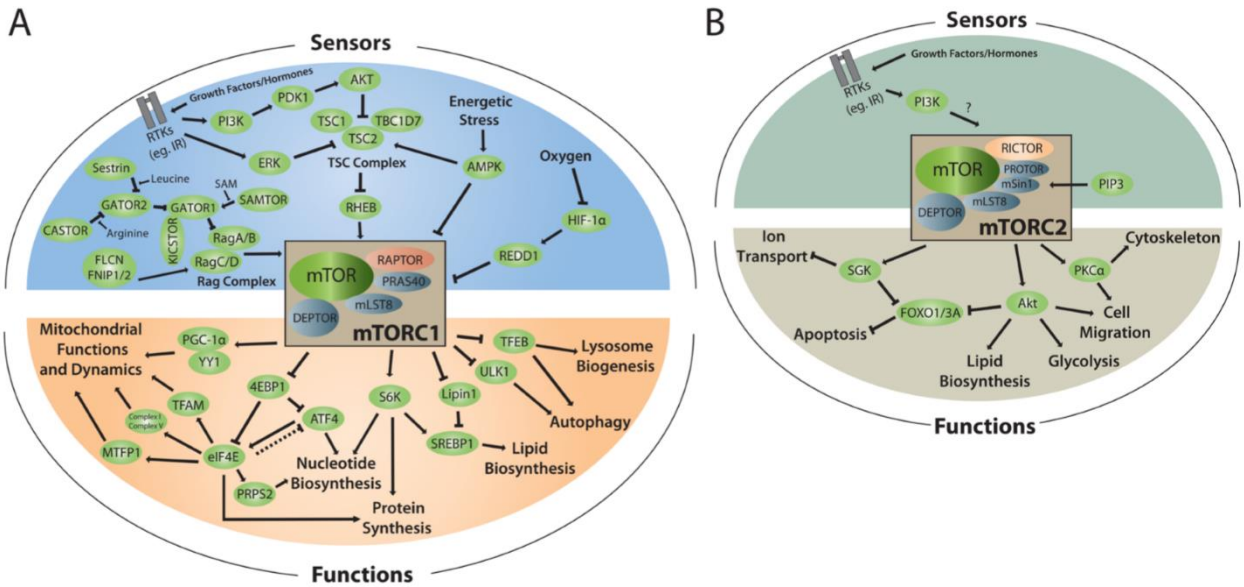


**Figure 2.** Adopted directly from (Papadopoli et al., 2019), a summary of events for the role of both mTORC1 (A) and mTORC2 (B).

## 2. Sirtuins

Sirtuins (SIRTs) are a family of $NAD^+$-dependent proteins that most often serve as protein deacetylases within the cell (Anderson, Green, Huynh, Wagner, & Hirschey, 2014). First discovered in yeast, eukaryotic cells are now known to contain seven different sirtuin proteins. SIRT1, SIRT6, and SIRT7 are of the most important focus in this paper, being the only sirtuin proteins located in the nucleus (Scher, Vaquero, & Reinberg, 2007). Sirtuins require $NAD^+$ as a

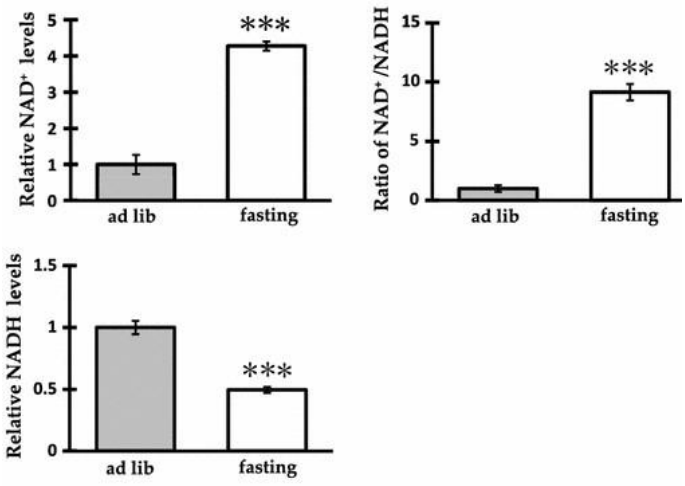


**Figure 2.** Mice fed under ad libitum (ad lib) and mice after a period of fasting for 24 hours, with water as the only intake, were compared for their NADH and $NAD^+$ levels. (Hayashida et al., 2010)

co-substrate in the reaction to remove acetyl groups from a protein and in the reaction to remove ribosyl groups. $NAD^+$ is hydrolyzed, removing the nicotinamide group, and absorbing the acetyl group, to ultimately form both O-acyl-ADP-ribose (byproduct) and nicotinamide (Zhu, Su, & Lin, 2013). In-vitro analysis has determined that sirtuin enzyme activity is determined not only by $NAD^+$ levels but by NADH levels as well. When NADH concentrations reach 10 mM and above, NADH will begin to compete for the active site of sirtuin, inhibiting the enzyme activity of sirtuin and thereby working as a competitive inhibitor. Although to be clear, since Sirtuins roughly have a 1000-fold higher affinity for $NAD^+$ than NADH, the concentration of NADH must reach far beyond the concentration of $NAD^+$ (Schmidt, Smith, Jackson, & Denu, 2004). Sirtuin levels fluctuate within a cell-based on metabolic states but the approximated concentration of Sirtuins in the nucleus is from 10 μm to 100 μm, while the concentration within the mitochondria is around 230 μm (Yang et al., 2007).

Sirtuins serve at the forefront of interaction with a cell's energy levels. $NAD^+$ levels will be elevated in periods of low energy and nutrients, while NADH will be favored in periods of high energy and nutrients because NADH is an abundant electron carrier in our cells. NAD+ levels are significantly increased in mice after fasting in comparison to normal fed mice. What's

more, NADH levels decrease significantly in the same mice, creating a significant difference in the $NAD^+$ to NADH ratio in the fasting mice compared to the regular diet mice (Figure 2).

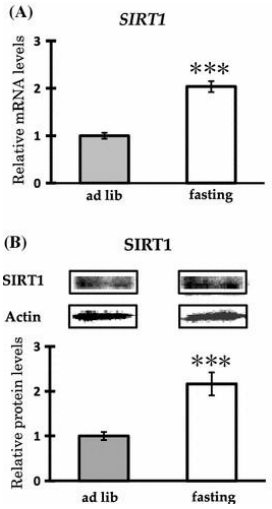


**Figure 3.** (A) mRNA levels from the hepatocytes of mice fed ad libitum (ad lib) and of mice that after 24 hours of fasting. (B) Western blot of total protein extracted from the mice hepatocytes. (Hayashida et al., 2010)

Since $NAD^+$ is an activating co-substrate for Sirtuins, the deacetylase activity of Sirtuins increases in proportion with the increase of NAD+ levels within a cell, and vice versa (Peek et al., 2013). Intermittent fasting, associated with lower energy availability, will then increase $NAD^+$ levels as well as increase the enzymatic activity of Sirtuins. More intriguing is the interconnected network of events regulating the expression of proteins for DNA stress responses, repair, and longevity. After fasting, mice hepatocytes have significantly increased mRNA levels of SIRT1 and significantly increased SIRT1 total protein levels as well (Figure 3). While fasting itself aside from the increased NAD+ levels is likely responsible for the increased SIRT1 expression and protein translation, its possible NAD+ works as a positive feedback to promote the increased gene expression and protein translation.

A deacetylase can remove an acetyl group from different protein products, for the case of Sirtuins, and specifically SIRT1, the protein target varies, ranging from histones, DNA, to cell cycle control proteins like p53. Histones are grouped as an octamer, with their positive charge attracting the negatively charged DNA to tight wrap around each histone, constituting a nucleosome. Nucleosomes wind our DNA together, regulating the condensation or loose conformation of chromatin as a result of modifications to the histones. An acetyl group carries a negative charge, the same charge of DNA. When an acetyl group is covalently added to a histone, the acetyl most often is bonded with the positively charged lysine tail. This interaction

rids the positively charged lysine from interacting favorably with the DNA, while the negatively charged acetyl group induces repulsion and therefore a looser conformation of DNA. Loosely associated chromatin is then more accessible to transcription factors that may promote gene expression. When the acetyl group is removed however, this repulsion ceases to exist, causing a more tightly associated wrapping of the histones and DNA due to the positive charge of lysine and negative charge of DNA. The removal of the acetyl group on lysine tails of histones is prominently under the control of Sirtuin proteins. The histone acetylation activity of sirtuins is directly increased in fasting subjects (Figure 4).

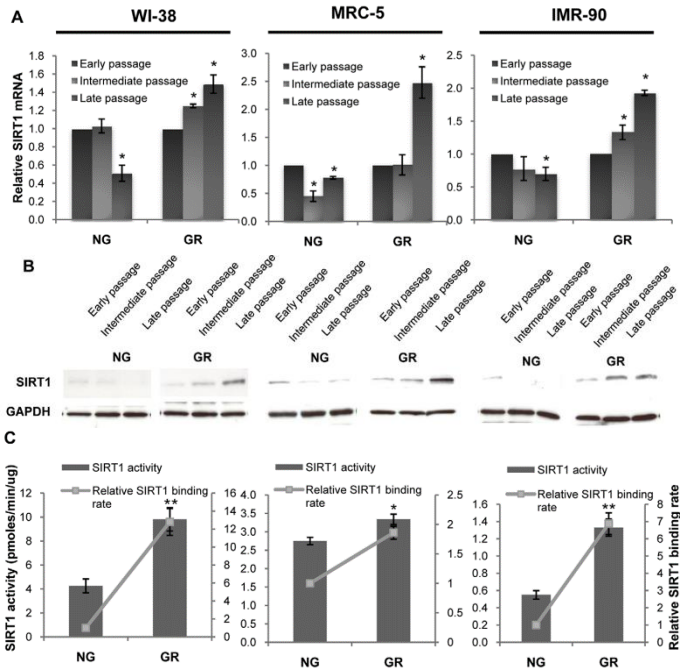


**Figure 4.** In-vitro demonstration of IF effects. Normal human fibroblasts (WI-38) and human fetal lung fibroblasts (MRC-5 and IMR-90) were fed either normal glucose (NG) or glucose restricted (GR) medium. mRNA extraction occurred at the intervals of early, intermediate, and late proliferation. (A) Analysis of SIRT1 mRNA extracted from the three fibroblasts via transcription-PCR. (B) Protein extract analysis of SIRT1 protein throughout cell proliferation stages, and with GAPDH as a control group. (C) HDAC activity of SIRT1 and the relationship to SIRT1 binding to p16 promoter. (Li & Tollefsbol, 2011)

Besides functioning as a DNA acetylase, SIRT1 repair double-stranded DNA breaks by homologous recombination and prevent nonhomologous end-joining, which is a more error-prone DNA repair mechanism (Uhl et al., 2010 2010). The ability of SIRT1 to promote homologous recombination makes this protein one of the most important proteins throughout our body in preserving the epigenetic structure of our chromatin and in preserving the integrity of our linear DNA genome. Homologous recombination suffices to repair DNA while preserving

the original nucleotide sequence. Other forms of DNA repair may lead to mutated sequences that do not match the original sequence. Of course, this may not always be a terrible issue, but in the chance that this unmatching sequence cannot base pair with the original strand of DNA, a bulge in the DNA will be created which throws off DNA replication machinery.

In a study performed by Jamshed, H., et al. (Jamshed et al.), a comparison was determined between two different fasting regimes. The group found early time-restricted feeding groups in contrast to regular fasting groups that underwent periods of no eating as they saw fit, had statistically significant increased gene expression of SIRT1 in the a.m., and increased expression in the p.m., although that was not shown to be statistically significant. If these groups were to be compared to individuals who undergo no specific or intentional fasting regime and at ad-lib, its only causal to suggest that the SIRT1 expression would be significantly higher at all times in a fasting cohort compared to the ad-lib cohort. A probable role could then be suggested that increased SIRT1 expression may increase longevity, exactly like was shown in animals (Mitchell et al., 2019 2019). The improved insulin sensitivity resulting from intermittent fasting may contribute as well to longevity through SIRT1. A study by Sun, C., et al. 2007 found SIRT1 to have a decreased gene expression in response to decreased insulin sensitivity, indicating the likely hood that improved insulin sensitivity promotes greater expression of SIRT1.

### 3. DNA Methylation

DNA methylation is a type of epigenetic modification which functions to silence a gene, while it also functions to allow recognition of the template or original strand in DNA replication and homologous recombination. From our years of childhood through middle-aged years, DNA

methylation is abundant and tightly regulated to contribute to an overall highly regulated genetic expression. Methylation most often occurs on cytosine residues of CpG dinucleotides located within CpG islands. Adding a methyl group to a cytosine forms a 5-methylcytosine. In addition to methylation of cytosine residues, adenine residues may be methylated as well, but often much less frequently.

As we age, we lose DNA methylation in an almost exact proportion to our chronological age (Gonzalo, 2010). DNA hypomethylation is most accounted for within highly repetitive genomic regions and genomic interspersed elements (Bollati et al., 2009; Zampieri et al., 2015). In addition to DNA hypomethylation, hypermethylation within gene promoters as we age contributes to the overall disruption of global DNA methylation patterns, causing an overall loss of the proper epigenetic information needed to maintain a healthy gene expression pattern (Bell

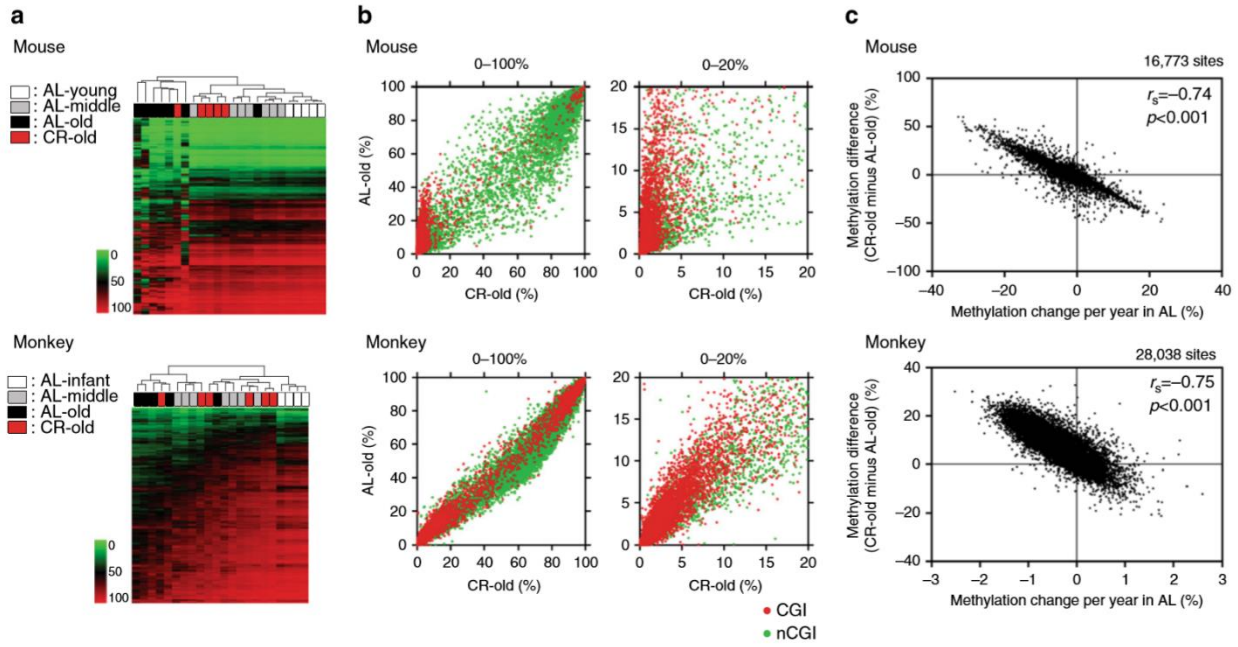


**Figure 5.** Methylation comparison of mice fed 40% CR diet from the age of 0.3 years old to the age of 2.7-3.2 years old (CR-old, n=12) and Rhesus monkeys fed a 30% CR diet from the age of 7-14 years old until 30 years old (CR-old, n=6, median age= 26 y) to the methylation of AL animals. (A) Green to red is the methylation values in percent across all genomic regions. (B) Full range (0-100%) and low range (0-20%) of DNA methylation levels at CpG sites. (C) Methylation difference between CR-old and AL-old at specific CpG sites (y-axis) compared to the percent methylation change per year in AL (x-axis).

et al., 2012; Christensen et al., 2009). The ability to characterize the "biological age" of an organism based on DNA methylation patterns is so effective that an "epigenetic clock" is the term now used to characterize the age of an individual based on these patterns (Horvath, 2013).

The alteration of or loss of DNA methylation patterns are not just coupled with aging, but likely give rise to accelerated aging. But the genetic information carried by the pattern of DNA methylation is different from the genetic information carried by DNA, which remains unalterable by our choice. On the other hand, it is possible to alter DNA methylation patterns favorably, to slow the aging process and the related onset of diseases. Caloric restriction remains at the forefront of feasible methods to retain and maintain the global genomic DNA methylation patterns.

A study by Maegawa, S., et al. 2017 utilizing Rhesus monkeys and mice as test subjects, demonstrated just how effective caloric restriction is for delaying DNA methylation drift. In both mice and monkeys, when the most highly methylated regions of the genome are preserved in the caloric restricted old groups compared to the ad libitum old groups (Fig. 5a). When fed ad libitum, there is a distinctive hypomethylation of the overall genome as both mice and monkey age (Fig 5a). The hypomethylation of non-CpG island genomic regions of ad libitum old in comparison to caloric restricted old (Fig. 5b) may occur at gene promoters such as a proto-oncogene, accelerating aging and the cell cycle altogether. And for every year that passes in an ad libitum subject, the difference in the percentage of methylation at identical genomic regions between ad libitum and caloric restricted subjects widens proportionally (Fig. 5c).

Maegawa, S., et al. 2017 further provided evidence for the credibility of caloric restriction to reduce the loss of global DNA methylation and to, therefore, slow the process of aging. In testing both mice and monkeys, calorically restricted old subjects have a statistically significant correlation with a lower predicted or "biological age" in comparison to their actual chronological age (Fig. 6). Unsurprisingly, the ad libitum old subjects had a predicted or "biological age" that was much more like their actual chronological age (Fig. 6). It is evident to conclude that caloric restriction will reduce the loss of DNA methylation patterns, this preventative practice will ensure a much more stable genome and telomere stability as well.

DNA methyltransferases are responsible for the addition of methyl groups to the 5' carbon of cytosine. There are three main types of DNA methyltransferases, but DNA methyltransferase 1 (DNMT1) is found in the highest amounts throughout the nucleus of a cell (Hermann, Gowher, & Jeltsch, 2004). DNMT1 is the most abundant DNA methyltransferase not by coincidence, but because DNMT1 prefers to localize to and interact with hemimethylated DNA often present at DNA replication forks and therefore also helps the cell to copy methylation patterns from the template strand to the daughter strand(Chuang et al., 1997; Egger et al.; Goll & Bestor, 2005). Aside from the preference of hemimethylated DNA, DNMT1

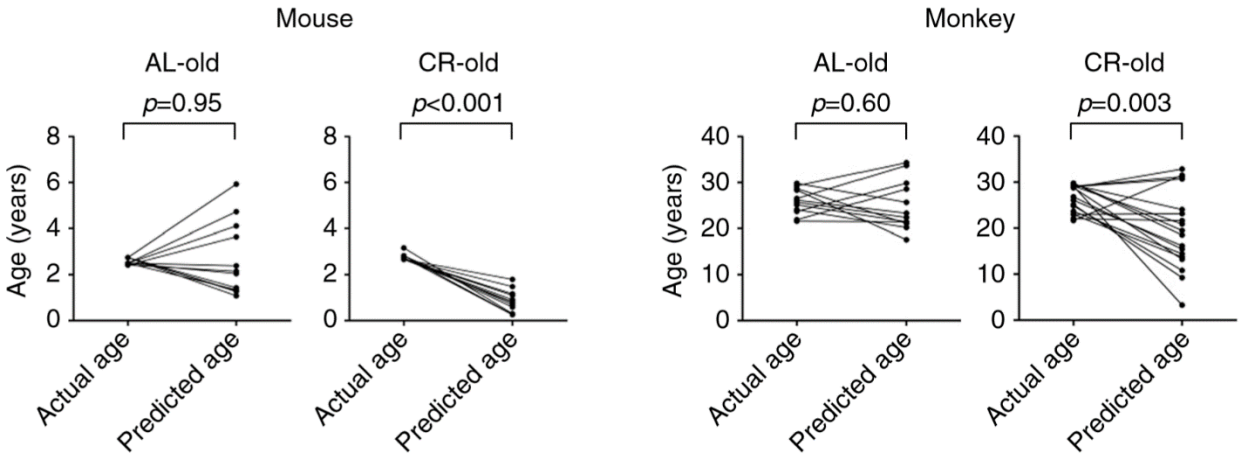


**Figure 6.** Comparison between the chronological age and biological age based on methylation percentage. Monkey CR-old (n=18) vs AL-old (n=12) and mouse CR-old (n=12) vs AL-old (n=12).

inhibits the expression of tumor suppressor genes through methylation and encourages cell survival (Egger et al., 2006). DNMT1 activity is regulated by post-translational modifications such as phosphorylation, ubiquitination, and acetylation. DNMT1 activity is inhibited when acetylated at either two of its lysine residues. Accordingly, SIRT1 which can deacetylate various proteins colocalizes with DNMT1 in the nucleus and removes the acetyl group to increase DNMT1 enzymatic activity (Peng et al., 2011). As we age DNMT1 enzymatic activity decreases in correspondence to decreased expression of DNMT1 as well (Ciccarone et al., 2016). When intermittent fasting increases the expression of SIRT1, we can now know that it also increases DNMT1 activity, further contributing to the maintenance of genomic stability to encourage longevity.

When DNA methylation patterns are altered by caloric restriction, the effects outlast the length of time spent practicing caloric restriction. Four-month-old mice that underwent one-month of caloric restriction had statistically significant changes in the expression of various genes in comparison to the ad libitum mice group which at ad libitum for five-months. The study further demonstrated that for the caloric restricted mice, 20 to 50% of the changes in gene expression were still in effect two-months after caloric restriction ended (Lopatina et al., 2002; Unnikrishnan et al., 2017).

In a study conducted by Belsky, D. W., et al. 2018 in nonobese humans, subjects which underwent a 25% caloric restriction had a not statistically significant rate of change of biological

aging over two years' time, meaning much greater retention of the DNA methylation patterns and genome stability (Fig. 7). On the other hand, subjects which at ad libitum experienced a statistically significant rate of change in biological aging over the two years' time period (Fig. 7).

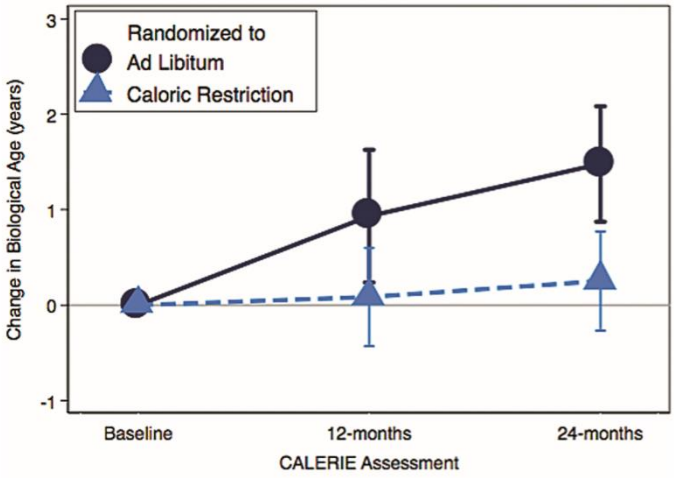


**Figure 7**. Mean values with 95% confidence intervals for change in biological age from baseline biological age. n=75 for AL and n=145 for CR. CR with a p-value of 0.353 and AL p-value of p=2.97 x 10-6. Difference between treatment arms in rate of biological aging is statistically significant with a p-value of 0.03.

## Conclusion

The epigenomic and metabolic molecular pathway alterations as a result of IF demonstrate a role in slowing the rate of biological aging of an individual. IF is therefore a practical intervention, when performed safely without malnutrition and in consideration of other health variables, may indeed promote longevity. In addition, IF promotes a "healthy" longevity in which the extended lifespan is fulfilled with much more healthy years, rather than a time of disease and suffering. In review, IF contributes to longevity by promoting the inhibition of the mTOR kinase to allow the recycling of dysfunctional cellular components through autophagy and conservation and restoration of the a more regulatory epigenome through upregulation of SIRT deacetylases and DNMTs.

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