The caloric restriction fallacy, rodent diet aids and the tumor suppression theory of aging

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This manuscript has been split into two parts now titled “Rodent diet aids and the fallacy of caloric restriction” (the previously first part of the manuscript), and “The tumor suppression theory of aging” (the previously second part of the manuscript).

Both manuscripts were accepted for publication (back-to-back) in the journal Mechanisms of Ageing and Development on October 5th 2021. Previous versions, their reviewer comments and my responses are available at the OSF preprints site. The final reviewer comments were just accepting the manuscripts as they were. I am flattered by the anonymous reviewer commending the tumor suppression theory of aging as a “solid, inspiring and thought-provoking piece of literature” (page 76), thank them for accepting it despite its “heavily opinionized” style and can only hope that at least some readers will be similarly enthusiastic about this attempt at a mechanistic theory of aging.

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1   **Rodent diet aids and the fallacy of caloric restriction**

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7

8   Abstract

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10   Understanding the molecular mechanisms of normal aging is a prerequisite to significantly improving human health span.
11   Caloric restriction (CR) can delay aging and has served as a yardstick to evaluate interventions extending life span.
12   However, mice given unlimited access to food suffer severe obesity. Health gains from CR depend on control mice being
13   sufficiently overweight and less obese mouse strains benefit far less from CR. Pharmacologic interventions that increase
14   life span, including resveratrol, rapamycin, nicotinamide mononucleotide and metformin, also reduce body weight. In
15   primates, CR does not delay aging unless the control group is eating enough to suffer from obesity-related disease.
16   Human survival is optimal at a body mass index achievable without CR, and the above interventions are merely diet aids
17   that shouldn't slow aging in healthy weight individuals. CR in humans of optimal weight can safely be declared useless,
18   since there is overwhelming evidence that hunger, underweight and starvation reduce fitness, survival, and quality of
19   life. Against an obese control, CR does, however, truly delay aging through a mechanism laid out in the following tumor
20   suppression theory of aging.

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1. Introduction

The last century has brought impressive gains in human life expectancy (Oeppen and Vaupel, 2002). Most of these gains have been achieved by reducing unnecessary deaths due to infectious disease, violence and injuries and massive improvements in standards of living, nutrition and sanitation. Most deaths in industrialized countries are now due to diseases of old age like cancer or cardiovascular disease, which cannot be prevented or delayed without understanding the underlying process of aging itself. Even though the phenomena of aging are familiar to all humans and can be well described, surprisingly little is known about the molecular mechanism(s) that drive the process of aging and, as a consequence, whether there is anything that might be done about it (Hayflick, 2021; Kirkwood, 2017).

Experimentally however, caloric restriction (CR) is hailed as a universal mechanism to delay aging (Madeo et al., 2019a). CR, the process of reducing caloric intake in a group of animals relative to the amount the animals would consume if given unlimited access to food but without malnutrition (i.e. while still maintaining an adequate supply of nutrients like vitamins and minerals) delays aging relative to a control group (which is given unlimited access to food) (Masoro, 2005). Discovered first in rodents more than a hundred years ago (McCay et al., 1935), it has since been reproduced in a variety of species ranging from invertebrates to higher mammals (Weindruch, 1996). This culminated in perhaps the longest and possibly most important experiment in aging research conducted so far: testing the validity of CR as an age-retarding intervention in primates (Colman et al., 2009; Colman et al., 2014a; Masoro, 2003; Mattison et al., 2012a). The results of the experiment, despite being somewhat disappointing (Maxmen, 2012), are generally interpreted as confirming the effectiveness of caloric restriction as an intervention that delays the aging process (Mattison et al., 2017).

Over the last one or two decades, several small molecules have been found to increase life span in mice, the most famous and promising of which could be considered to be resveratrol, rapamycin, increasing cellular levels of nicotinamide mononucleotide and metformin. All of these are seen as well-established pathways relevant to the human aging process (Campisi et al., 2019). How these substances prolong life is an active area of research, but there is a broad consensus that all of them produce changes at least partially resembling those seen under caloric restriction, and this is interpreted as a validation of their anti-aging effects (de Cabo et al., 2014a; Fontana and Partridge, 2015). Mice are evolutionary and metabolically close to humans; it is commonly assumed that translation of these findings to humans is feasible and merely a question of time and technology.

I will argue here that caloric restriction is merely preventing the excessive obesity-related mortality seen in most experimental animals given unlimited access to food. Furthermore, most of the above pharmacologic interventions that purportedly delay aging are mere weight loss compounds, and there is little evidence that they affect the root cause of aging other than through weight loss. Their positive effects on life span originate from their ability to reduce proliferative signaling and body weight gain and the excess mortality associated with it in mice. Weight loss is frequently downplayed in investigations of how these compounds work, but a uniting feature of resveratrol, rapamycin, metformin and NAD⁺ supplementation.

2. Results

2.1 Obesity and Health in Rodents

In a natural environment, food scarcity is the primary constraint on survival and reproductive success. Food supply is highly variable in the wild, so the natural behavior of most animals is to consume and store as much energy as possible (Berry and Bronson, 1992). Laboratory animals with constant access to unlimited food eat much more than they

would be able to in the wild. Mammals store available excess energy mostly in fat deposits, and laboratory mice and rats are grossly obese compared to their brethren living in the wild (Austad and Kristan, 2003; Martin et al., 2010). Mice taken from their natural environment weigh only about half as much as common laboratory strains (Mutze et al., 1991), and are much lighter than wild-derived mice (mice that have been caught in the wild but kept in a laboratory for a few generations) (Austad and Kristan, 2003). Laboratory mice have been bred in captivity for hundreds of generations. Captive breeding can lead to further adaption and selection for traits such as fast growth and early fertility even though mice caught from the wild or held in captivity for a short time are equally passionate eaters (Austad and Kristan, 2003). While mice caught from the wild had body fat levels between 3 and 5 %, mice in captivity had between 9 and 22 % body fat, with the popular C57BL/6J inbred strain having a whopping 22 % body fat. Part of the obesity seen in laboratory mice might therefore be due to selection and adaption to the sedentary lifestyle in a laboratory cage (Harper et al., 2006), but their general behavior seems to have stayed the same. How far overeating in captive mice might shorten life span is therefore an important factor to consider in order not to over-extrapolate mechanism of aging in excessively obese rodents to rodents of a healthy weight or non-obese humans.

The effect of obesity on mouse survival is illustrated in Supplementary Figure 1. Mouse body mass index (BMI, at 13 weeks age) significantly correlates with life span (Supplementary Fig. 1b; $p = 0.011$, Pearson correlation coefficient -0.53) and survival in strains with a lower BMI is especially improved in mice that survived beyond the median lifespan (Supplementary Fig. 1a), emphasizing the negative effect of obesity on mouse maximum life span. Life span is also significantly correlated to BMI at 6 and 12 months of age (both $p < 0.05$) (Ackert-Bicknell et al., 2016). Similar plots have been created previously by (Bogue et al., 2016). Measurements of body size to enable the calculation of mouse BMI are relatively rare, but weight measurements are common. Increased mouse body weight is associated with shorter life spans, and the correlation is significant for a range of ages and genders (Supplementary Fig. 2a-h). Body weight at younger age is also negatively associated with life span in 31 strains of ILXISS recombinant inbred mice (Supplementary Fig. 2i) (Bennett et al., 2005; Liao et al., 2010). Beyond middle age however, the capacity to maintain body weight becomes a predictor of life span, and the negative correlation between body weight and life span disappears (Liao et al., 2011). This demonstrates a robust negative association of weight and obesity with life span in a wide variety of inbred mouse strains. Obesity is seldom appreciated as a determinant of longevity in mice, even though early weight gain has been found to be an important determinant of life span (with bigger mice dying earlier) (Goodrick, 1977; Miller et al., 2002). In rats, dietary behavior and growth responses early in life accurately predict longevity (Ross et al., 1976), and growth in general negatively impacts life span in rodents (Rollo, 2002).

2.2 Caloric Restriction in Mice

The original discovery of the life-prolonging effects of caloric restriction were reported about 85 years ago (McCay et al., 1935) and, over the years, confirmed in other rodents (McDonald and Ramsey, 2010). Also called dietary restriction, food restriction or energy restriction, CR fits well with the rate of living theory of aging (Pearl, 1928), which is supported by the both convincing and intuitively satisfying relation of body size and metabolic rate to life span (Austad and Fischer, 1991). As CR obviously reduces energy consumption per animal, a fixed life-long allowance for energy consumption should lead to the observed life span increase. The National Institute of Aging Biomarkers of Aging Program evaluated more than 60.000 rodents, and confirmed CR as a “gold standard” for evaluating intervention strategies in aging (Turturro et al., 1999). The magnitude of the life-span extending effects of CR, however, differed significantly between both strains and gender. When weight gain during adulthood is measured as the area under the weight curve, the magnitude of life span extension afforded by CR is beautifully correlated to the weight gain in control mice fed *ad lib* (Sohal and Forster, 2014). DBA/2 mice exhibit less weight gain after maturity even with unlimited access to food and respond little to CR (Hempenstall et al., 2010). Differences in body weight between *ad libitum* and calorie-restricted animals are small, and decrease with increasing age (Forster et al., 2003). Body fat was almost the same in older *ad lib*

and calorically restricted DBA/2 mice, and it was concluded that life span extension by CR depends on a positive imbalance between energy intake and energy expenditure, i.e. significant overeating (Sohal et al., 2009).

Calorically restricted laboratory mice weigh significantly more than wild mice and have far higher body fat levels (Austad and Kristan, 2003). Mice consuming a normal rodent diet (which provides 10% of calories from fat) and kept under standard conditions are already morbidly obese and poor models of healthy human aging (Martin et al., 2010). High fat diets designed to maximize obesity deliver much more calories from fat (60% for example in (Baur et al., 2006)) (Speakman, 2019). The purpose of feeding high fat diets to mice is often purportedly to mimic a human diet high in fat, but high fat diets seem to activate the reward system and are a sure method to increase obesity to a level probably unimaginable for a wild mouse (Hu et al., 2018; Salmon and Flatt, 1985). Reducing this morbid obesity without serious toxicity must be expected to improve health and life span significantly.

2.3 Alternative dietary interventions in mice

Like CR, protein and particularly methionine restriction, but not carbohydrate or lipid restriction, increases maximum longevity in rodents (Sanchez-Roman and Barja, 2013). Amino acids, and branched-chain amino acids (BCAA) like leucine in particular (Wolfson et al., 2016), are key signals for mTOR activation and insulin release (Chotechuan et al., 2009; Jewell et al., 2013; Neishabouri et al., 2015; Proud, 2004; Yang et al., 2010), and high branched-chain amino acid intake is central to the development of obesity-associated insulin resistance via chronic phosphorylation of mTOR (Newgard et al., 2009). Low protein content induces compensatory overeating (Le Couteur et al., 2016; Solon-Biet et al., 2014) and the opposite, an exome-matched diet (which adjusts food protein content to the exome amino acid composition), reduced mouse ad libitum food intake despite improved growth and higher lean and fat mass (Piper et al., 2017). In rats, methionine restriction stops growth completely despite higher food and calorie intake on a body weight basis (Orentreich et al., 1993). Body weight was halved, and testis and seminal vesicle weights were reduced to around one fourth to one sixth of controls (Orentreich et al., 1993), but both median and maximum life span was increased significantly in 4 strains of rats when methionine was reduced from 0.86% to 0.17% (Orentreich et al., 1993; Zimmerman et al., 2003). Methionine restriction below 0.12% was lethal within 1 month (Orentreich et al., 1993). In mice, about 25% of methionine-restricted (0.1-0.15% vs. 0.43% in controls, from 6 weeks of age) mice died within the first year, but the survivors managed to gain weight at about one fourth the rate of controls (Miller et al., 2005). The methionine-deficient diet lowered serum IGF-1, insulin and glucose, and body weights averaged ~65% of controls between 6 and 18 months of age. The surviving methionine-restricted mice lived significantly longer, with both median and maximum life span increased (Miller et al., 2005). Starting methionine restriction later prevented early mortality and increased longevity less, but body weight was unfortunately not reported (Sun et al., 2009). Similarly, a low BCAA diet (leucine, isoleucine and valine reduced to 33% of control) reduced weight and, consistent with weight loss, promoted metabolic health and decreased frailty in aged mice (Richardson et al., 2021). Low BCAAs also reduced weight in young mice, but longevity increased only in males, not in females or when BCAA restriction was started in middle age (Richardson et al., 2021).

2.4 Interventions in mice - Resveratrol

The first pharmacological intervention to achieve widespread attention was resveratrol with its promise of “guilt-free gluttony” (Kaeberlein and Rabinovitch, 2006). Consumption of resveratrol at a not unreasonable dose improved health and survival in C57BL/6 mice fed a 60% fat diet (Baur et al., 2006). Previously known for its cancer-preventive activity (Jang et al., 1997) and shown to increase life span in yeast (Howitz et al., 2003), *C. elegans* (Viswanathan et al., 2005) and short-lived fish (Valenzano et al., 2006), resveratrol also came with the story of the “French paradox”, a low

incidence of coronary heart disease despite high dietary intake of cholesterol and saturated fats in France(Renaud and de Lorgeril, 1992).

Resveratrol-mediated weight loss was clearly shown, even though it was downplayed by pointing out that the weight loss lost statistical significance at old age (Baur et al., 2006). Weight loss due to resveratrol supplementation was replicated by Lagouge et al., who also showed a remarkable decrease in body fat (Lagouge et al., 2006). Since then, many others have also reported weight loss due to resveratrol supplementation (Andrade et al., 2014; Cho et al., 2012; Milton-Laskibar et al., 2017; Soyoung et al., 2011; Sung et al., 2017; Um et al., 2010; Wang et al., 2020), with weight loss generally bigger and/or dependent on a high fat diet(Milton-Laskibar et al., 2017). Moderate Sirt1 overexpression also reduced weight gain under a high fat diet, despite increased food intake(Pfluger et al., 2008). Sometimes weight or weight gain in resveratrol treatment groups is not reported(Price et al., 2012). Lifespan extension by resveratrol is not observed when nutrients are restricted in *C. elegans* and *Drosophila melanogaster*, which shows that the pathways of CR and resveratrol supplementation might overlap (Wood et al., 2004). The NIA Interventions Testing Program, established to evaluate agents hypothesized to increase life span, found that resveratrol at doses twofold (0.3 g/kg food) and eightfold (1.2 g/kg food) higher than those showing a beneficial effect in C57BL/6 mice on a high-fat diet did not significantly extend life span in genetically heterogeneous UM-HET3 on a normal diet, when started at 12 months age(Miller et al., 2011). There seemed to be, however, a clear trend towards increased median and 90th percentile survival, at the higher resveratrol concentration, especially in males(Miller et al., 2011). Unfortunately, body weight was not reported for both resveratrol concentrations(Miller et al., 2011). When the lower resveratrol concentration (0.3 g/kg food) was started earlier (at 4 months of age), it led to a small, insignificant increase in median survival, with no discernible change in survival at the 90th percentile (Strong et al., 2013). Body weights were only slightly and insignificantly decreased in males, but there was a clearer trend towards lower body weights in females, which was nevertheless significant only at 12 months(Strong et al., 2013). Others also report improvements resembling CR, but insignificant effect on weight and life span with a normal diet(Barger et al., 2008; Pearson et al., 2008), and a small increase in longevity and weight with a low dose of resveratrol in mice on a high fat diet(Pearson et al., 2008).

Resveratrol neither causes weight loss nor acts as a CR mimetic when administered intraperitoneally, i.e. when the gut is bypassed (Pallauf et al., 2019), supporting evidence that obesity reduction by resveratrol might be partially mediated by gut microbiota (Wang et al., 2020), which can consume dietary fatty acids(Agans et al., 2018). Absorption of fats from the small intestine is dependent on the enzyme pancreatic (triglyceride) lipase (Lowe, 1997), and pancreatic lipase is effectively inhibited by resveratrol (Sergeant et al., 2012), even though fecal lipid content is unchanged (Baur et al., 2006; Lagouge et al., 2006; Wang et al., 2020). Polyphenolic pancreatic lipase inhibitors contained in tea also reduce body weight and body weight gain in mice fed a high fat diet by decreasing fat uptake(Basu and Lucas, 2007; Chan et al., 1999; Grove et al., 2012; Yang et al., 2016). This possible explanation for the weight loss is supported by the finding that weight loss is, like life span extension, not observed when animals are fed normal chow(Milton-Laskibar et al., 2017), as for green tea(Kitani et al., 2007; Strong et al., 2013).

Resveratrol activates the fuel-sensing AMP-activated kinase (AMPK), which is activated under conditions of low energy, or more specifically, by starvation or physical exercise. Mice lacking AMPK did not show resveratrol-induced weight loss(Um et al., 2010). This led to the conclusion that many of the effects of resveratrol are due to AMPK activation, which, like resveratrol, reduces fat accumulation, and increases glucose tolerance, insulin sensitivity, mitochondrial biogenesis and physical endurance (Price et al., 2012; Um et al., 2010), supporting the interpretation that the starvation-like response seen with resveratrol is induced by exactly that: “relative” starvation compared to high fat diet controls. Resveratrol’s mechanism of action remains controversial(Pacholec et al., 2010; Park et al., 2012), but activation of sirtuins still seems to be seen as the main mechanism and a promising strategy for testing in humans(Longo et al., 2015). A 2016 review lists dozens of clinical trials for resveratrol and other sirtuin-activating compounds(Bonkowski and Sinclair, 2016), none of which seem to have been successful (as of February 2021).

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195 **2.5 Interventions in mice - Rapamycin**

196 Rapamycin, an immunosuppressant drug approved for use in humans, extends mouse lifespan when fed late in
197 life(Harrison et al., 2009). This created a lot of excitement, because all previously discovered interventions had to be
198 started early in life for a significant effect. Rapamycin inhibits mammalian target of rapamycin(mTOR), a regulator of cell
199 metabolism, growth and proliferation(Laplane and Sabatini, 2009). Its activation is crucial in rapid proliferation and
200 clonal expansion of T cells in the adaptive immune response. Because of this, rapamycin is an immunosuppressant and
201 used to prevent the rejection of transplanted organs(Saunders et al., 2001). Because of its antiproliferant action, it also
202 inhibits angiogenesis and is approved for the treatment of advanced renal cell carcinoma(Motzer et al., 2008).

203 Rapamycin (14 mg per kg food) was encapsulated in an enteric coating material that led rapamycin to be released in the
204 small intestine rather than in the stomach and reach blood concentrations approximately tenfold higher than non-
205 encapsulated rapamycin(Nadon et al., 2008). Body weight was reportedly not changed, but the authors probably meant
206 “not significantly changed” and did not show the data (Harrison et al., 2009). Mice given rapamycin at the same dose
207 but from 9 months age were lighter than controls(Miller et al., 2011). Rapamycin-induced weight loss was dose-
208 dependent, with higher doses leading to more weight loss and larger increases in median and 90th percentile life span
209 (Miller et al., 2014). Another study found reductions in weight, lean and fat mass when rapamycin treatment was started
210 both in midlife or late in life(Neff et al., 2013). A rapid onset, significant decline in body weight was also apparent during
211 transient (3 month) rapamycin treatment(Bitto et al., 2016).The weight loss remained significant for several months
212 after cessation of rapamycin treatment and did not seem to disappear (Bitto et al., 2016). During the early phase of
213 transient rapamycin treatment (2 to 6 weeks, where weight loss is not yet apparent) only the detrimental effects of
214 rapamycin treatment are observed, but once rapamycin-induced weight loss becomes large enough, beneficial effects
215 start to be observed (Fang et al., 2013). When rapamycin was administered intermittently (2 weeks per month) from an
216 early age, it increased lifespan and reduced body weight and tumorigenesis in female mice(Anisimov et al., 2011). In rats,
217 rapamycin treatment reduced body weight gain by 86%, which was partly attributed to reduced food intake, but mostly
218 due to increased energy expenditure, decreased food efficiency and decreased plasma leptin levels (Houde et al., 2010).
219 Side effects of rapamycin treatment are not limited to immunosuppression. Rapamycin treatment promotes insulin
220 resistance, severe glucose intolerance, and increased gluconeogenesis by impairing lipid deposition in rat adipose tissue
221 (Houde et al., 2010). Cataracts were increased and testicular degeneration was observed in 83% of rapamycin-treated
222 male mice (Wilkinson et al., 2012). All stages of spermatogenesis were lost and the location where sperm should be was
223 filled with multinucleate giant cells, dead cells from different stages, and debris. The full effects was apparent even in
224 those mice receiving the lowest rapamycin dose(Wilkinson et al., 2012).

225 Deletion of a component of the mTOR signaling pathway, ribosomal S6 protein kinase 1 (S6K1), led to increased lifespan
226 and resistance to age-related pathologies such as bone, immune and motor dysfunction and loss of insulin
227 sensitivity(Selman et al., 2009). S6K1 knockouts also weighed only two-thirds of controls, fat tissue was reduced to one
228 third of controls, and the authors emphasize the parallels to changes seen with CR (Selman et al., 2009).

229 As both CR and rapamycin inhibit mTOR, it has been suggested that the beneficial effects of rapamycin might be
230 attributed to its ability to mimic caloric restriction(Kaerberlein and Kennedy, 2009). In particular, the striking dose-
231 response relationship for both life span extension and weight loss (Table 1) suggest weight loss might be central to the
232 effects of rapamycin (Miller et al., 2014).

233

234 **2.6 Interventions in mice - NAD+**

235 Sirtuins are NAD⁺-dependent deacetylases, and raising the concentration of the cosubstrate NAD⁺ promotes sirtuin
236 activation(Mouchiroud et al., 2013). NAD⁺ seems to decline with age and limit sirtuin activity (Imai and Guarente, 2014),
237 and several strategies to raise NAD⁺ levels have been reported to activate sirtuins similar to resveratrol and other
238 sirtuin-activating compounds (Imai and Guarente, 2016). NAD⁺ also plays a central role in redox reactions, where the
239 reduced forms NADH and NADPH supply electrons to reduce a variety of metabolic substrates(Jones and Sies, 2015).

240 The NAD⁺ precursor nicotinamide riboside reduced body weight in both old and young mice, accompanied by improved
241 mitochondrial and stem cell function over a short treatment period(Zhang et al., 2016a). The authors maintained that
242 body mass remained comparable among all groups, demonstrated enhanced life span when NAD⁺ repletion started at 2
243 years of age, but weight and gender was not reported for this part of the study (Zhang et al., 2016a). Feeding mice the
244 NAD⁺ precursor NMN also suppressed body weight gain by accelerating metabolism(Mills et al., 2016). The NIA
245 Interventions Testing Program could replicate the weight loss, but not the life extension with NR
246 supplementation(Harrison et al., 2021). The age-related decrease in NAD⁺ is mediated by increased expression of the
247 NADase CD38 (Camacho-Pereira et al., 2016). CD38-deficient animals are smaller, lighter, have a higher metabolic rate
248 and are protected against high-fat diet-induced obesity (Barbosa et al., 2007), pointing to weight reduction as the
249 central mechanism of how NAD⁺-mediates mouse health improvements.

250 Overexpressing two NAD⁺-producing enzymes in mice also mimicked caloric restriction and promoted health (Diaz-Ruiz
251 et al., 2018). The mice had lower body weight between the age of 2 and 12 months, but were heavier at old age, when
252 age-related terminal weight loss started to appear (Diaz-Ruiz et al., 2018). Similarly, mice moderately overexpressing
253 human glucose-6-phosphate dehydrogenase (G6PD), an enzyme producing the reductive fuel NADPH, had higher levels
254 of NADPH, lower ROS-derived damage, lower body weight and were protected from aging-associated functional decline,
255 including extended median lifespan in females (Nobrega-Pereira et al., 2016). In humans, G6PD deficiency is the most
256 common gene mutation. An estimated 400 million affected individuals are mostly asymptomatic with no known effect
257 on life span(Beutler, 2008).

258

259 **2.7 Interventions in mice - Metformin**

260 Mice treated with metformin (0.1% in food) weighed less than controls despite consuming more food, and a variety of
261 beneficial effects, including increased lifespan, resembled caloric restriction (Martin-Montalvo et al., 2013). In
262 apparently mammary tumor-prone short-lived HER-2/neu transgenic mice(Blagosklonny and Campisi, 2008) metformin
263 (100mg/l in drinking water) extended the life span and increased food consumption without changing body weight, but
264 the weight data was not shown(Anisimov et al., 2005). In a second, identical study with fewer animals, metformin
265 decreased body weight and extended life span, but maximum life span was decreased(Anisimov et al., 2010). In female
266 SHR mice, another very short-lived, unknown outbred strain, metformin led to remarkable increases in both median and
267 maximum longevity, without affecting body weight(Anisimov et al., 2008). On the other hand, the NIA Interventions
268 Testing Program found no life span extension with 0.1% metformin in food, but did not report weight(Strong et al.,
269 2016). In rats, metformin reduced body weight but did not increase life span(Smith Jr et al., 2010). Intermittent high (1%
270 in food) metformin reduced body weight and reduced food intake when mice were on metformin(Alfaras et al., 2017).
271 Orally administered metformin also reduced mouse body weight by decreasing energy intake(Kim et al., 2013). The
272 anorectic cytokine GDF15(Emmerson et al., 2017; Mullican et al., 2017; Yang et al., 2017) is increased significantly by
273 metformin in both normal chow and high fat diet groups(Day et al., 2019; Ouyang et al., 2020) and metformin reduced
274 food intake, body mass, fasting insulin and glucose intolerance in mice consuming a high fat diet(Day et al., 2019; Matsui
275 et al., 2010), in which the effects of metformin to reduce body weight were reversed by a GDF15 receptor-antagonist
276 antibody(Coll et al., 2020).

277

278 2.8 Other Treatments in Mice

279 Besides the above “mainstream” pharmacologic treatments widely assumed to slow aging in mice, several other
280 substances have also been reported to extend life span in mice. Combined statin and angiotensin-converting enzyme
281 inhibitor treatment increased median but not maximum life span of male mice, but also decreased body weight, as did
282 statin treatment alone (Spindler et al., 2016). Consumed calories were unchanged, suggesting that altered energy
283 utilization was responsible for the weight loss (Spindler et al., 2016). Nordihydroguaiaretic acid (NDGA) extended the life
284 span of flies and mice, in the latter in a narrow therapeutic window. Treatment was accompanied by weight loss and an
285 increased incidence of tumors (Spindler et al., 2015; Strong et al., 2008). NDGA reduced weight previously (Miller et al.,
286 2007), but some authors later insisted it was not (Strong et al., 2008). NDGA prevented weight gain in mice on a
287 unhealthy diet (Chan et al., 2018) and caused weight loss in dyslipidemic rats (Zhang et al., 2016b). Life span extension
288 with NDGA was replicated again by the NIA Interventions Testing Program, but weight data was, unlike for the other
289 interventions reported at the same time, not shown (Harrison et al., 2014; Strong et al., 2016). In a rare case where an
290 “antioxidant” was beneficial, N-acetylcysteine improved muscle function in a mouse model of Duchenne muscular
291 dystrophy. But 2% in drinking water also reduced body weight gain both in the model mdx mice as well as in control
292 C57Bl/10ScSn mice (Pinniger et al., 2017), confirming previous results of weight loss and life span extension with 1% and
293 0.5% N-acetylcysteine (Flurkey et al., 2010). Chronic N-acetylcysteine treatment also reduced circulating IGF-1 by 20%–
294 50%, depending on mouse strain (Ackert-Bicknell et al., 2004). The estrogen 17- α -estradiol extended male median
295 lifespan (by 19%) when started late in life (16 and 20 months), but also caused a 20% weight drop (Harrison et al., 2021).
296 Similar but smaller weight loss and life span extension was found at a lower dose of 17- α -estradiol (Strong et al., 2016).
297 The α -glucosidase inhibitor acarbose reduces postprandial serum glucose and insulin concentrations and is used for the
298 treatment of diabetes mellitus in humans (Chiasson et al., 2002). Acarbose also reduced weight, IGF-1 and insulin and
299 extended life span, more in male than in female mice (Harrison et al., 2019; Harrison et al., 2014). Mice eating a diet low
300 in advanced glycation end products gain less weight than controls and live longer (Cai et al., 2007). Body weight
301 reduction as the straightforward explanation for increased life span (as with the ACE inhibitor enalapril in rats), is rare
302 (Santos et al., 2009).

303 For genetic interventions, reduced MYC (Hofmann et al., 2015), overexpressing FGF21 (Zhang et al., 2012) or the
304 autophagosomal protein Atg5 (Pyo et al., 2013) and deletion of adenylyl cyclase type 5 (Yan et al., 2007) reduced weight
305 and increased longevity. Liver-specific inactivation of the IGF-I gene decreased body weight and increased mean life span
306 in mice, and the authors deserve recognition for openly stating that reduced body weight could be one mechanism for
307 the prolonged life span (Svensson et al., 2011). Increasing FGF21 up to 17-fold using an adeno-associated virus-based
308 gene therapy caused rapid weight loss and improved comorbidities of obesity compared to the control group consuming
309 a high-fat diet (Davidsohn et al., 2019). Positive effects in combinations of up to 3 genes were dependent on weight loss
310 relative to the high-fat diet consuming control mice. Gene therapies not resulting in weight loss were ineffective
311 (Davidsohn et al., 2019).

312 Table 1 summarizes studies that report both life span extension using pharmacological methods and body weight in
313 mice. To limit the table size, genetic interventions, studies that did not report body weight or full life span data and
314 studies not using mice were omitted. Calorie and protein restriction studies are included for comparison. Weight loss is
315 ubiquitous, and the weight loss is generally larger in treatments with bigger effects on life span.

316

317 2.9 Obesity and life expectancy in non-standard species

Besides laboratory animals, not many species have unlimited access to food, but some kept by humans as pets seem to do. Even though access to food is normally controlled by their owners, some cats and dogs are able to eat much more than what is healthy for them. Excess body weight in middle-aged cats is associated with increased health risks and early mortality(Scarlett and Donoghue, 1998). Obese cats also have a shorter life span and decreased survival when grouped according to a body condition score, which corresponds well to the percentage of body fat (Teng et al., 2018). Even moderately overweight dogs have a higher risk of early morbidity (Laflamme, 2012). Genetic selection for food motivation in Labrador retrievers, which results in good trainability, seems to be partly responsible for the high frequency of obesity in these dogs(Raffan et al., 2016). Middle-aged Labrador retrievers given unlimited access to food weighed about 32 kg, of which 10 kg was body fat, and lived a median 11.2 years. Litter-paired Labrador retrievers restricted to 75% of what their *ad libitum* sibling ate the previous day weighed about 24 kg, of which only 4 kg was body fat (Lawler et al., 2008). Nine of 24, or 37.5%, of CR dogs remained alive by the time all *ad lib* dogs had died, and median survival was increased to 13 years. Despite eating 25% less, CR retrievers had only about 10% less lean body mass as adults, suggesting that this level of restriction is not stunting growth(Lawler et al., 2008). And while Labrador retrievers are seen as especially motivated by food, other breeds do not seem to be significantly less motivated by it(Raffan et al., 2015). The tendency to overeat has become a major problem in pet health care(German, 2006), and loss of health and life span due to excess calorie intake is not limited to rodents and humans.

2.10 Caloric Restriction in Primates

Starting in the late 1980s and early 1990s, two long term studies in rhesus monkeys conducted by the Wisconsin National Primate Research Center (WNPRC) and the National Institute on Aging (NIA) sought to settle once and for all whether caloric restriction is beneficial in longer-lived species(Masoro, 2003). In both studies, CR monkeys were kept on 30 percent fewer calories for more than 20 years. Both provided evidence that CR reduced the incidence of age-related conditions, such as cancer, heart disease, and diabetes. But in what was meant to be two independent replications of the same hugely important experiment, the WNPRC found an extension of lifespan(Colman et al., 2009), while the NIA did not (Mattison et al., 2012b). There were, in hindsight, several significant differences between the two studies, including monkey breed, type of food, and most importantly, the control groups(Mattison et al., 2017). Lifespan extension was found only when the control group was given unlimited access to food. If food was allotted to approximate *ad libitum* intake based on age and bodyweight without overfeeding, which means the control group was “restricted” to 100% of a normal food intake, lifespan extension disappeared. This means that rhesus monkeys (who live about 40 years maximum) do not live significantly longer when their calorie intake is reduced from 100% down to 70% of the amount an average monkey would consume. This finding was reinterpreted as to that the control group in the NIA study was “effectively undergoing caloric restriction”(Colman et al., 2014b). I would absolutely agree. One group was served a balanced meal every day, but never ever allowed to eat more for their whole life. The other had an all-you-can-eat buffet every day. It is not at all surprising that this makes a big difference, given that (at least in worms) a psychological component is responsible for almost half the life extending effect of CR(Libert et al., 2007). But is there a point in salvaging caloric restriction as an anti-aging intervention by insisting that the control group has to be sufficiently obese? Without doubt, control group monkeys at both NIA and WNPRC were much heavier than what would be best for their survival.

2.11 Caloric Restriction in Humans

Voluntary caloric restriction in humans increases life span by 8.4 years in men and 6.1 years in women when started at between 20 and 39 years of life (Grover et al., 2015). The caveat is that these improvements are only achieved against a

control group of humans with a BMI above 35 (Grover et al., 2015). By the same reverse logic, voluntary CR (just a healthy food intake) increases health span by two to four times the increase in life span, for up to twenty years (Grover et al., 2015). Is this proof that caloric restriction slows aging in humans? Or is it not OK to use an obese control group? Compared against humans of a healthy weight, the life-shortening effects of obesity and overweight are striking. Overweight decreased life expectancy by 3 years and obesity by 6 and 7 years in men and women, respectively (Peeters et al., 2003). Years of life lost increase further with more severe obesity (Fontaine et al., 2003). Obesity affects so many hallmarks of aging that it may be apt to call it accelerated aging (Cutler, 1982; Salvestrini et al., 2019). Obesity accelerates the onset of cardiovascular disease (Silva et al., 2006), and epigenetic aging in liver (Horvath et al., 2014), thymus (Yang et al., 2009) and blood of middle aged, but notably not old humans (Nevalainen et al., 2017). CR-induced weight loss improves cardiovascular health also in healthy normal weight humans (Kraus et al., 2019). Weight loss alone improves conduit and resistance artery endothelial function in young and old overweight or obese adults (Pierce et al., 2008). Very large scale human studies have established that risk of death is lowest in humans with a body mass index around 25 (Adams et al., 2006; Berrington de Gonzalez et al., 2010; Di Angelantonio et al., 2016; Zheng et al., 2011). Whether slight overweight (a body mass index of between 25 and 30) is beneficial or detrimental relative to a lower body mass index seems to be controversial (see for example (Flegal et al., 2013)), but seems to protect against communicable disease, a significant cause of mortality in the elderly (Bhaskaran et al., 2018). The BMI associated with lowest mortality risk is higher in older individuals (Bhaskaran et al., 2018), when loss of body mass due to sarcopenia (age-related loss of muscle mass) and other age-related illnesses is called terminal weight loss (Masoro, 2011). Within the healthy range, a slight increase in BMI with age improves survival over staying skinny throughout life (Zheng et al., 2021). Nir Barzilai, even though advocating weight loss through metformin use (Kulkarni et al., 2020), actually finds centenarians “a chubby bunch” (Maxmen, 2012).

Effective CR is also almost impossible to achieve in humans (Kraus et al., 2019). Laboratory animals undergoing CR are “convinced” that food is scarce. Perception of food scarcity seems crucial for CR to work, as worms given the impression of abundant food by the smell of yeast lost almost half of the life extension provided by CR (Libert et al., 2007). Even if they would be able to adhere to CR (which they are almost certainly not (Kraus et al., 2019)), humans would still know that food is abundant and therefore lose the beneficial effects regulated by psychology. Dieting is therefore a particularly unattractive and ineffective intervention to increase lifespan in humans. To partially circumvent the unbearable constant hunger that dooms every effort to achieve laboratory animal levels of CR in humans, alternate day fasting has been promoted to mimic caloric restriction. Alternate day fasting reduced fat mass (particularly trunk fat) and depleted methionine on fasting days (Stekovic et al., 2019). Another fasting-mimicking diet improved almost all parameters in participants at risk for disease (i.e. obese patients), but far less in healthy individuals (Wei et al., 2017). Extremely obese patients often struggle to reduce their weight through changes in their diet or behavior. Bariatric surgery is a dangerous and drastic intervention to reduce weight in morbidly obese patients with no other viable option to improve their seriously compromised health (Buchwald et al., 2004). Compared to dietary interventions, loss of body weight is rapid and sustained, and resembles forced reduction in calorie intake in laboratory animals. As such, bariatric surgery can be viewed as a human equivalent of CR in rodents, and the improvement of many parameters of metabolic youth in patients after bariatric surgery underlines the similarities between CR in rodents and bariatric surgery in humans: mortality due to disease was decreased almost by half (Adams et al., 2007). Bariatric surgery reduced markers of metabolic syndrome (Rega-Kaun et al., 2020), senescence-associated secretory proteins, telomere oxidation and increased circulating leucocyte telomere length (Hohensinner et al., 2018). Drastic improvements not only in metabolic but also in many social factors like appearance, quality of life and social and economic opportunities (Buchwald et al., 2004) illustrate that surgically enforced weight loss constitutes a profound rejuvenation in several not only metabolic biomarkers of aging. But despite massive improvements, most patients still remain in the obese weight category and the doubling of suicide risk after gastric bypass surgery suggests that this intervention is often not a happy one (Adams et al., 2007).

405

406 **2.12 Caloric Restriction Mimetics in Humans – rapamycin**

407 Rapamycin derivatives are standard therapy for organ transplant recipients, some cancers, tuberous sclerosis complex (a
408 genetic disease causing aberrant mTOR activation) as well as several other diseases. As a consequence, the side effects
409 of long term rapamycin treatment are well known. Various mild or moderate infections as a side effect of
410 immunosuppression are common, but life-threatening sepsis can also be observed (Trelinska et al., 2015). Wound-
411 healing complications are common, as wound healing requires expansion of cells for tissue regrowth. Similar to mice,
412 hyperglycemia, diabetes, hypercholesterolemia, hypertriglyceridemia, dyslipidemia and gonadal dysfunction are also
413 seen in humans (Kaplan et al., 2014; MacDonald, 2001). This would be expected to promote atherosclerosis and these
414 side effects are often seen as too severe to seriously consider rapamycin treatment in humans (Bitto et al., 2016).
415 Rapamycin-induced insulin resistance was reported to be mediated by mTORC2 disruption (and therefore uncoupled
416 from longevity effects) (Lamming et al., 2012), and more specific mTORC1 inhibitors are seen by some as a possible
417 avenue to reduce some of these side effects (Mahoney et al., 2018; Schreiber et al., 2019). Unexpectedly, six weeks of
418 treatment with the rapamycin analogue RAD001 was reported to improve adaptive immune function in elderly
419 people (Mannick et al., 2014). However, an oral, selective mTORC1 inhibitor, RTB101, failed to meet the primary goal in a
420 clinical trial in older patients, increasing rather than decreasing risk of respiratory illness (Kaeberlein, 2020), as would be
421 expected from an immunosuppressant. The drug later disappeared from the pipeline of the company that acquired its
422 developer, resTORbio. Like in mice, mTOR inhibition interferes in human spermatogenesis and causes infertility, which
423 can be reversed by treatment withdrawal (Deutsch et al., 2007; Oliveira et al., 2017), even though not in all cases (Zuber
424 et al., 2008).

425

426 **2.13 Caloric Restriction Mimetics in Humans – NAD supplementation**

427 Raising NAD⁺ concentration is the most recent intervention to receive widespread interest as a potential treatment to
428 increase life or health span. In humans, it is still too early to tell if raising NAD⁺ levels might have any long-term
429 beneficial effects, whether through weight loss or otherwise. Nevertheless, short term studies are available, and
430 increasing circulating NAD⁺ levels using various supplements seems to be safe and well tolerated, with no significant
431 effects on a variety of physiological parameters, including weight (Conze et al., 2019; Dollerup et al., 2018; Martens et al.,
432 2018). Caution however, is appropriate: a combination of nicotinamide riboside (NR, a NAD⁺ precursor, 500 mg per day)
433 and pterostilbene (PT, a resveratrol analogue and SIRT1 activator, 100 mg per day), increased total and LDL-cholesterol
434 (“bad” cholesterol) in both normal and overweight individuals by a highly significant 0.48 mmol/L ($p < 0.001$) (Dellinger
435 et al., 2017). Lowering LDL-cholesterol by 1.0 mmol/L reduces the risk of major vascular events (fatal and non-fatal
436 myocardial infarctions, strokes, etc.) by approximately 20% (Cholesterol Treatment Trialists et al., 2012), so this
437 purportedly safe and effective way to increase NAD⁺ levels (Dellinger et al., 2017) would be expected to increase major
438 vascular events by about 10% at the above dose. As about one in four people die of heart disease in the US (Kochanek et
439 al., 2011), long term consumption of this supplement would be expected to kill about 1 in 40 of long-term consumers at
440 the above dose, and 1 in 80 at its recommended dose. Since the increase in LDL-cholesterol is probably attributable not
441 to NR but to pterostilbene (Brenner and Boileau, 2019), this potential public health nightmare is avoidable and not tied
442 to increasing NAD⁺ levels.

443

444 **2.14 Caloric Restriction Mimetics in Humans – metformin**

Metformin has been in use for diabetes for several decades (Clarke and Duncan, 1968), so despite being a more recent addition to the catalogue of CR mimetics, its long history of human use puts it in the forefront of candidates for trials in humans. Billions of prescriptions have produced irrefutable evidence of its beneficial effects in type 2 diabetes patients, where it produces durable weight loss (Stumvoll et al., 1995; UKPDS Group, 1998). The molecular mechanism of metformin action is still a matter of debate (Kalender et al., 2010), but is most often attributed to reduced hepatic glucose production caused by its inhibition of complex I of the mitochondrial respiratory-chain, which activates AMPK (Viollet et al., 2012; Zhou et al., 2001). The weight loss effects of metformin are well known (Lachin et al., 2007), and metformin is an effective weight-loss drug also in non-diabetic humans (Knowler et al., 2002; Lexis et al., 2015; Pedersen, 1965; Preiss et al., 2014; Seifarth et al., 2013). The percentage of weight loss was independent of age and body mass index. Heavier patients lost more weight and insulin-resistant patients lost more weight than insulin-sensitive patients (Seifarth et al., 2013). Decreased food intake with metformin has been reported (Lee and Morley, 1998; Malin and Kashyap, 2014), and recently, anti-diabetic effects of metformin and its ability to lower body weight have been attributed to suppressing food intake by elevating the anorectic peptide hormone GDF15 (Coll et al., 2020; Kleinert and Müller, 2019), as in mice.

Targeting Aging with Metformin (TAME) is a clinical trial to test whether metformin can delay the onset of several age-related diseases, including cancer and cardiovascular disease (Barzilai et al., 2016). Given that this trial is held in a population with a high prevalence of obesity, improvements in variety of biomarkers of aging, resembling the changes seen with caloric restriction in mice or fasting in humans, along with weight loss, are expected. The TAME study was developed to obtain a new FDA indication to target aging and to allow industry to justify the development of next-generation drugs to target aging (Barzilai, 2017). It is highly questionable whether opening the door to weight-loss drugs pretending to extend health span is a good idea. The gastrointestinal side effects of metformin (Flory et al., 2019) might well outweigh the benefit, even if there were any in healthy weight humans. On the other hand, the median BMI for middle aged humans in the U.S. is approaching 30 (Fryar et al., 2018), so most Americans would benefit from weight loss. The risk of developing a range of tumors is increased in type 2 diabetes (López-Suárez, 2019), and metformin treatment reduces this risk (Evans et al., 2005), especially for colon and pancreatic cancers (Currie et al., 2009). Whether through weight loss or other effects, metformin can prevent diabetes (Knowler et al., 2002) and might very well reduce cancer in a non-diabetic but overweight population as well (Renehan et al., 2008).

3. Discussion

A 2019 anniversary review of aging research lists seven interventions to increase health span and/or lifespan (Campisi et al., 2019). Five of them (CR, rapamycin, NAD precursors, sirtuin-activating compounds and metformin) might be little more than rodent diet aids. Exercise is the sixth, and certainly an intervention with almost no downside. Exercise undoubtedly improves health and survival (Nocon et al., 2008; Vina et al., 2012), but there seems to be little evidence that, once corrected for the associated lower body weight, it has any significant impact on the rate of aging (Holloszy, 2000). Lack of exercise contributes to chronic diseases (Booth et al., 2011), but there seems to be no enrichment of athletes or physical laborers in centenarians (Centenarians, 1997). Exercise had no effect on the fundamental rate of aging in mice (Garcia-Valles et al., 2013) or rats (Holloszy et al., 1985), despite lowering body weight. The beneficial effects of food restriction and exercise on survival are not additive or synergistic in rats (Holloszy, 1997). Most researchers justifiably roll their eyes upon the suggestion to prevent aging with more exercise.

Doubts about CR have certainly been raised previously. CR is questioned at length (though not dismissed) by Austad (Austad, 1997). According to Austad and Kristan (Austad and Kristan, 2003), several books seem to have speculated that the effects of CR on rodent aging might be artefacts of overfeeding under captive conditions (Cutler, 1982). But even though they show that laboratory mice are extremely obese compared to wild mice, which have 8-9 times less body fat

than C57BL/6J, but up to three times higher metabolic rates per g body weight(Austad and Kristan, 2003), they conclude that, because *ad libitum* laboratory mice eat no more than wild mice after correcting for body mass, CR restricts energy consumption beyond that of mice in nature(Austad and Kristan, 2003). As if a sedentary 200 kg person eating 4 pizzas is more calorically restricted than a 55 kg marathon runner eating one. Minority opinions like that the correct interpretation of caloric restriction experiments in mice may be that overfeeding reduces longevity(Hayflick, 2010) have been dismissed with distractions about yeast and worms, and the fantastic sleight of hand of relabeling healthy nutrient intake as CR(Fontana et al., 2010). Dismissing CR mimetics as mere diet aids does not mean that they have no effect besides weight loss. Testing the above treatments against an at least moderately calorie-restricted control group might clarify whether any of the above compounds can delay aging in healthy subjects through pathways unrelated to growth retardation and preventing preventable weight gain. Interestingly, the 2020 cohort of the NIA Interventions Testing Program includes 2,4-dinitrophenol, a mitochondrial uncoupler and famous weight loss agent with significant acute toxicity and risk of death(Grundlingh et al., 2011). What should be clear though is that weight loss-mediated “reversal of aging” (when measured with biomarkers), however lifesaving (in case of bariatric surgery) or healthy (in case of dieting, fasting, or other weight loss in overweight individuals), is ignorant of the mechanism of aging in healthy weight organisms, and should not be expected to inform us about how to prevent or delay healthy human aging.

Why is CR so well accepted as an age-retarding intervention? The beauty of CR and most of its appeal as a fundamental method to retard aging lies in its ability to delay most phenotypes of aging in such a way that it is appropriate to state that CR delays aging in general(Masoro, 1985, 1988, 2005; van den Boogaard et al., 2020; Yu et al., 1985), not only one or a few specific aspects of aging. CR really does delay aging against an obese control group. Why is obesity so bad? I think looking at why the alleviation of obesity, or the endocrine retardation of growth (Bartke, 2019; Bartke et al., 2013) slows aging tells us something about the fundamental mechanism of aging. The underlying aspect both obesity and growth have in common is that they accelerate the pace of cell division: CR decreases proliferation, while IGF-1 reverses it back to normal(Dunn et al., 1997). Disrupted GH/IGH-1 signaling is associated with small size and increased longevity in many vertebrate species(Berryman et al., 2008). Many long-lived dwarf mice are relatively obese(Berryman et al., 2014), and genetically obese mice on a CR diet, despite having almost 50% body fat, live longer than normal, less obese mice given unlimited access to food, showing that fat deposits by themselves are not an important determinant of longevity (Harrison et al., 1984). Elevated IGF-1 is a risk factor for several human cancers(Sonntag et al., 1999), as is obesity(Renehan et al., 2008). Effects of CR are generally the opposite of IGF-1, with dramatic reductions in many types of murine cancers(Speakman and Mitchell, 2011), reduced pathologies and increased life span(Sonntag et al., 1999). Severe stunting, low IGF-1, but increased longevity with protein restriction despite compensatory overeating (Miller et al., 2005; Solon-Biet et al., 2014) supports anabolic growth and proliferation, but not calorie intake or adipose tissue mass, as the key determinants of longevity, even in humans(Levine et al., 2014). The obvious explanation why lowered intake of particular proteins and specific amino acids rather than overall calories regulate aging(Fontana and Partridge, 2015) is because these nutrients regulate proliferative signaling through mTOR(Laplane and Sabatini, 2012). Cell proliferation is necessary to build up body mass during increased nutrient availability, and the resulting increase in fitness and resilience is key to achieving higher reproductive success. In the following article, I argue that somatic mutations accumulating mostly during cell division are the fundamental driver of aging, and that the pace and the genetic precision with which cell division is accomplished mostly determines the rate of aging in higher organisms. Malignant growth is a fundamental problem for large and long-lived mammals, and tumor suppression mechanisms had to evolve together with the preservation of genomic integrity, an observation known as Peto’s paradox. The tumor suppression theory of aging (originally part of this article(Wolf, 2021a) and briefly mentioned in(Wolf, 2021b)) proposes that only oncogenic mutations driving clonal expansion are relevant for aging, and that most aging phenotypes are the consequence of tumor-suppressive replicative exhaustion and cell loss and due to apoptosis, differentiation and senescence.

4. Conclusion

Obesity is common and predicted to increase further with economic development (Ward et al., 2019). Obesity contributes to hypertension and related cardiovascular, renal and metabolic disorders (Hall et al., 2015). Weight loss drugs are very attractive “anti-aging” treatments in an overweight population, and several CR mimetics might be approved as drugs to treat obesity rather than aging. Manipulation of nutrient absorption (Holman et al., 2017; Yang et al., 2014) and satiety (Frias et al., 2018; Husain et al., 2019; O’Neil et al., 2018) are also attractive avenues to achieve rejuvenating weight loss (Kushner, 2014). Finding out why even slight overweight seems to increase risk for so many apparently unrelated ailments (Gallagher and LeRoith, 2015) is very valuable. Increased proliferation might explain accelerated aging, but is probably only one of many factors leading to increased mortality in obese patients. Progeria model mice show impressive extension of life span under caloric restriction (Vermeij et al., 2016), suggesting that in the unusual condition of increased mutation accumulation per cell division, curbing replication as far as possible with a diet that might be stunting for normal children might be beneficial in children with progeria. In such patients (and possibly even in normal aging), controlled suppression of proliferation through, for example, inhibition of mTOR, might yield benefits that outweigh the costs.

What is clear however is that excessive growth suppression has overwhelmingly negative consequences on fitness, and is thoroughly unattractive as a candidate pathway for life extension in otherwise healthy individuals (Churgin et al., 2017; Guevara-Aguirre et al., 2011; Hur et al., 2020; Jenkins et al., 2004; Podshivalova et al., 2017). Rapamycin suppresses crucial cell replication and, for this reason, has probably the harshest side effects among the CR mimetics (Bitto et al., 2016; Kaplan et al., 2014). On the other hand, enhancing growth usually increases fitness. Endocrine messengers like growth hormone (Rudman et al., 1990), young blood (Conboy et al., 2013; Rando and Chang, 2012; Villeda et al., 2014; Wyss-Coray, 2016) or anabolic steroids (Franke and Berendonk, 1997) do so and might rejuvenate epigenetic (Fahy et al., 2019; Mendelsohn and Larrick, 2019), and other (Srinivas-Shankar et al., 2010) biomarkers of aging as well. But like obesity, they will, through the stimulation of cell division, probably accelerate aging rather than delay it (Anisimov and Bartke, 2013; Bartke, 2019; Bartke et al., 2013), illustrating the double edged nature of anabolic stimulation (Ratajczak et al., 2017). Nevertheless, age-appropriate nutrition in old adult humans is very different from what one would conclude when looking at longevity in mice (Levine et al., 2014). Suppressing proliferation through pharmacology or diet might slow aging, but in close to optimal weight humans, potentially positive aspects of doing so seem currently not separable from a heavy loss of fitness and quality of life. Weight loss is not known to be beneficial and therefore not recommended to normal weight humans (Initiative, 1998).

The lack of success and current sorry state of aging research is due to the repeated failure to see caloric restriction as what it is: nothing more than the prevention of obesity. Especially the failure to correctly interpret and accept the outcome of the monkey CR studies (Mattison et al., 2017) is striking. Of course CR improves health and survival of rhesus monkeys (Mattison et al., 2017) if you mean >obese< rhesus monkeys. Of course, CR mechanisms are likely translatable to human health (Mattison et al., 2017) if you mean >obese< humans. Early 20th century anti-aging interventions are now ridiculed as quackery, but at that time were state of the art and endorsed by many leading scientists (Hansson et al., 2020; Kahn, 2005; Rubin, 1923; Sengoopta, 2003). Maybe we don’t have to age (Sinclair and LaPlante, 2019), but mistaking the manipulation of obesity for the successful deceleration of aging has produced a fallacious optimism (Campisi et al., 2019; de Cabo et al., 2014b; López-Otín et al., 2016; Madeo et al., 2019b; Partridge et al., 2020) and commercial entities selling the hope that, as a consequence, pharmacologic control of human aging is within reach (De Magalhães et al., 2017) or already there and we just have to open our mouths and wallets (Dellinger et al., 2017). But slower aging is a highly valuable trait (Gurven et al., 2006; Lahdenperä et al., 2004; Shanley et al., 2007) and it is unlikely

that something that requires little investment of energy like increasing a metabolite or blocking a protein, can improve life span by a measurable amount(Kirkwood, 2005). Viewing aging not as inevitable but as a treatable condition is to be welcomed, but while investors salivate over the projected sales figures of a treatment for a disease that affects 100% of the population, progress in aging research is prevented by the continued investigation of rodent weight loss compounds. The aging process might seem much more malleable than we used to think(Kirkwood, 2017), but, at least in my opinion, remains as malleable as a block of tungsten carbide. The tumor suppression theory of aging described in the following article, if correct, would conclude that slowing aging is the same as preventing cancer. Both is, for now, essentially impossible.

Author contributions

A.M.W. wrote the paper.

Conflict of interest statement

The author declares no competing interests.

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Treatment	Dose	Initiation age	Strain & Gender	Body weight	Lifespan change	Reference
				Change Age	Median 90th Percentile	
Sirtuin activators						
Resveratrol	0.1g/kg HFD	12 mo	C57BL/6	m	+8.5% avg	+10.7% +4.98% Pearson 2008
Resveratrol	0.4g/kg HFD	12 mo	C57BL/6	m	-4.3% avg	+7.5% +3.1% Pearson 2008
Resveratrol	0.3g/kg SD	4 mo	UM-HET3	m	-1.5% 12 mo	+3.4% NC Strong 2013
Resveratrol	0.3g/kg SD	4 mo	UM-HET3	f	-5.3% 12 mo	+4.7% NC Strong 2013
SRT2104	1.33 g/kg SD	28 wks	C57BL/6J	m	-7.3% 18 mo	+9.7% +4.9% Mercken 2014
SRT1720	1.33 g/kg SD	28 wks	C57BL/6J	m	-11.7% avg	+8.8% NC Mitchell 2014
SRT1720	2 g/kg HFD	28 wks	C57BL/6J	m	-11.7% avg	+21.7% NC Mitchell 2014
SRT1720	0.6 g/kg HFD	56 wks	C57BL/6J	m	-3.7% 82 wks	+9.96% +3.1% Minor 2011
SRT1720	2 g/kg HFD	56 wks	C57BL/6J	m	-9.3% 82 wks	+22.4% +7.5% Minor 2011
mTOR inhibitors						
Rapamycin	14 mg/kg SD	20 mo	UM-HET3	m	NC -	+9% +9% Harrison 2009
Rapamycin	14 mg/kg SD	20 mo	UM-HET3	f	NC -	+13% +14% Harrison 2009
Rapamycin	14 mg/kg SD	9 mo	UM-HET3	m	-10.6% 24 mo	+10% +16% Miller 2011
Rapamycin	14 mg/kg SD	9 mo	UM-HET3	f	-4.9% 24 mo	+18% +13% Miller 2011
Rapamycin	4.7 mg/kg SD	9 mo	UM-HET3	m	-4.2% 24 mo	+3% +6% Miller 2014
Rapamycin	4.7 mg/kg SD	9 mo	UM-HET3	f	-1.3% 24 mo	+16% +5% Miller 2014
Rapamycin	14 mg/kg SD	9 mo	UM-HET3	m	-11.2% 24 mo	+13% +8% Miller 2014
Rapamycin	14 mg/kg SD	9 mo	UM-HET3	f	-2.8% 24 mo	+21% +11% Miller 2014
Rapamycin	42 mg/kg SD	9 mo	UM-HET3	m	-18.2% 24 mo	+23% +8% Miller 2014
Rapamycin	42 mg/kg SD	9 mo	UM-HET3	f	-19.4% 24 mo	+26% +11% Miller 2014
Rapamycin	42 mg/kg SD	20 mo	UM-HET3	m	-12% 24 mo	+11% +9% Strong 2020
Rapamycin	42 mg/kg SD	20 mo	UM-HET3	f	-13% 24 mo	+15% +12% Strong 2020
Rapamycin, cycles	42 mg/kg SD	20 mo	UM-HET3	m	-9% 24 mo	+9% +8% Strong 2020
Rapamycin, cycles	42 mg/kg SD	20 mo	UM-HET3	f	-7% 24 mo	+8% +10% Strong 2020
Rap. 1.5 mg/kg SC 3x weekly 2 wks/mo 2mo~			129/Sv	f	-4.7% 24 mo	+4.3% +7.8% Anisimov 2011
Rapamycin 8 mg/(kg bw*day) IP, day 600-690			C57BL/6JNia	m	-12.4% 23 mo	+14% +11.8% Bitto 2016
Rapamycin 8 mg/(kg bw*day) IP, day 600-690			C57BL/6JNia	f	-14.3% 23 mo	NC -8.3% Bitto 2016
Rapamycin 126 mg/kg SD, day 600-690			C57BL/6JNia	m	NC 23 mo	+14% +5.2% Bitto 2016
Rapamycin 126 mg/kg SD, day 600-690			C57BL/6JNia	f	NC 23 mo	+9% +2.8% Bitto 2016
NAD+ repletion						
Nicotinamide riboside	0.4 g/kg SD	2 yrs	C57BL/6JRj	?	-3.6% ⁽¹⁾ ?	+4.7% +6.9% Zhang 2016
Nicotinamide riboside	1 g/kg SD	8 mo	UM-HET3	m	-3.8% 24 mo	-3% -3% Harrison 2021
Nicotinamide riboside	1 g/kg SD	8 mo	UM-HET3	f	-13.0% 24 mo	NC +2% Harrison 2021
Metformin						
Metformin	1 g/kg SD	1 year	C57BL/6	m	-5% 18 mo	+5.8% +5% Martin-Montalvo 2013
Metformin	1 g/kg CRD	1 year	B6C3F1	m	NC	+4.15% NC Martin-Montalvo 2013
Metformin	0.1 g/kg DW	2 mo	HER-2/neu ⁽²⁾	f	NC, NS -	+8.0% +13.1% Anisimov 2005
Metformin	0.1 g/kg DW	2 mo	HER-2/neu ⁽²⁾	f	-12% 8 mo	+8.8% -11.1 Anisimov 2010
Metformin	0.1 g/kg DW	3 mo	SHR ⁽²⁾	f	NC -	+37.9% +20.8% Anisimov 2008
Protein restriction						
0.1-0.15% Methionine Diet		6 wks	CB6F1	f	-35% avg	+15.2% +11.2% Miller 2005
Low BCAA Diet (1/3 of controls)		0.75 mo	C57BL/6J	m	-17.8% 12 mo	+31.8% +12.3% ⁽³⁾ Richardson 2021
Caloric restriction						
23% CR (N/N85)	CRD	21-28 d	C3B10RF1	f	-23.2% 24 mo	+16.0% +13.8% Weindruch 1986
55% CR (N/R50)	CRD	21-28 d	C3B10RF1	f	-52.8% 24 mo	+54.0% +52.1% Weindruch 1986
64% CR (N/R40)	CRD	21-28 d	C3B10RF1	f	-54.5% 24 mo	+62.0% +59.0% Weindruch 1986
Others						
Acarbose	1 g/kg SD	4 mo	UM-HET3	m	-14% 12 mo	+22% +11.1% Harrison 2014
Acarbose	1 g/kg SD	4 mo	UM-HET3	f	-22% 12 mo	+5% +8.9% Harrison 2014
17 α -estradiol	4.8 mg/kg SD	10 mo	UM-HET3	m	-11% 12 mo	+12% +4.9% Harrison 2014
17 α -estradiol	4.8 mg/kg SD	10 mo	UM-HET3	f	-6% 12 mo	NC -0.4% Harrison 2014
17 α -estradiol	14.4 mg/kg SD	16 mo	UM-HET3	m	-18.9% 24 mo	+19% +7% Harrison 2021
17 α -estradiol	14.4 mg/kg SD	20 mo	UM-HET3	m	-18.9% 24 mo	+11% +5% Harrison 2021
N-acetylcysteine	5 g/kg DW	7 mo	UM-HET3	m	-23.7% 17 mo	+13.3% +55.9% Flurkey 2010
N-acetylcysteine	5 g/kg DW	7 mo	UM-HET3	f	-20.0% 17 mo	+1.9% +6.6% Flurkey 2010
N-acetylcysteine	10 g/kg DW	7 mo	UM-HET3	m	-30.1% 17 mo	+26.7% +54.9% Flurkey 2010
N-acetylcysteine	10 g/kg DW	7 mo	UM-HET3	f	-28.3% 17 mo	NC +18.0% Flurkey 2010

Abbreviations: SD, standard diet;HFD, high fat diet;DW, drinking water;CRD, caloric restriction diet;SC, subcutaneous;IP, intraperitoneal;avg, average over life span;NC, no change;NS, not shown. Arrows indicate the magnitude of changes (above 2.5%), one arrow per 10% change. Semitransparent arrows represent changes <5%. Studies performed by the NIA Interventions Testing Program are filled with a beige background. (1) Separate treatment group, weight loss after 6 weeks of rapamycin treatment, body weight in longevity cohort not reported. (2) Short-lived strains. (3) 75th percentile.

Table 1. Pharmacologic treatments extending life span in mice and their effects on body weight. Caloric (Weindruch et al., 1986) and protein restriction is shown for comparison.

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292

Highlights

- Caloric restriction extends life span mostly by reducing excess mortality due to obesity
- Most pharmacologic interventions to slow aging, including resveratrol, rapamycin, NAD⁺ and metformin, also cause weight loss
- Dietary restriction and weight loss is beneficial only for obese subjects.
- Investigating mechanisms of caloric restriction will not help prevent or slow aging in healthy weight humans.

Supplementary Materials for

Rodent Diet Aids and the Fallacy of Caloric Restriction

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Supplementary References

Supplementary Figure 1. Dependence of life span and survival on obesity in common mouse strains. (a) Kaplan-Meier plots for 22 strains of mice stratified and color-coded according to their body mass index. Relatively obese mouse strains are colored red and relatively lean mouse strains are colored green. Intermediates are in yellow. (b) Correlation of median life span against body mass index for the 22 strains of mice shown in (a). Pearson correlation coefficient -0.52, $p = 0.011$.

Supplementary Figure 2. Dependence of life span on body weight in common mouse strains. (a-h) Scatter plots illustrating the dependence of mouse strain average life span (x-axis, shared in (a) – (h)) (Yuan et al., 2012) on body weight at different ages. Pearson's correlation coefficient ρ and p values are shown in each graph. (a) female body weight at 13 weeks age for $n=27$ strains (CDGPheno1 data set) plotted against strain average life span (b) females body weight at 6 months of age for $n=30$ strains (Ackert1 data set) (c) females at 6-9 weeks age, $n = 13$ (Boon et al., 2011) (d) males at age 6 weeks, $n = 24$ strains (Courtney and Massett, 2012) (e) 8-10 weeks old females, 23 strains (Church et al., 2014) (f) 6-8 week old females, 24 strains (Harrill et al., 2012) (g) 6 month old females, $n=31$ strains (Leduc et al., 2010; Yuan et al., 2009) (h) 8-11 week old males, 26 strains (Wiltshire et al., 2015). (i) Life span versus body weight in 31 ILXISS mouse strains.

Supplementary Methods

Mouse body mass index and survival (Sup. Fig. 1a & 1b)

Individual male mouse life spans reported by Yuan et al. (Yuan et al., 2012) were obtained from the Mouse Phenome Database (MPD). BMI for the different strains was calculated from the Center for Genome Dynamics Multi-system survey of mouse physiology (CGDpheno1 data set, also from the MPD), which was taken at 13 weeks of age. Kaplan-Meier survival curves were plotted for all 22 mouse strains overlapping both data sets. Microsoft Excel was used to derive t values from the Pearson correlation coefficient and the number of strains assuming a bivariate normal distribution. p values were determined using a two-tailed Student t-distribution and n-2 degrees of freedom, with n being the number of strains.

Mouse weight versus life span (Sup. Fig. 2a-i)

Data sets containing strain-specific mouse weights at different ages were obtained from the MPD. Eight data sets contained at least 13 strains of mice that were also contained in the mean life span data from Yuan et al. (Yuan et al., 2012). The body weight data sets and number of overlapping strains used were Ackert1 (30 strains, females at 6 months of age), Boon1 (13 strains, females at 6-9 weeks age)(Boon et al., 2011), CDGPheno1 (27 strains, females at 13 weeks of age), Harrill1 (23 strains, females, 8-10 weeks old)(Church et al., 2014), Harrill2 (24 strains, females, age 6-8 weeks)(Harrill et al., 2012), Massett1 (24 strains, males, age 6 weeks)(Courtney and Massett, 2012), Tarantino1 (26 strains, males, 8-11 weeks old)(Wiltshire et al., 2015) and Yuan1 (31 strains, females, 6 months old)(Leduc et al., 2010; Yuan et al., 2009). The lifespan of ILSXISS recombinant inbred mice (Williams et al., 2004) is from (Liao et al., 2010) and their body weight at 90 days of age is from (Bennett et al., 2005). p values were calculated as for Supplementary Figure 1b.

Supplementary References

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Rodent diet aids and the fallacy of caloric restriction

Reviewer #1: The author did a very good job addressing the critique, polishing the style and improving the content of the manuscript.

Major comments:

The only thing, which this reviewer does not find suitable or justified for this specific article is a mention in the highlights and abstract that "Obesity accelerates and caloric restriction decelerates aging through their effect on cell proliferation rate". With a single sentence in the manuscript referring to this notion I disagree that it should be used in the highlights/abstract. Not to mention that the sentence/statement in the text is supported by a single reference (which is Dunn et al., 1997), that actually describes growth of cancer under DR and has little to do with proliferation rate in healthy tissues of DR or obese animals. This subject has been described much better in the follow-up/parallel paper (The tumor suppression theory of ageing) but does not directly relate to the subject "Rodent diet aids and the fallacy of caloric restriction". As such, I would recommend that this highlight/part of the abstract and mentions on this subject in the text are removed.

I thank Reviewer #1 for alerting me to the fact that I have removed the argumentation necessary to support the statements about cell proliferation rate from this paper. As a consequence, these statements should have been removed as well, and I have done so now.

Minor comments:

There is a number of editing issues including double spaces, double comas and a small number of grammar errors that are probably resultants of corrections/changes applied on the previous version. These small issues should be addressed before the article is released.

I hope I have found and corrected all of them. None of the corrections changed any meaning.

I also made 3 small other corrections:

I deleted a duplicate "in a wide variety of species" in line 35 (it is repeated in the next sentence).

I added "gastric" in line 403 to clarify that I mean gastric (and not coronary) bypass surgery.

I added "and protein" in line 608, since protein restriction was also included in the table for comparison, even though it is not a pharmacologic intervention.

Reviewer #2: The author has responded constructively to all reviewer and editor comments and I now recommend publication

1   **The tumor suppression theory of aging**

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10

11   **Abstract**

12   Despite continued increases in human life expectancy, the factors determining the rate of human biological aging
13   remain unknown. Without understanding the molecular mechanisms underlying aging, efforts to prevent aging are
14   unlikely to succeed. The tumor suppression theory of aging introduced here proposes somatic mutation as the
15   proximal cause of aging, but postulates that oncogenic transformation and clonal expansion, not functional
16   impairment, are the relevant consequences of somatic mutation. Obesity and caloric restriction accelerate and
17   decelerate aging due to their effect on cell proliferation, during which most mutations arise. Most phenotypes of
18   aging are merely tumor-suppressive mechanisms that evolved to limit malignant growth, the dominant age-related
19   cause of death in early and middle life. Cancer limits life span for most long-lived mammals, a phenomenon known
20   as Peto's paradox. Its conservation across species demonstrates that mutation is a fundamental but hard limit on
21   mammalian longevity. Cell senescence and apoptosis and differentiation induced by oncogenes, telomere shortening
22   or DNA damage evolved as a second line of defense to limit the tumorigenic potential of clonally expanding cells, but
23   accumulating senescent cells, senescence-associated secretory phenotypes and stem cell exhaustion eventually
24   cause tissue dysfunction and the majority, if not most, phenotypes of aging.

1. Introduction

Prevention of premature deaths from violence, poor hygiene and curable diseases has increased human life expectancy well past 80 years (Oeppen and Vaupel, 2002). However, the underlying cause(s) of human aging remain largely unknown, and the rate of biological aging essentially unchanged. Years of life gained from further progress in the treatment of diseases is therefore spent in a state of ever increasing frailty and dependence, making the further extension of human life expectancy less and less desirable. Modern medicine has undoubtedly improved quality of life for most elderly humans (Vaupel, 2010), but the prevention of death without the prevention of aging promises little more than additional years spent in dehumanizing geriatric care, which is by many understandably seen as a nightmare rather than a medical goal worth pursuing.

Evolutionary theories of aging developed in the 20th century explain very well why we age (the reason) (Kirkwood and Austad, 2000), but unfortunately not how. Despite centuries of efforts to understand how we age, aging has essentially remained a disjointed collection of phenotypes (Finkel, 2015; López-Otín et al., 2013). Biogerontology can describe what is happening, but is unable to explain why or how the many molecular mechanisms presumably involved ultimately determine the rate of human aging. At least when looking at the practical application of aging research, the last century has produced little more than an illustrious queue of self-aggrandizing quacks and lunatics (Sengoopta, 2003). There are few sound reasons why encouraging observations in rodents, without any understanding of etiology (Campisi et al., 2019), should suddenly lead to anything useful.

Single cause theories of aging remain important in aging research. One obvious reason for this is the need for simplification. Another reason is the necessity to at least break up the aging process into potentially treatable parts, even if no single treatment can be expected to do much (Kirkwood, 2005). Efforts in the last 70 years to elucidate the molecular mechanism of aging have been one big disappointment, partially because during the same time, caloric restriction (CR) has become widely accepted as a universal mechanism to delay aging across the phylogenetic tree, and CR “mimetics”, pharmacological agents that increase life span by inducing CR-like effects, are seen as the most promising and realistic avenue to prevent or slow aging. In the preceding article, I argued that CR does little more than prevent the excessive obesity present in most laboratory animals. Agents like metformin, NAD⁺, resveratrol and rapamycin, but many other genetic and pharmacologic interventions as well, induce weight loss, and there is little reason to assume that they prevent aging in any way other than causing a beneficial reduction in body weight. This fallacy of caloric restriction has survived so long because, against an obese control, CR really does delay aging. In this article, I will argue that obesity accelerates and CR decelerates aging against an obese control mostly through its effect on cell proliferation rate. Proliferation drives aging because most mutations arise during cell division when DNA damage is converted into irreparable mutations.

2. The tumor suppression theory of aging

2.1 Overview

Somatic mutations have long been proposed as a cause of aging (Failla, 1958; Szilard, 1959) and genomic instability is one of the four primary hallmarks of aging (López-Otín et al., 2013). The tumor suppression theory of aging outlined here differs from previous theories in that clonal expansion and malignancy is proposed as the relevant consequence of somatic mutation and that impairment, loss of cellular function, or cell death as a consequence of somatic mutation is largely irrelevant. To counter the tumorigenic potential of clonally expanding cells, we have evolved tumor suppression mechanisms that remove or limit proliferation of stem cells. Accumulating senescent cells and loss of capacity for self-renewal and repair eventually cause the phenotypes we experience in very old age. This theory of aging, briefly mentioned in (Wolf, 2021b) and illustrated in Figure 1, evolved out of an analysis of the effects of obesity and CR on cell proliferation and aging, and was originally part of the preceding article about the fallacy of caloric restriction (Wolf, 2021a).

The idea of DNA as the store of a human age is obvious: our biological age has to be stored somewhere. Most of our tissues renew themselves at a considerable rate (Milo and Phillips, 2015; Post and Clevers, 2019), and, besides long-lived proteins (Toyama and Hetzer, 2013), little to store age remains besides the accumulated, irreversible mutations in DNA. Aging is irreversible: in billions of humans, there has been no case of spontaneous rejuvenation ever. Nor are there any people who, by some genetic, metabolic, nutritional or other wonder, have slowed down their rate of aging by any significant amount. Many interventions that increase somatic mutations strikingly resemble aging. Exposure to mutagenic UV-containing sunlight causes premature skin aging (Yaar and Gilchrest, 2007). Genotoxic stress causes premature hair greying (Inomata et al., 2009). Smoking causes not only cancer (Hecht, 2003) but also premature aging in exposed organs (Choukrallah et al., 2020; Morita et al., 2009; Okada et al., 2013). Radiation and mutagenic chemotherapy leads not only to therapy-related secondary cancer (Allan and Travis, 2005), but also to premature frailty, multi-morbidity and increased mortality (Armstrong et al., 2014; Cupit-Link et al., 2017; Robison and Hudson, 2014), which has been attributed to the system-wide persistent accumulation of senescent cells (Short et al., 2019). Premature aging syndromes invariably destroy genomic integrity: almost all of them are disorders of DNA repair or maintenance of genomic integrity (Hoeijmakers, 2009). DNA repair is a longevity assurance system (MacRae et al., 2015). Somatic mutations can also explain why men die earlier than women: men lack a second X chromosome. When males are the heterogametic sex (like in mammals), they die earlier than females, but when females are the heterogametic sex (like in birds), males live 7.6% longer than females (Xirocostas et al., 2020), and male birds seem to have similar or longer lifespans than female birds (Austad and Fischer, 2016; Clutton-Brock and Isvaran, 2007). Biallelic expression of tumor suppressors escaping X-inactivation (Dunford et al., 2017) can explain why the heterogametic sex (XY in mammals, ZW in birds) dies, on average, earlier than its homogametic counterpart across a range of taxa (Xirocostas et al., 2020).

2.2 The somatic mutation theory of aging

Somatic mutations as the cause of aging were proposed (among others) by Szilard (Szilard, 1959) because the phenotype of animals exposed to non-lethal amounts of mutation-inducing radiation resembled aging so much (Curtis, 1964). Why does the somatic mutation theory of aging, despite all the above evidence, receive so little support? First, for a long time, it was difficult to quantify mutations in somatic cells, so estimating the functional consequences of somatic mutations was difficult. Second, radiation-induced aging did not resemble normal aging very well: radiation mostly affects dividing cells, whereas aging seemed to happen mostly in non-dividing cells (Strehler, 1959). Furthermore, the amount of mutations necessary to shorten life span was much more than that acquired during normal aging. To match the observed age-dependent exponential increase in mortality, mutations had to be assumed to cause aging mainly by damaging recessive elements, but the rate of aging did not depend on ploidy (Strehler, 1986). Rose states that “by the end of the 1960s, somatic mutation was dead as a general theory of aging” (Rose, 2005), which appears to be more or less true up to the present, at least for its original form. Recently, Ferrucci et al. conclude that “solid evidence that the accumulation of somatic mutations during normal aging is associated with the phenotypes of aging is lacking” (Ferrucci et al., 2020).

In my opinion, the most important and crucial flaw of the somatic mutation theory of aging, and in current thinking about the role of somatic mutations in aging in general, is that it is mistaken about the consequence of somatic mutations. Mutations were and, up to this day, are universally thought of as damaging and pathogenic (Freitas and De Magalhães, 2011; Kennedy et al., 2012; López-Otín et al., 2013; Vijg and Dong, 2020; Vijg et al., 2017; Vijg and Montagna, 2017), leading to “cell functional loss and human disease” (Vijg and Dong, 2020), like in the Hallmarks of Aging, where genomic instability is viewed as “resulting in dysfunctional cells that... may jeopardize tissue and organismal homeostasis” (López-Otín et al., 2013). Aging itself is characterized by a loss of function, and so it is common thinking that the mechanistic cause of aging must also result in a loss of function (see for example the introduction in (Kinzina et al., 2019)). Looking at individual somatic mutations, I think this is mistaken. Elimination and replacement of damaged cells is ubiquitous (Milo and Phillips, 2015) and preserves function rather than impairing it (Clevers et al., 2014; Post and Clevers, 2019). Despite our vastly improved knowledge of somatic

116 mutations in adult tissues, there is little evidence for any significant deterioration of function due to somatic
117 mutations(Vijg and Dong, 2020).

118 Cancer is caused by mutations in oncogenes and tumor suppressor genes (Fearon and Vogelstein, 1990; Hoeijmakers,
119 2001; Michor et al., 2004; Stratton et al., 2009). Cancer genome sequencing has unequivocally revealed the
120 “mountains” (frequently altered genes) and “hills” (less frequently altered genes) of positive selection in the cancer
121 genome(Vogelstein et al., 2013). The opposite however, the relative absence of mutations in essential genes, is not
122 observed (Bakhoun and Landau, 2017; Martincorena et al., 2017; Yadav et al., 2016). And while this might not be
123 surprising given that humans have two copies of most genes, that most mutations are recessive(Wilkie, 1994), and a
124 pervasive robustness of biological systems(De Visser et al., 2003; Félix and Barkoulas, 2015; Kim and Fernandes,
125 2009; Wagner, 2013), this absence of negative selection is strong evidence that somatic mutations do not cause
126 significant negative selection or elimination of cells, further contradicting the currently prevailing dogma of
127 functional impairment as the consequence of somatic mutation.

129 **2.3 The fundamental problem of malignant growth**

130 RNA and DNA as the storage medium for the blueprint of life originated approximately 4 billion years ago in short-
131 lived prokaryote life forms(Betts et al., 2018; Brasier et al., 2015) who depended little on the ability to store
132 information reliably for decades. Only very recently (some 500 million years ago), possibly prompted by an
133 abundance of high-energy oxygen(Marshall, 2006), did single-celled organisms rapidly evolve into multicellular life
134 forms and then into even larger bony fish. These grew lungs and started to walk on land all within the few dozen
135 million years of the Cambrian explosion(Erwin et al., 2011; Marshall, 2006). At that time, however, all life had firmly
136 settled on DNA as a now inconveniently fragile storage medium. The sudden rapid increase in organism size and life
137 span was and had to be accompanied by concurrent improvements in genome maintenance and protection against
138 newly appearing mutagenic oxygen radicals(Fridovich, 1975; Halliwell, 1978).

139 Together with increasing size and life spans, uncontrollable cell growth became a fundamental problem for large,
140 long-lived multicellular organisms. Cell sizes are roughly the same in big and small animals(Savage et al., 2007), so,
141 from cell number alone, cancer risk can be assumed to scale with body size. Cancer risk increases with age as
142 mutations accumulate, so long lived animals face a further increase in cancer risk. As a consequence, long-lived
143 animals, including humans, evolved mechanisms to limit malignant growth together with life span and body size, a
144 phenomenon called Peto’s paradox (Caulin and Maley, 2011; Hiatt et al., 1977; Peto et al., 1975), which states that
145 cells of large, longer-lived animals have a much lower risk of turning cancerous over a certain time than those of
146 smaller animals(Abegglen et al., 2015). And even though a decline of metabolic rate (and most probably cell division
147 and mutation accumulation rate as well) with body size (Glazier, 2005; Savage et al., 2004) makes a calculated 3
148 trillion-fold difference in the per gram of tissue cancer risk between mice and humans(Peto, 2015) unlikely, species-
149 specific per-cell cancer risk differences are undoubtedly enormous. The presence of cancer and the evolution of
150 cancer resistance by themselves demonstrate that malignant growth is an important factor limiting lifespan in
151 apparently most long-lived vertebrates.

153 **2.4 Life-history characteristics in large animals**

154 Cancer is a significant cause of death not only in mice and humans, but in most animals where cause of death has
155 been investigated(Leroi et al., 2003; Madsen et al., 2017). Apocryphal stories about supposedly cancer-resistant
156 species such as sharks(Lane, 1992; Mathews, 1992) or naked mole rats(Tian et al., 2013) exist, but are not
157 true(Braude et al., 2021; Delaney et al., 2016; Finkelstein, 2005; McCutcheon, 1997; Ostrander et al., 2004; Taylor et
158 al., 2017). Among the few other species largely protected from extrinsic mortality, cancer accounted for 39% of
159 deaths in long-lived dog breeds and almost half of deaths in old dogs(Bronson, 1982). Breed-specific cancer mortality

ranged from 15 to 56%, and tended to increase with body size(Dobson, 2013). Cancer accounts for the vast majority of deaths in laboratory mice(Blackwell et al., 1995; Lipman et al., 2004; Miller and Chrisp, 2002; Pettan-Brewer and M. Treuting, 2011; Storer, 1966; Ward, 2006).

2.5 Human cancer epidemiology

Peto's paradox underlies one of the strongest arguments for the tumor suppression theory of aging, so cancer needs to be a valid and important driver in the evolution of human life span, not only in modern humans, but also in the prehistoric environment that shaped the genetically determined current human maximum life span.

Supplementary Figure 1a shows the age-dependent cause of death for the US population from 1999-2019. Accidents and other extrinsic mortality like infections dominate in early adulthood up to middle age, when absolute mortality is lowest. When non-age-related sources are removed, cancer and cardiovascular disease are the two dominant causes of mortality, and cancer is the dominant cause of death in children (Supplementary Figure 1b). Despite most cancers of childhood now being successfully treated in medically advanced societies like the US, cancer makes up 40%, 34% and 28% of deaths in the 5-9, 10-14 and 15-19 year old groups.

Approximately 80% of childhood cancers are cured in high income countries, whereas mortality in low income countries is above 90%, so prehistoric childhood cancers were probably about five times more lethal than with access to modern treatment(Lam et al., 2019). For adults, cancer incidence is about threefold cancer mortality(Siegel et al., 2020). On the other hand, cardiovascular disease (CVD) is significantly associated with risk factors rare in prehistoric societies: diabetes, smoking, high blood pressure and elevated cholesterol levels(Berry et al., 2012; Lloyd-Jones et al., 2006). In turn, diabetes is associated with obesity and sedentary lifestyle(Lascar et al., 2018), high blood pressure is associated with obesity (again, accounting for 65% to 75% of the risk)(Hall et al., 2015) and high intake of alcohol and salt. High blood cholesterol is associated with a diet high in saturated fats. Since primitive humans consumed mostly low fat, plant-based food, above CVD risk factors were probably more or less inexistent during prehistoric evolution. Whereas most North Americans falls into highest two of five CVD risk categories(Berry et al., 2012), prehistoric CVD mortality was probably close to that observed in groups having none of the above risk factors, which results in a profoundly lower risk for all kinds of CVD(Berry et al., 2012; Lloyd-Jones et al., 2006). Mortality with all CVD risk factors optimal is about one fourth of that in the most populous risk category, those with one major risk factor(Berry et al., 2012).

If, to mimic prehistoric conditions, cancer mortality is increased 5-fold for ages 1-19 and 3-fold for ages above 20, and death from cardiovascular disease reduced by 75%, cancer becomes the most common age-related cause of death by far, accounting for the majority of age-related deaths up until age 80-84 (Supplementary Figure 1c). In contrast, deaths from other ICD-10 chapters, including deaths from CVD, stay around or below 10%.

Despite cancer being the dominant age-related cause of death in early and middle life, absolute risk of death in high income societies like the US is very low up until late middle age. Age-related mortality from non-infectious disease is negligible in those under 40 years old(Supplementary Figure 2). Cumulative survival from all causes except accidents, infections and congenital malformations does not drop markedly before the 50's and 60's, and remains above 50% up into the 70's (Supplementary Figure 2). Nevertheless, cancer on average precedes other age-related causes of death such as cardiovascular disease and typical end-of-life ailment such as dementia, pneumonia and age-related organ failure, irrespective of whether or not adjusted for prehistoric mortality. Adjusting for prehistoric increased cancer mortality and lower CVD incidence shifts peak mortality to younger ages by about 3-4 years, as expected if cancer precedes other causes of age-related mortality (Supplementary Figure 2).

2.6 The (evolutionary) value of longevity

It is still a common fallacy that aging (or cancer) is rarely observed in the wild, due to opinions resembling Peter Medawar's careless assumption that wild animals "simply do not live that long" (Medawar, 1952). This is not true (Nussey et al., 2013). Humans, many flying animals and other species have evolved remarkable longevity after managing to escape high extrinsic mortality. Intelligent, long-lived birds show increased reproductive success as they get older, bigger, gain more experience and learn more tricks (Lecomte et al., 2010). Ravens, albatrosses and other birds learn skills like food source patterns or appropriate nest site selection over years (Dunk et al., 1997; Nevoux et al., 2007; Reed et al., 1999). Reproductive success is often best in experienced, veteran breeding pairs that have mastered a set of key skills crucial for survival and raising offspring (Angelier et al., 2007), and aging is often a primary natural limit on reproduction in these successful individuals (Bouwhuis et al., 2012; Nussey et al., 2013; Reed et al., 2008; Weimerskirch, 1992). Reproductive success in larger and/or long-lived species appears to be skewed towards a few highly productive individuals not only in birds, but also terrestrial animals such as seals (Le Boeuf et al., 2019) and humans (Favre and Sornette, 2012), and these few highly successful individuals disproportionately profit from longer lifespans.

Humans have evolved a rate of aging that assures their performance into late middle age. This is not only reflected in the retirement age of most industrialized societies, but also in the hunting performance of traditional societies (Walker et al., 2002), and the age of menopause, which results in the presence of dependent children well in the 50's of their parents. Furthermore, once humans survived childhood, their mortality was remarkably low, and significantly lower than that of closely related primates (Finch, 2010; Gurven and Kaplan, 2007). After reaching puberty, prehistoric humans entered a phase of low mortality, whose overall reproductive payoff probably depended significantly on the length of this period of adulthood marked by health, high performance and low mortality, especially in males (Favre and Sornette, 2012). A quantitative understanding of the prehistoric relative evolutionary value of preventing death at different ages is lacking, but it is not unreasonable to conclude that malignancy was an important age-related source of mortality at the upper end of human reproductive activity, the edge at which the reproductive value of additional health span drops below the cost associated with achieving it, and age-related mortality (Supplementary Figure 2) starts to become significant.

229

230 **2.7 The wonderful consequences of Peto's paradox**

In my opinion, this annoying fragility of DNA (Duncan and Miller, 1980), and the necessity to copy it with ever lower error rates (Caulin and Maley, 2011) has become the most important choke point in achieving even longer life spans. Peto's paradox wonderfully affirms and substantiates the tumor suppression theory of aging. Not because it shows that cancer limits mammalian lifespan, and the irrelevance of body size and life span to cancer risk (Peto, 2015), but because it proves the centrality and difficulty of maintaining genomic integrity as the cause of aging. Cancer is caused by mutations (Fearon and Vogelstein, 1990; Stratton et al., 2009). If preventing them would be easy, we would not have evolved cell senescence and other costly mechanisms of premalignant cell elimination that exhaust our stem cell pools. We would simply have evolved (much) better genomic maintenance to prevent mutations and cancer altogether. Of course this is just possible if evolving better genome maintenance would be easy. Or cheap (in terms of evolutionary investment). But we didn't. And we didn't, because all the DNA repair, maintenance and damage prevention mechanisms we evolved to counter the annoying fragility of DNA is already costly, complex or difficult to improve, and must make up a large, if not the largest, fraction of the lifespan-limiting maintenance and repair functions (Kirkwood, 2005).

This validation might be speculative, but more or less has to be true because single cause mechanistic theories of aging are inherently stupid: evolutionary life-history theories of aging (chiefly the disposable soma theory of aging) tell us that evolution should quickly "fix" dominant causes of aging. The only way to save the tumor suppression theory of aging from this crushing argument is to conclude that the many components of the sophisticated

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machinery to protect, repair and maintain DNA integrity constitute the majority of the lifespan-limiting maintenance and repair functions(Kirkwood, 2005). If somatic mutation would be fixable, it should have been fixed, to eliminate cancer. But not only in humans, but probably in thousands of other long-lived species as well, cancer has not been fixed.

Simple models predict evolutionary pressure to eliminate age-related diseases only up to the peak reproductive and child-caring years, but not much beyond(Hamilton, 1966; Williams, 1957). For humans, this might be more or less true, but for other species, it seems wrong. Mice have a generation time of less than 3 months, yet a maximum life span of more than 3 years(Weindruch et al., 1986). Mouse mothers can meet their great-great grandchildren within a year while still being quite fertile(Harman and Talbert, 1970; Talbert, 1971). Female mouse fertility starts to decline significantly after one year, yet birth remains possible up until 2 years(Talbert and Krohn, 1966). Even though this is vanishingly unlikely in the wild, old mice can easily survive more than ten generations of offspring. Mouse maximum lifespans are therefore somewhat in discrepancy with the evolutionary theory that longevity is mostly a trade-off with fertility(Kirkwood and Holliday, 1979; Williams, 1957) and that higher fertility will lead to high rates of senescence(Hamilton, 1966). Why have mice evolved such a long maximum life span? The tumor suppression theory offers an answer: because in small animals, tumor suppression must be relatively cheap. Aging and death are obstacles to reproduction, after all. Despite their small body size and short life span, the mouse germline and somatic mutation rate is less than an order of magnitude higher than in humans(Milholland et al., 2017). Longevity is a function of optimal resource allocation(Kirkwood, 2017a), and species maximum longevity should be determined by the actual cost of longevity as well, not only age of maturity and external mortality(Jones and Vaupel, 2017). Maturation might not always mark the onset of significant senescence as predicted(Williams, 1957) if, due to a small size, even a low allocation of resources towards protecting genomic integrity and tumor suppression might assure maximum lifespans many multiples the age of maturity, and without marked tradeoffs in fertility(Bouwhuis and Vedder, 2017; Jones et al., 2014; Wensink et al., 2017).

2.8 Mechanisms and drivers of somatic mutation accumulation

In a profound and fundamental study, Tomasetti and Vogelstein demonstrate that the number of stem cell divisions explains two thirds of the variation in cancer risk(Tomasetti and Vogelstein, 2015) of a tissue. This is an old idea (Albanes and Winick, 1988; Preston-Martin et al., 1990) based on the finding that the majority of oncogenic mutations arise during normal cell division(Ames et al., 1993), when repairable DNA damage is fixed into irreparable mutations during DNA replication. The number of stem cell divisions is the number of stem cells times the rate of stem cell division in a certain tissue, and this finding firmly establishes cell division as the key quantitative driver of mutagenesis. Tomasetti and Vogelstein's conclusion that most cancer risk is due to random mutations arising during DNA replication in normal, noncancerous stem cells leads to the obvious conclusion that any factors that can modulate stem cell division rates would obviously have a large impact on cancer risk.

Decreased proliferation under CR is almost universal(Hursting et al., 2003). Moderate (20%) CR caused a more than six fold decrease in mouse bladder cell proliferation, while IGF-1 treatment caused an ~7-fold increase in proliferation (relative to 20% CR) (Dunn et al., 1997; Hursting et al., 2003). Organ-dependent differences are obviously large, but marked (up to 10-fold) drops in proliferation rate are common and generally quite significant with even low or moderate levels of CR (Hsieh et al., 2005; James and Muskhelishvili, 1994; Lok et al., 1988; Lok et al., 1990). Treating cultured cells with CR sera reduced cell proliferation, and IGF-1 and insulin supplementation reversed it(de Cabo et al., 2003). High fat diet-induced obesity promotes colonic stem cell expansion during cancer initiation(DeClercq et al., 2015). Cancer, in particular hematopoietic neoplasia (lymphoma), is extremely common and the dominant cause of death in mice(Blackwell et al., 1995; Lipman et al., 2004; Miller and Chrisp, 2002; Pettan-Brewer and M. Treuting, 2011; Storer, 1966; Ward, 2006). The powerful effect of nutrient and growth signaling on proliferation not only explains the large gains in rodent longevity with CR. It is probably not unreasonable to suspect that the, from an evolutionary viewpoint, unnaturally high prevalence of malignancy in many strains of laboratory

mice is a consequence of the unnaturally high food intake and the resulting obesity. Tumor incidence is decreased three- to six-fold in mice eating 40% less(Harbison et al., 2016). Cancer mortality is also associated with increased body mass in 82 breeds of dogs(Fleming et al., 2011), where breed differences in body size are determined significantly by a single IGF-1 allele(Sutter et al., 2007). IGF-1 is a powerful stimulator of cell growth and replication and elevated IGF-1 is a risk factor for several human cancers(Sonntag et al., 1999). Plasma IGF1 levels at 6 months age inversely correlate with median mouse lifespan across strains(Yuan et al., 2009) and human height is associated with longevity and risk of death from cancers unrelated to smoking(He et al., 2014; Samaras and Storms, 1992; Smith et al., 2000).

Caloric restriction is so well accepted as an age-retarding intervention because it delays almost all phenotypes of aging in such a coordinated fashion (Masoro, 1985, 1988, 2005; van den Boogaard et al., 2020; Yu et al., 1985). The ability of CR to delay aging might derive largely from its ability to reduce cell proliferation rate via evolutionary conserved nutrient signaling pathways. CR produces not only dramatic reductions in murine cancers(Speakman and Mitchell, 2011) but also reduced pathologies and increased life span(Sonntag et al., 1999). Severe stunting, low IGF-1, but increased longevity with protein restriction despite compensatory overeating (Miller et al., 2005; Solon-Biet et al., 2014) suggest anabolic growth through mTOR-controlled proliferation(Dowling et al., 2010), but not metabolism or calorie intake as the key determinant of organismal aging rate(Levine et al., 2014). Cell proliferation rates are exquisitely sensitive to nutritional status(Hsieh et al., 2005), especially proteins that regulate mTOR(Richardson et al., 2021). The acceleration of cell division by obesity and somatotrophic growth signaling (Bartke, 2019; Bartke et al., 2013) and its deceleration by CR(Dunn et al., 1997) and disruption of IGF-1 signaling(Berryman et al., 2008) powerfully implicate proliferation and accompanying mutation accumulation as the fundamental driver of aging.

2.9 The inner moat of carcinogenesis

Besides maintaining DNA integrity, long-lived life forms evolved a second line of defense against tumorigenesis: the neutralization of cells showing signs of malignant transformation. At least three of these tumor-suppressive mechanisms exist: cell senescence, differentiation and apoptosis(Fig. 1). Cell senescence artificially limits a cell's capacity for self-renewal (Campisi, 2013) and the robust association of senescent cells with cancer illustrates that cellular senescence primarily works as a barrier to malignant tumorigenesis *in vivo* (Burd et al., 2013). For the organism, stable cell-cycle arrest is not a bug, it's the feature of senescent cells and probably a cheap (in terms of necessary extra energy expenditure) and therefore effective way of limiting cancer growth. Telomere shortening, another hallmark of aging (López-Otín et al., 2013), is sometimes seen as a cause of aging by itself(Chakravarti et al., 2021; Whittemore et al., 2019). It is not. Telomerase is a powerful oncogene(Nault et al., 2013), as it provides unlimited replicative potential(Cong et al., 2002). Telomere shortening is insignificant in the stem cell compartment, where telomerase maintains telomere length(Flores et al., 2008), like in the germline. Unfortunately, most publications about "telomere length" investigate just circulating leucocyte telomere length(Epel et al., 2004), a convenient yet (in my opinion) largely meaningless parameter reflecting the amount of telomere shortening during hematopoietic stem cell differentiation, which might reflect inflammatory status but not aging(Carulli et al., 2016; Jongbloed et al., 2019; Laimer et al., 2016). Telomeres shorten rapidly during differentiation(Flores et al., 2008), and this is a highly effective mechanism to limit the tumorigenic potential of differentiating and expanding clones of cells.

Senescent cells can be seen as frozen cancer cells, arrested in their development before they could potentially kill the organism by turning into a cancer. Clonally expanded cells can make up significant portions of various organs and, despite none of them being cancerous, they form what seems like perfectly normal tissue (Martincorena et al., 2018; Martincorena et al., 2015; Tomasetti, 2019; Yokoyama et al., 2019). Growth arrest alone does not explain the functional decline seen in aging, as senescent cell burden probably remains low in many organs at an age when many phenotypes of aging are already apparent. But even few senescent cells can cause physical dysfunction (Xu et al., 2018) via the senescence-associated secretory phenotype (SASP)(Coppé et al., 2008) and bystander effects(Nelson et al., 2012). This is not unreasonable. The SASP is beneficial in wound healing (Demaria et al., 2014),

and the pleiotropic role of senescence(Campisi, 2005) and the associated SASP explain why apparently excessive chronic inflammation appears even when relatively few senescent cells are present(Xu et al., 2018): the inflammatory response protects the body against infection and injury, which was an extremely valuable function in prehistoric times(Finch, 2010). Widespread senescence only appears in the evolutionary shadow of old age. The possibly vicious circle of ubiquitous senescence, widespread SASP and chronic inflammation might be an evolutionary accident brought about by the unforeseen, unprecedented surge in human longevity.

For effective tumor suppression, cell senescence has to kick in well prior to tumor formation. Mutations are stepping stones to the, on average, four or five driver mutations present in a full-blown cancer(Cieslik and Chinnaiyan, 2020). Proliferation vastly increases the probability that any of its progeny will acquire additional mutations, not only by increasing the number of cells already harboring a critical mutation, but also by having increased rates of mutation-prone cell replication (Jaiswal et al., 2014). Cell senescence resembles a prison for attempted murder: any perceived attempt is (and has to be) treated as guilty. The number of genes able to drive clonal expansion and cancer is probably a few hundred (Bailey et al., 2018; Futreal et al., 2004; Tamborero et al., 2013) and more than 1% of all human genes are by mutation implicated in carcinogenesis (Sondka et al., 2018). Due to this diversity of oncogenes, senescence has to be triggered by the phenotype of (excess) proliferation and is broadly activated by both oncogene-driven as well as anabolic growth-driven replication. The term “oncogene-induced senescence” is somewhat of a misnomer. A cell cannot identify mutated genes, let alone classify them as oncogenic. Oncogene-induced senescence is established by phenotype, after the hyper-replicative phase following oncogene expression, and the hyper-replication causes DNA damage, which induces DNA damage response (DDR)-mediated senescence(Di Micco et al., 2006). The multiple stimuli that trigger senescence(Campisi and Di Fagagna, 2007; Hernandez-Segura et al., 2018) are those that were effective in preventing malignant growth. Evolving a mechanism that can reliably distinguish between dangerous, malignant and benign, physiological replication is probably impossible.

Whereas mechanisms to maintain genome integrity are classic MRFs which cost only energy, cell senescence is clearly pleiotropic. High ability for self-renewal allows for a youthful organism but favors cancer. Too little ability for self-renewal protects against cancer but leads the many hallmarks of aging. In groups with marginal rates of obesity like Japanese women (where average BMI is 21.7)(WHO, 2015), malignant growth is (despite low rates of smoking) the most common cause of death by far (~30%), outnumbering obesity-driven cardiovascular disease(Twig et al., 2016) approximately twofold(Ministry of Health, 2018). Life expectancy in Japanese women is 87.5 years(Ministry of Health, 2019). In contrast, in the US, heart disease slightly outnumbers cancer(Murphy et al., 2021), with obesity lowering life expectancy (to 76.2 and 81.2 years for males & females in 2018)(Murphy et al., 2021) and shifting the leading cause of death away from cancer. 50% deaths from cancer would be the “ideal balance” between too much and too little cell senescence that would be expected in a population aging according to the tumor suppressor theory of aging. However, cancer deaths stay below 50% due to effective cancer treatment, of which radiotherapy and chemotherapy, like endogenous cancer suppression, work primarily by inducing DDR-mediated apoptosis and cell senescence(Jackson and Bartek, 2009; Ou and Schumacher, 2018).

2.10 The tumor suppression theory of aging – appraisal of a hypothesis

The tumor suppression theory of aging postulates that most of the seemingly complex phenotype of aging emerges from mechanisms of tumor suppression, chiefly cellular senescence, but also apoptosis and differentiation (Fig. 1). These have evolved to limit the further proliferation of clonally expanding cells, or remove such cells from the regenerating stem cell pool.

An important conundrum in aging research is the apparent incompatibility of the metabolic malleability of aging with the concept of damage accumulation as the cause of aging. Aging represents, or is at least seen as, a collection of phenomena(Finkel, 2015) reflecting damage accumulation, as predicted from evolutionary arguments (Kirkwood,

2005, 2017b). Molecular mechanisms of damage, however, should diverge between organisms whose life span differs by several orders of magnitude, so pathways affecting aging should diverge as well. This is not easily reconciled with the observed malleability of aging via conserved pathways from yeast to mammals. This has led to controversial, programmed or quasi-programmed theories of aging that require regular debunking (Blagosklonny, 2006, 2013; de Magalhães, 2012; Gems and de la Guardia, 2013; Goldsmith, 2014; Kowald and Kirkwood, 2016; Libertini, 2014; Longo et al., 2005; Zimniak, 2012) or the postulated existence of “longevity programs” (Longo, 2019). Cell proliferation rates in mice are extremely sensitive to changes in caloric intake (Hsieh et al., 2005), and via the modulation of cell proliferation, the tumor suppressor theory of aging can explain the apparent metabolic regulation of aging with a mechanistic explanation, somatic mutations being a form of damage supported by convincing evolutionary as well as experimental evidence. The tumor suppression theory of aging also resolves another conundrum, the apparent mismatch of the disposable soma theory, which suggests life span to be limited by energy available for maintenance, with the effects of caloric restriction, which increases longevity despite less energy being available (Shanley and Kirkwood, 2000). Put more simply, why would the body not repair when energy is available, but instead do so when energy is scarce (Mitteldorf, 2001)? The solution, of course, is that when energy is available, the body invests heavily in growth and fitness to maximize reproduction, and this is achieved through accelerated replication. Accelerated aging is at this point only a minor, evolutionary irrelevant, side effect. On the other hand, CR forces a reduction of energy-intensive cell proliferation and, as a consequence, in the rate of aging.

2.11 Aging - really just tumor suppression?

Of the highly influential Hallmarks of Aging (López-Otín et al., 2013), only four (genomic instability, telomere attrition, epigenetic alterations and loss of proteostasis) are upstream, primary hallmarks that would qualify as underlying, proximal causes of aging. As explained above, telomere attrition is an elegant tumor suppression mechanism, but not a serious candidate for a proximal cause of aging. Epigenetic alterations accumulate with age (Rando and Chang, 2012) and can be used to derive impressive biomarkers of aging (Horvath and Raj, 2018), but depend on other causative factors such as DNA damage to explain their accumulation over time (Kane and Sinclair, 2019). Epigenetic alterations are involved in many of the changes observed with aging (Pal and Tyler, 2016), but the plasticity and reprogrammability of epigenetic markers, while encouraging as a target of interventions (Lardenoije et al., 2015; Ren et al., 2017), is poorly compatible with the observed irreversibility of aging. Steady state DNA damage or genotoxic stress can result in cellular functional decline (Vermeij et al., 2014) and impinges on many secondary hallmarks of aging, most notably by its ability to drive cells into a DNA damage response, apoptosis and senescence (Yousefzadeh et al., 2021a). Lumped together with somatic mutations, DNA damage can explain many aspects of aging and cancer (van den Boogaard et al., 2020). However, like epigenetic alterations, DNA damage is reversible (Hoeijmakers, 2009). Mechanisms of DNA damage are mostly chemical and species-independent and steady state nuclear DNA damage is not correlated with species life span (Barja and Herrero, 2000), so explaining the heterogeneity of life spans with DNA damage is difficult.

The ability of enhanced tumor suppression to induce premature aging (Beausejour and Campisi, 2006; Campisi, 2005; Donehower, 2002; Ferbeyre and Lowe, 2002; Keyes et al., 2005; Sharpless and DePinho, 2007; Tyner et al., 2002) powerfully supports the tumor suppression theory of aging. The ability to induce premature aging (Kujoth et al., 2005; Trifunovic et al., 2004), however, doesn't necessarily prove a significant involvement in normal aging (Vermulst et al., 2007). At least with the current, unreliable markers of senescence, senescent cells do not seem to accumulate to high proportions (Wang et al., 2009; Yousefzadeh et al., 2020), and some cell types, notably epithelia, do not seem to senesce (at least when applying standard markers of senescence) (Giangreco et al., 2008; Stern and Bickenbach, 2007), but instead die or differentiate (Gandarillas, 2012; Gandarillas and Watt, 1997; Ying et al., 2018) to remove them from the stem cell pool (Fig. 1). Methods to detect and quantify mutations and evaluate their spread in tissues due to clonal expansion are still rapidly developing (Laurenti et al., 2021). A definitive consensus on the extent of clonality and its organ-specific development with age is still some time ahead. Insufficient quantitative evidence as to how and where mutations cause disease-relevant stem cell exhaustion is for the moment

certainly an important weakness in the tumor suppression theory of aging, but downright stem cell depletion is in many cases not necessary to explain impaired stem cell function. Senescence represents many diverse cellular states united only by stable proliferative arrest (Van Deursen, 2014). Consistent markers are lacking (Sharpless and Sherr, 2015), but this is not surprising when keeping in mind that senescence is triggered by diverse signs of impending tumor formation. There are large gaps in tracking senescent cells *in vivo*, let alone quantifying which kind of cells die, and why, during natural turnover or senolytic treatment. Several attempts to mark senescent cells with a specific promoter driving expression of red fluorescent protein markers for *in vivo* imaging did not seem to result in any presentable fluorescence images (Jeon et al., 2017; Liu et al., 2019). Due to differences in cell division history and frequency, organ- and cell-specific variations in the load of somatic mutations are large. Skin, an organ exposed to mutagenic UV radiation, accumulates such huge numbers of mutations that in aged, sun-exposed yet anatomically normal epidermis, expanded clones make up large patches of tissue (Martincorena et al., 2015). Notch inactivating mutations not only trigger immortalization, but also promote differentiation of adjacent wild type cells, effectively replacing entire sections of epithelium (Alcolea et al., 2014). Patches of seemingly normal but essentially premalignant epithelium are continuously eliminated by the immune system, as revealed by the more than 100-fold increase in skin cancer rates after long-term immunosuppression (Euvrard et al., 2003). Such patches can be recognized by the immune system because the number of mutations they carry (Martincorena et al., 2015) is higher than most human cancers (Alexandrov et al., 2013) and enough to allow distinction from self. Driver mutations seem to promote growth only during the initial expansion of mutant clones (Martincorena et al., 2016), hinting at the induction of a growth-limiting form of cell senescence in the absence of classical senescence markers. How fast powerful mutagens like UV radiation drive cell senescence and aging is apparent in the perfection of skin in small children (despite injury and regeneration alternating at astonishing speeds), which is lost even before reaching adulthood. In esophageal epithelium, expanded clones make up 40 to 80% of tissue in patients over 60. Like in skin, Notch-mutated clones can colonize large patches of normal epithelium, and driver mutations can be acquired in infancy as an inevitable consequence of normal aging (Martincorena et al., 2018; Yokoyama et al., 2019). Notch-mutations were more common in normal tissue than in esophageal cancer, suggesting that vigorous growth in notch-mutated clones might trigger tumor-suppressive mechanisms that effectively inhibit later carcinogenesis (Martincorena et al., 2018). In older liver, mean and median expanded clone volumes were quite large (1.14 mm^3 and 0.59 mm^3) and increased with fibrosis and tissue injury (Zhu et al., 2019). The nodular architecture of cirrhotic liver restrained clonal growth (Zhu et al., 2019). Like in esophagus, some recurrent mutations were more frequent in normal tissue than in liver cancer, suggesting a potentially tumor-suppressive effect of certain kinds of mutation-driven rapid proliferation, some of which might even increase hepatic fitness (Zhu et al., 2019). In colonic epithelium, only 1 % of crypts harbored driver mutations. Crypt fission was rare and not associated with driver mutations, indicating that the peculiar architecture of the colon is a barrier to the spatial expansion of clones (Lee-Six et al., 2019). On the other hand, clones of respiration-deficient cells harboring near homoplasmic mtDNA mutations constitute above 10 and 15% of crypts in those over 70 and 80, respectively (Taylor et al., 2003), induce crypt fission (Greaves et al., 2006) and a metabolic rewiring that favors malignant transformation (Nikkanen et al., 2016; Smith et al., 2020). So at least in colon, not only nuclear, but mtDNA mutations as well might contribute to organismal aging by driving clonal expansion and senescence (Wolf, 2021b).

Clonal expansion by itself (without necessarily depleting a pool of stem cells) seems to cause many phenotypes of aging as well. Selection of preleukemic clones with mutations imparting growth or survival advantages is assumed to underlie aging (Rossi et al., 2008) and, of course, carcinogenesis (Xie et al., 2014) in the hematopoietic stem cells (HSC) compartment. The diminished reconstituting ability of HSCs from elderly donors shows the decay in HSC function (Sharpless and DePinho, 2007), even though the HSC compartment compensates for a decreased regenerative capacity and skewed differentiation (Geiger et al., 2013; Rossi et al., 2005) with increased self-renewal (Signer and Morrison, 2013; Sun et al., 2014) and elevated absolute HSC numbers (de Haan and Lazare, 2018; de Haan et al., 1997). Impaired hydroxylation of 5-methylcytosine caused by TET2 mutation (Ko et al., 2010) leads to increased HSC self-renewal, splenomegaly (Moran-Crusio et al., 2011), clonal expansion, myeloid transformation and malignancy (Quivoron et al., 2011), and increased risk for coronary heart disease (in humans) (Jaiswal et al., 2017), all very much resembling the changes seen in aging (de Haan and Lazare, 2018; Geiger et al., 2013). Clonal expansion of

481 a plasma cell manifesting in monoclonal gammopathy is present in 3.2 percent of patients over 50, increases with
482 age (Kyle et al., 2006), and is an important risk factor for and precursor to multiple myeloma(Weiss et al., 2009).
483 Clonal hematopoiesis without other hematologic abnormalities (CHIP), almost doubles risk for coronary heart
484 disease(Jaiswal et al., 2017). Even though only $\approx 20\%$ of leukocytes are of clonal origin on average, CHIP carriers have
485 a markedly increased risk of myocardial infarction, stroke, perhaps venous thrombosis and pulmonary
486 embolism(Libby and Ebert, 2018) and higher all-cause mortality(Zink et al., 2017). Detection technology determines
487 sensitivity and therefore estimated prevalence and extent of clonal hematopoiesis, which has been estimated from
488 more than 10% of persons older than 70 years(Jaiswal et al., 2017), to about 25% in 65-75 year olds and more than
489 half in those over 85, when using whole genome data(Zink et al., 2017). The senescence marker p16INK4a
490 exacerbates age-associated HSC dysfunction(Janzen et al., 2006) and a single nucleotide polymorphism (SNP)
491 adjacent to p16INK4a increases risk of myocardial infarction 1.64 times(Helgadottir et al., 2007). p16INK4a inhibition
492 has been proposed to improve aged tissue injury repair (Janzen et al., 2006) and senescent HSC removal could
493 reportedly rejuvenate aged HSCs in mice(Chang et al., 2016). Systemic aging induced by a senescent HSC
494 compartment suggest wide-ranging cell non-autonomous effects promoting aging in non-lymphoid
495 organs(Yousefzadeh et al., 2021b).

496 Human mesenchymal stem cell numbers in bone marrow decline with age(Stolzing et al., 2008). Supporting
497 senescence as a cause of stem cell depletion, muscle stem cell numbers decline with age(Sousa-Victor et al., 2015),
498 and p16INK4a switches them from quiescence to senescence(Sousa-Victor et al., 2014), leading to a shrinking stem
499 cell pool. A SNP near p16INK4a is associated with severely limited physical function in older people(Melzer et al.,
500 2007). Extensive proliferation of a low proportion of highly plastic vascular smooth muscle cells (VSMCs) results in
501 VSMC accumulation after injury and in atherosclerotic plaque formation (Chappell et al., 2016; Wirka et al., 2019).
502 Atherosclerotic plaques derive from clonal expansion of a single(Misra et al., 2018) or few (Jacobsen et al., 2017)
503 VSMCs that give rise to all the smooth muscle-derived cells comprising the majority of cells in an advanced
504 plaque(Jacobsen et al., 2017; Misra et al., 2018). Whether clonal expansion is causally involved in human
505 atherosclerosis as well is certainly a key question in understanding this extremely important human pathology.

506 DNA damage is a, if not the, major determinant of mutation accumulation, so DNA damage obviously play a central
507 role in driving the aging process. Associations of DNA repair with life span have been observed long ago(Hart and
508 Setlow, 1974). The DDR plays a central role in determining cell fate as one of several triggers that eliminate cells
509 from the stem cell pool through senescence(Hernandez-Segura et al., 2018), apoptosis(Roos and Kaina, 2013) and
510 differentiation(Harris, 1990; Inomata et al., 2009). These mechanisms evolved to limit tumorigenesis, but might also
511 eliminate damaged cells and exhaust the stem cell pool without clonal expansion. It is somewhat premature to
512 speculate about the relative contribution of replication-dependent mutation accumulation versus replication-
513 independent DNA damage-mediated effects to organismal aging. Naturally, accumulating DNA damage is better at
514 explaining aging in post-mitotic tissues and infrequently dividing cells.

515 Induction of replication in normally quiescent stem cells increases ROS, which trigger cell senescence (Sharpless and
516 Sherr, 2015). ROS activating p38 MAPK limit the lifespan of hematopoietic stem cells (HSCs)(Ito et al., 2006). ROS
517 cause telomere shortening and limit the carcinogenic potential of expanding cells by inducing telomere-dependent
518 senescence (Passos et al., 2007). Activation of quiescent HSCs leads to ROS-mediated DNA damage and stem cell
519 attrition(Walter et al., 2015). mTOR-mediated expansion of T cells also induced ROS (Mak et al., 2017; Sena et al.,
520 2013), and loss of APC in intestinal stem cells triggers RAC1-driven ROS production during colorectal cancer initiation
521 (Myant et al., 2013). ROS production therefore seems to be a shared physiological response occurring during stem
522 cell exit from dormancy and proliferation, resulting in DNA damage and cell senescence that is maintained by a
523 feedback loop (Passos et al., 2010; Takahashi et al., 2006) designed to trap cells in a senescent state and limit the
524 proliferation of cells once outside a carefully maintained stem cell niche. This tightly connected interplay of ROS,
525 DNA damage and telomere shortening triggered by cell division and expansion illustrates the obsessive-compulsive
526 preoccupation of long-lived mammalian cells with tumor suppression. Much of the DNA damage deciding cell fate
527 might be elicited as a consequence of proliferation and replication stress(Di Micco et al., 2006; Gaillard et al., 2015;

Zeman and Cimprich, 2014), so that while DNA damage certainly plays a central roles in the aging process(Schumacher et al., 2021), DNA damage is a signaling intermediate(Flach et al., 2014).

DNA damage is used as a primary driver of tumor suppression mechanisms like senescence and apoptosis because of a fundamental problem in cell fate decision: mutations are invisible. Once a mutation is established during cell division, it is indistinguishable from its genuine, non-mutated counterpart. To limit malignant transformation, cells have evolved DNA damage as a proxy for potentially oncogenic DNA lesions mostly out of necessity (telomere shortening is another proxy). This is the reason why DNA damage is integrated into so many elaborate mechanisms of tumor suppression. Because DNA damage is a useful sign of dangerous damage and excess proliferation, it contributes to aging as a mediator of cell elimination despite not being the true cause of aging.

Further arguments against DNA damage (opposed to mutation-driven clonal expansion or excess proliferation in general) as a proximal cause of aging include that short- and long-lived species differ in mutation rates(Milholland et al., 2017) but not in nuclear DNA damage rates(Barja and Herrero, 2000). CR does not reduce DNA damage significantly in humans(Heilbronn et al., 2006). In mammals, long-lived and/or larger species tend to induce DDR-mediated apoptosis or senescence to prevent malignancy, whereas short-lived species tolerate genomic instability(Croco et al., 2017). Peto's paradox(Peto, 2015) and the centrality of mutation and proliferation in cancer(Tomasetti et al., 2017) and tumor suppression(Campisi et al., 2001; Franceschi, 1989) also argue against a simple age-related increase in DNA damage as the proximal cause of aging. Rapamycin is probably the most powerful age-retarding pharmacological intervention because mTOR sits at the end of several proliferative signaling chains (Sudarsanam and Johnson, 2010), including that from IGF-1 to PI3K, PTEN, Akt and finally mTOR (Chappell et al., 2011; Singh et al., 2019). Rapamycin is something like an anti-proliferative kill switch(Murakami et al., 2004), since mTOR integrates diverse environmental cues to control growth and proliferation, processes that use large amounts of energy and nutrients(Laplane and Sabatini, 2012). Rapamycin would therefore, despite scary side effects(Johnson and Kaeberlein, 2016; Laplane and Sabatini, 2012; Salmon, 2015), be efficient in retarding aging mediated by mutation and clonal expansion(Johnson et al., 2013; Martinet et al., 2014). Protein restriction reduces mTORC1 signaling only in male mice(Richardson et al., 2021), and it's male-only sex-specific effects resemble that of rapamycin(Strong et al., 2020), suggesting that proliferative mTOR signaling is the key component of nutritional life span extension, at least against an obese control. If DNA damage would cause aging, increased cell proliferation would, at least in proliferating tissues, be expected to slow aging, since replication is accompanied by DNA repair and stringent cell cycle checkpoints.

2.12 Limitations of the tumor suppression theory of aging

The tumor suppression theory of aging certainly cannot explain all aspects of aging. The theory cannot explain aging of invertebrates such as yeast or postmitotic worms. Long-lived proteins such as the lens (protein) in the human eye age independent of cell replication. Turnover of human cardiomyocytes(Bergmann et al., 2009) and adipocytes(Spalding et al., 2008) is slow. Similarly, neurons are essentially postmitotic and might lose function with age independent of mutation and cell replication. However, recent, and therefore still potentially unreliable results in mice suggest that some loss of brain function with age might be caused by cell senescence in supporting cells other than neurons(Bussian et al., 2018; Zhang et al., 2019).

Mechanisms of aging mediated by steady state DNA damage probably complement replication- and clonal expansion-mediated aging, but are also sufficiently different conceptually to treat and think of them as separate mechanisms. DNA damage-mediated loss of function might certainly play an important role in the age-related functional decline of post-mitotic tissues(Lu et al., 2004). Old cells exist in a variety of organs(e Drigo et al., 2019), and even though their functional significance is not yet clear, their influence would be independent of mutation and clonal expansion. Loss of genomic integrity (if this is meant to include both DNA damage and somatic mutations) is probably the most important causal mechanism of aging, if there is any remotely dominant single cause. And while a

lot of DNA damage determining cell fate decisions might be a consequence of proliferation (Di Micco et al., 2006), and stem cell exhaustion the consequence of tumor suppression mechanisms (Janzen et al., 2006), DNA damage-mediated cell elimination might be antagonistically pleiotropic: it eliminates cancer during early and middle life, but in late life, it might in many cases be triggered “by mistake”, i.e. due to DNA damage that has accumulated with age but in the absence of oncogenic mutations, and therefore contribute to the exhaustion of stem cells (Fig. 1).

Replication-independent aging might nevertheless be a relatively minor problem in the evolution of long lifespans. Despite lens proteins being much the same in humans and mice and experiencing similar wear and tear, temperatures, oxygen concentrations and exposure to external sources of damage, mice develop cataracts even though they age much faster than humans (Wolf et al., 2000). Why do human lenses last ~ 20 times longer? Good eyesight is valuable so natural selection has eliminated the earlier cataract formation we inherited from our short-lived ancestors, and selected those that make better lenses so that the decay of lens protein is slowed enough to make the lens last the human lifetime. Age-related hearing loss displays similar characteristics, appearing in old mice (Brown et al., 2008; Johnson et al., 1997) despite a mouse cochlea being built much the same as a human cochlea. Long-lived proteins such as lenses, dermal collagen or joints, and organs like the cochlea are relatively small parts of the human body, and the energy cost necessary to make them last longer is probably negligible compared to the benefit of slower aging.

The tumor suppression theory of aging postulates cancer and aging as the direct and indirect consequence of somatic mutations, with increased cancer and premature aging as the two principal outcomes of defective genome maintenance. Why one can dominate over the other seems to depend on the specific molecular nature of the defect. Elevated carcinogenesis seems to be the dominant phenotype of defective (error-prone) DNA replication, which probably produces somatic mutations in the relative absence of DNA damage (Heitzer and Tomlinson, 2014; Prolla et al., 1998). On the other hand, elevated DNA damage and the specific molecular nature of it might favor the induction of tumor suppression and cell elimination, leading to premature aging rather than cancer (Yousefzadeh et al., 2020), as is observed after artificially elevated tumor suppression (Ferbeyre and Lowe, 2002). Predicting whether cancer or aging is the penetrant phenotype of defective genome maintenance remains difficult (De Renty and Ellis, 2017) and whether cancer or aging is the dominant outcome probably also depends on organ-specific properties such as replication frequencies, the tissue microenvironment and other unknown factors.

2.13 Consequences for delaying aging

Removal of senescent cells would currently seem to be the only widely discussed intervention that offers reasonable promise to alleviate some aspects of aging. The large variation in the pace of tissue- and cell-specific mutation accumulation (Wang et al., 2009; Yousefzadeh et al., 2020) suggest that aggressive, global removal of senescent cells might have unpredictable side effects, but also that stem cells with no or few oncogenic mutations should remain in most organs, and these cells might regenerate the organ after senescent cells are removed. Removing senescent cells can apparently reverse some aspects of aging (Baar et al., 2017; Baker et al., 2016; Baker et al., 2011) and seems to have convinced many that senescent cells are responsible for many, if not most, of the aging phenotypes (Bhatia-Dey et al., 2016; Borghesan et al., 2020; McHugh and Gil, 2018). Even in the brain, an organ in which even partially preventing or reducing aging is seen as particularly difficult due to the central role of largely post-mitotic neurons, clearing senescent astrocytes and microglia could reportedly prevent pathology and preserve cognitive function (only in mice, of course) (Bussian et al., 2018). Removal of senescent oligodendrocyte progenitor cells was also reported to ameliorate cognitive deficits in a model of Alzheimer's disease (Zhang et al., 2019). Given that preserving brain function is so indispensable for successful aging, these findings are certainly cause for optimism. On the other hand, prominent clinical failures (Dolgin, 2020) show that, despite the same substance being very effective in pre-clinical research (Jeon et al., 2017; Villanueva, 2017), in the real world, senescent cells and their deleterious effects might not melt away like the catchy name “senolytic” suggests. This can have many reasons, but given the in some cases additional commercial incentive to represent results as encouraging as possible (De Magalhães et al.,

2017), one can only assume that some longevity research is even less reproducible than life science in general (Drucker, 2016). Independent evaluation of small molecule senolytics in the NIA Interventions Testing Program (Nadon et al., 2017) might, if successful, add welcome reassurance and credibility to senescent cell removal as a viable anti-aging intervention. Furthermore, small molecule agents might be a blunt weapon of questionable specificity against senescent cells. If surface markers specific for even fractions of senescent cells do exist, eliminating key populations of senescent cells using CAR T cells might be a more targeted approach with less side effects (Amor et al., 2020).

Senescence is not the only way to deal with malignant and premalignant cells: eliminating them by apoptosis is an alternative option. Apoptosis seems to be favored by animals with very large body sizes (Abegglen et al., 2015; Sulak et al., 2016; Vazquez et al., 2018). Nudging pre-malignant cells towards apoptosis rather than senescence might be one way of reducing the burden of senescent cells and retard some phenotypes of aging. The increased cancer resistance that many large animals had to evolve independent of humans (Vazquez and Lynch, 2021) might very well give valuable hints at how to suppress cancer in humans as well (Keane et al., 2015; Seluanov et al., 2018).

Why did large, long-lived animals develop additional tumor suppression, but not better DNA repair and copying mechanisms? Gene duplications are a common genetic event (Reams and Roth, 2015), so enhanced tumor suppression via duplication of tumor suppressors (Vazquez and Lynch, 2021) might be genetically more pliable (Wagner, 2011). Better DNA repair might also be quite costly, since top level stem cells constitute only a small part of the body, whereas unnecessary maintenance and removal of DNA damage in already differentiated cells might constitute a considerable waste of energy.

If cancer and aging are both driven by somatic mutation, slowing it down is the only plausible way to prevent aging in healthy weight humans. Like for the primary prevention of cancer (Tomasetti et al., 2017), reducing the error rate of our DNA polymerase or more DNA repair prior to cell division would be expected to help. Also annoying is the unavoidable temperature-sensitive spontaneous deamination of cytosine residues to uracil or thymidine (Duncan and Miller, 1980; Nabel et al., 2012), which has to be constantly repaired (Krokan and Bjørås, 2013) and might contribute significantly to glioblastoma, prostate, colorectal and other cancers (Martincorena and Campbell, 2015; Poulos et al., 2017). But even if primary prevention of most somatic mutations seems impossible for now, this does mean that all humans age at the same rate. Relatively little insight into the complex genetic determinants of human longevity and the large genetic diversity of humans also suggest that many factors determining human maximum lifespan remain undiscovered. The moderate yet significant heritability of longevity proves that genes produce variations in the individual rate of aging (Brooks-Wilson, 2013; Kaplanis et al., 2018; Robine and Allard, 1998; Vijg and Suh, 2005). Genome-wide association studies using the rate of mutation accumulation rather than achieved life span might be much more sensitive towards revealing genetic variations associated with better maintenance of genomic integrity in humans and should be feasible given the exploding amounts of genetic data available (Kaiser, 2015; Turnbull et al., 2018).

In addition, strategies to reduce DNA damage would certainly be an important tool to slow aging as they would reduce the rate of somatic mutation per cell division. Transcriptional regulation of maintenance functions such as DNA repair might be modulated favorably by hormesis. Efforts to increase the activity of protective transcription factors like NRF2 have been ongoing for years (Maher and Yamamoto, 2010; Martín-Montalvo et al., 2011; Strong et al., 2016), but the unwanted promotion of metastasis seems to be a reoccurring side effect of increased resistance to oxidative stress (Piskounova et al., 2015; Schafer et al., 2009; Sporn and Libby, 2012) and explains the cancer-promoting effect of increased NRF2 (de la Vega et al., 2018) and anti-oxidant supplementation (Albanes et al., 1996; Bjelakovic et al., 2007; Group, 1994; Lignitto et al., 2019; Wiel et al., 2019).

There is certainly potential for human life extension. Many humans have access to an essentially unlimited supply of food, and would be able to invest heavily in genome maintenance without starvation. Spending more energy on maintaining DNA integrity might even allow some weight loss without having to exercise. The immune system (a critical factor for early survival) might be modulated and chronic sterile inflammation subdued to increase health

span or even reverse some aspects of aging(Adler et al., 2007; Yousefzadeh et al., 2021b). Except during a viral pandemic, reducing activity of cytidine deaminase (Vieira and Soares, 2013), which converts cytosine to uracil in viral, but also in exposed single-stranded loops of normal DNA and leads to characteristic G->A mutations (Petljak et al., 2019), might be worthwhile, as such mutations can make significant contributions in cervical, bladder, head-neck and lung cancer(Martincorena and Campbell, 2015).

3. Conclusion

The current sorry state of aging research is attributable to two major problems. As laid out in detail in the preceding publication (originally part of this one(Wolf, 2021a)), caloric restriction has probably been misinterpreted as delayed aging. This might seem surprising given the lack of insight and consensus on what causes aging, but is understandable, since, against an obese control, caloric restriction really does delay most aspects of aging. And the vast majority of laboratory animals given unlimited access to food are very obese. But weight loss induced through pharmacologic or genetic means is also often ignored or mistaken for a reduction in the rate of aging. Quite a number of chemicals can, at an optimized, non-toxic dose, induce weight loss, and the accompanying increase in life span is often attributed to their effects on specific pathways rather than to generic weight loss(Wolf, 2021a). Over the past two or three decades, many academics working on retarding aging were apparently convinced that great strides have been made in tackling, or even solving the scientific problem of aging due to a mistaken exuberance about what appeared to be breakthroughs in the manipulation of mammalian aging(Campisi et al., 2019; Partridge et al., 2020). This has led to the impression that human longevity should be malleable using pharmacologic or even nutritional interventions(Fontana and Partridge, 2015; Madeo et al., 2019).

The second problem is the lack of progress on elucidating the molecular mechanisms of aging. This is, in my opinion, not due to a lack of effort, as some seem to think(Hayflick, 2021). I think it is more due to the pervasive acceptance of CR as delayed aging, and the resulting focus on elucidating its molecular mechanisms(Sciences, 2009). Medicine can be successful without understanding causality, and discover life-saving interventions by careful observation and trial and error. This convenient strategy, the ignorance of obesity and the convenience of mice and letting them eat how much they want, resulted in the acceptance of CR and other weight loss agents as interventions that prevent aging, not just obesity. To be worthy of public trust and funding, aging research really needs to open its eyes to what has been in plain sight for many years now, the failure of CR to delay aging in anything else than gluttonous obese laboratory animals(Mattison et al., 2012) and their human counterparts. But rather than those trying to defend CR with invertebrate distractions and linguistic trickery(Fontana et al., 2010), Planck's principle looks more likely to eliminate the sceptic(Hayflick, 2010).

On the other hand, thanks to the high prevalence of obesity in regions of middle and high incomes, many of the CR mimetics discovered in mice might turn out to be excellent weight loss drugs. Metformin, for example, can, through the induction of weight loss, prevent diabetes and many other ailments related to excess body weight in a high risk (i.e. obese) population(Knowler et al., 2002). Whether this is labelled prevention of aging, prevention of disease, or simply prevention of obesity might be important for politics, marketing or funding, but is irrelevant from a medical perspective. Of course, CR mimetics might have positive effects beside weight loss, but these should be convincingly demonstrated in a non-obese cohort before insinuating any beneficial impact on health or life span in normal weight individuals.

The disposable soma theory of aging, with its (correct) prediction of damage to macromolecules as the ultimate cause of aging(Kirkwood and Holliday, 1979) might have led many to think of (besides cancer, of course) damage and loss of function as the primary consequence of somatic mutation(Vijg and Dong, 2020; Vijg et al., 2017; Vijg and Montagna, 2017). The tumor suppressor theory of aging, if it were true, would put forward three new concepts in

thinking about aging: First, mutations driving excess cell division are proposed as the main, proximal cause of aging and the relevant type of somatic mutation, not (like the prevailing view) those causing loss or impairment of function. Mutations arise mostly during proliferation, so second, the pace of stem cell division is concluded to determine the rate of aging, together with the mutation rate per mitosis. This makes the tumor suppression theory of aging compatible with findings linking longevity to growth, nutrition and obesity through their effects on the rate of cell division. And third, dedicated tumor suppression mechanisms like cell senescence are postulated to cause most of the phenotypes of functional decline that characterize aging. Cancer and aging would be seen as the side effects of too much and too little capacity for self-renewal, the latter having evolved to suppress the further. Because it rests significantly on evolutionary arguments, the tumor suppression theory of aging, like the antagonistic pleiotropy and disposable soma theories of aging, explains primarily why we age: because the positive effect of tumor suppression through senescence and cell elimination was large enough to improve reproductive success. Since the etiology of cancer is well understood(Michor et al., 2004; Vogelstein et al., 2013), it is concluded that somatic mutations are, albeit indirectly, responsible for aging as well. Germline mutation accumulation rates, a reasonable proxy for the genetic accuracy of cell division in individual humans, predict longevity and reproductive life span(Cawthon et al., 2020). Human subjects in the top and bottom quartile seemed to have an approximately two-fold difference in their germline mutation rate, and a 4.7 year difference in life span(Cawthon et al., 2020).

4.1 An inconvenient truth

If the tumor suppression theory of aging would be correct, the only way to retard human aging would be a reduction of somatic mutation. Preventing of aging would be the same as preventing cancer. Even before the Tomasetti and Vogelstein death knell(Tomasetti et al., 2017), primary prevention of cancer was an uphill struggle. For example, despite its notoriousness in nutrition research, there is limited evidence for the carcinogenicity of red meat in humans(Bouvard et al., 2015), and a complete overhaul of western dietary habits is only “likely” to result in a modest (~ 10%) reduction in risk for certain cancers(Bouvard et al., 2015; Chan and Giovannucci, 2010). As suggested (Tomasetti et al., 2017), secondary prevention measures like, for example, colonoscopic polypectomy (Winawer et al., 1993; Zauber et al., 2012) are more realistic and offer better returns.

Unfortunately, reduction or prevention of somatic mutation is something that remains thoroughly out of reach of current medical technology. The problem of aging will probably defy the assault of human ingenuity for some time to come.

In the meantime, removal of senescent cells seems to offer a reasonable chance of alleviating many phenotypes of very old age(Amor et al., 2020). Cell senescence is antagonistically pleiotropic, and accumulation of senescent cells probably an evolutionary accident brought about by the unforeseen increase in average human life expectancy. Together with a second antagonistically pleiotropic phenomenon, the age-related emergence of systemic and excessive chronic sterile inflammation(Furman et al., 2019; Ridker et al., 2017), these two phenomena might be mutually reinforcing evolutionary accidents responsible for many of the pathogenic processes promoting the irreversible functional decline of very old age. Evolution shaped human physiology, and survival of childhood was the biggest hurdle for individual reproductive success (Finch, 2010; Volk and Atkinson, 2008). A vigorous immune system helps enormously to survive childhood, but probably haunts modern humans now regularly surviving into the evolutionary shadow(Yousefzadeh et al., 2021b). Even though they do not prevent aging per se, in terms of looking at realistic strategies for increasing human health span, these two processes are, compared to the primary prevention of mutation, probably lower hanging fruit and offer plentiful possibilities in postponing the dreaded symptoms of old age.

753 **Author contributions**

754 A.M.W. wrote the paper.

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756 **Conflict of interest statement**

757 The author declares no competing interests.

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Figure 1. The dual fate of cells acquiring oncogenic mutations. As mutations (x) inevitably accumulate with age, malignancy or its suppression is the ultimate fate awaiting both individual cells and the organisms as a whole. The tumor suppressor theory of aging proposes that (what we experience as) aging is the consequence of tumor suppressive mechanisms that have evolved to counter the threat of malignancy in expanding clones of cells by inducing cell senescence, differentiation and apoptosis.

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Highlights

- Somatic mutations accumulating during cell division are the cause of aging and cancer
- Nutrient signaling paces aging through its effect on cell proliferation rate
- Only oncogenic mutations causing proliferation and clonal expansion are important, functional impairment is mostly irrelevant.
- The phenotypes of aging are mostly the consequence of the tumor suppressive mechanisms of (stem) cell senescence, apoptosis and differentiation
- Cancer and aging are the consequence of too much and too little capacity for self-renewal, and the latter has evolved to suppress the further

Supplementary Materials for

The Tumor Suppression Theory of Aging

Prepared July-August 2021 & submitted to Mechanisms of Ageing and Development

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Supplementary Figure 1.

(a) Age-dependent underlying cause of death in the USA in 1999-2019.

(b) Cause of death after removing infectious and parasitic diseases (ICD-10 codes A00-B99), congenital malformations, deformations and chromosomal abnormalities (Q00-Q99), conditions originating in the perinatal period (P00-P96) and external causes of morbidity and mortality (V01-Y89, which are mostly accidents and suicides).

(c) Estimated age-dependent cause of death to after adjusting for assumed prehistoric cancer mortality (5-fold increase for ages 1-19, 3-fold increase for ages above 20) and death from cardiovascular disease (age-independent 75% reduction).

Supplementary Figure 2. Risk of death from non-infectious disease during each 4 or 5-year interval (dotted lines) and cumulative survival before (as in Supplementary Figure 1b) and after (as in Supplementary Figure 1c) adjustment for prehistoric cancer survival and cardiovascular disease incidence.

Supplementary Methods

Underlying cause of death data were obtained from the United States Centers for Disease Control and Prevention CDC WONDER database (<https://wonder.cdc.gov/Deaths-by-Underlying-Cause.html>; “1999-2019 Underlying Cause of Death by Bridged-Race Categories”) (Friede et al., 1993). The mortality data are based on information from all death certificates filed in the fifty states and the District of Columbia. Deaths and population were grouped into ICD-10 chapters and five-year age groups from all regions, races and genders from years 1999 to 2019. All times and places of death were included, and deaths were counted regardless of whether an autopsy was performed or not. Data was adjusted and figures were created using Microsoft Excel.

Data Source

United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS), Underlying Cause of Death 1999-2019 on CDC WONDER Online Database, released 2020. Data are compiled from data provided by the 57 vital statistics jurisdictions through the Vital Statistics Cooperative Program. Accessed at <http://wonder.cdc.gov/ucd-icd10.html>.

Supplementary References

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The tumor suppression theory of aging

Reviewer #1: Once again I am positively surprised how well the manuscript has been developed since its original submission. It now stands as a solid, inspiring and thought-provoking piece of literature with a robust set of literature backing up the hypotheses quite well (in most places). At times, the author managed to provide a well-balanced discussion between different concepts and hypothesis on ageing, as well as to describe pieces of evidence, which are inconvenient for his hypothesis, which is admirable.

The manuscript is heavily opinionized, but in contrast to the previous piece (on the DR fallacy), here this style is better justified. One thing I would suggest is to change the type of the paper from "research paper" to "opinion paper", or "perspective paper" (I regret that categories such as "theory paper" or "conceptual paper" are missing in many journals).

The Guide to Authors for Mechanisms of Ageing and Development specifies only “research paper” and “review” as the two choices available. Opinion articles are by invitation only, and a “perspective paper” category does not seem to exist.

I would be happy to change the type of the paper if the editors offer a way to accommodate this request, but article type does not seem to appear in the final print layout anyway. The only distinction made between article types seems to be a designation in the table of contents. Currently this designation would be “research article”. I would be perfectly fine with this, as this designation is only a very minor point. I am not sure if changing this is really worth the hassle.

Important is only that style and structure of the article are appropriate for its content. As indicated below, this journal seems to be flexible and accommodating towards structuring the manuscript like a theory paper, which I hope to have done as requested below.

One final thing I would recommend is to change the structure of the manuscript. There must be a better way to divide it than into: 1. Introduction (~half page), 2. Tumor suppression theory of aging (~7 pages) and 3. Discussion (~7 pages) and 4. Conclusions (~2 pages). As the discussion is usually reserved for the research manuscript, I believe all the pieces from that part could be incorporated into the main part of the manuscript. Then the manuscript could consist of introduction, "body" (including 12 of its sub-chapters) and conclusions.

The current structure including the “Discussion” subheading was adopted merely to follow the Guide to Authors instructions for the category “research paper” (which suggest Introduction-Material and methods-Results-Discussion-Conclusions-Appendices for original research papers).

Looking at other articles in Mechanisms of Ageing and Development, I found that subheading titles can apparently be changed or removed to reflect article content. Closely following the suggestion of reviewer #1, I have removed the (unnecessary and somewhat arbitrary) division between the main parts, merging them by replacing “3. Discussion” with “2.10 The tumor suppression theory of aging – appraisal of a hypothesis”, since at this point, the manuscript switches from outlining the theory to its appraisal/discussion. The manuscript now consists of introduction, "body" (titled “The tumor suppression theory of aging” as before, with 13 sub-chapters) and conclusion, almost as suggested.

I have made no changes to the text except for renumbering the sub-chapters, fixing a garbled sentence in line 120 by inserting "that", inserting "%" in line 192, and fixing an incoherent past tense in line 219. I also weakened two statements that were too strong by inserting "might" in line 301 and "postulated" in line 387, removed an unnecessary comma in line 445, and inserted an "s" and removed a punctuation error in line 556. I also moved the introduction of the abbreviation for DNA damage response (DDR) to its first occurrence in line 355 (from line 411), added the clarification of "(error-prone)" in line 589, and removed the words "cells" in line 614 and "of" in line 688.

As soon as these minor comments are incorporated I believe the manuscript is ready for publication.

Reviewer #2: The author has responded constructively to all reviewer and editor comments and I now recommend publication