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Mediterranean diet and the hallmarks of ageing

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ABSTRACT

Ageing is a multifactorial process associated with reduced function and increased risk of morbidity and mortality. Recently, nine cellular and molecular hallmarks of ageing have been identified, which characterise the ageing process and, collectively, may be key determinants of the ageing trajectory. These include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. Healthier dietary patterns reduce the risk of age-related diseases and increase longevity and may influence positively one or more of these hallmarks. The Mediterranean dietary pattern (MedDiet) is a plant-based eating pattern that was typical of countries such as Greece, Spain, and Italy pre-globalisation of the food system and which is associated with better health during ageing. Here we review the potential effects of a MedDiet on each of the nine hallmarks of ageing, and provide evidence that the MedDiet as a whole, or individual elements of this dietary pattern, may influence each hallmark positively – effects which may contribute to the beneficial effects of this dietary pattern on age-related disease risk and longevity. We also highlight potential avenues for future research.

INTRODUCTION

Ageing is defined as a time-dependent, progressive decline in physiological functions [1], which is associated with an increased risk of numerous chronic diseases and mortality [2]. The ageing process is multifactorial and López-Otín et al. [1] proposed nine cellular and molecular hallmarks of ageing, which are believed to contribute to the ageing process, and collectively determine the ageing trajectory. These are genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication (Figure 1). Each of these hallmarks satisfies the criteria that: 1) it occurs during normal ageing; 2) its experimental exacerbation accelerates ageing; and 3) its experimental amelioration slows ageing and, consequently, increases lifespan.

The ageing trajectory is plastic, and may be modulated by dietary and other lifestyle factors [3,4]. One dietary approach that has attracted particular attention in this regard is the Mediterranean dietary pattern (MedDiet), which was characteristic of countries such as Greece, Italy, and Spain in the 20th century before globalisation of food production, processing and distribution [5]. The MedDiet is rich in plant-based foods such as fruits, vegetables, olive oil, legumes, grains, nuts and seeds, and also comprises a high intake of fish, and a moderate intake of red wine around mealtimes. Conversely, red meat, high-fat dairy products, and highly processed foods are consumed infrequently [5,6]. This dietary pattern contains an abundance of bioactive compounds, including a range of vitamins and minerals, polyphenols, fibre, nitrate, and monounsaturated and polyunsaturated fatty acids [7–9], many of which have been shown, individually or when combined, to elicit beneficial health effects [10–14]. Indeed, higher adherence to the MedDiet has been associated with reduced risk of several age-related chronic diseases, including cardiovascular disease (CVD) [15], type II diabetes [16], neurodegenerative

diseases [17], and cancer [18]. In addition, epidemiological evidence has demonstrated increased longevity with higher adherence to the MedDiet [19,20], whilst a meta-analysis of over 1.5 million participants reported a 10% decrease in overall mortality for a 2-point increase in MedDiet adherence score on a 9-point scale [21]. In this review, using the López-Otín et al. [1] *Hallmarks of Ageing Model* as a framework, we discuss the potential mechanisms through which the MedDiet may modulate the ageing process. The findings of this review are likely to be of relevance to researchers and nutritional practitioners, by advancing understanding of potential mechanistic pathways through which the MedDiet may influence health, and by providing information which could help inform the design of future randomised controlled trials (RCTs) and nutritional guidelines. We also identify limitations to the current body of evidence, which may serve as inspiration for future research in this area.

METHODS

This review provides a narrative synthesis of studies which assess the effects of the MedDiet on the hallmarks of ageing. The included studies were retrieved from searches of online databases or relevant individual journals, as well as scrutinising reference lists of relevant articles. Searches were conducted up to 14th April 2020 with no restriction on study publication date, using keywords for each respective hallmark combined with MedDiet or Mediterranean diet or Mediterranean lifestyle. Both medical subject headings (MeSH) terms and free text searches were used, and the searches were restricted to publications in the English Language. Where evidence was not available for MedDiet as a whole, potential effects of individual MedDiet components were explored (Table 1).

GENOMIC INSTABILITY

Genomic instability, defined as greater susceptibility to genomic alterations (such as mutations, DNA damage and chromosomal abnormalities), results from the combined effect of oxidative stress, epigenetic alterations, and inadequate DNA repair and telomere maintenance [22]. The MedDiet contains a multitude of bioactive compounds such as melatonin, phytosterols, carotenoids and polyphenols (e.g. resveratrol and hydroxytyrosol) [8], many of which exert a protective effect against genomic instability by preventing DNA damage, enhancing DNA repair, or attenuating telomere shortening (see *Telomere Attrition*). These effects appear to be related to the anti-inflammatory effects of the MedDiet (see *Altered Intercellular Communication*), but also may be due to changes in gene expression induced by the MedDiet directly [23–25], or via epigenetic mechanisms [26].

Reactive oxygen species (ROS) and/or reactive nitrogen species (RNS), by-products of the cell's oxidative metabolism, generate oxidative stress resulting in damage to biological macromolecules, including DNA. Of the four DNA bases, guanine oxidized metabolites, such as 8-oxo-2'-deoxyguanosine (8-OHdG), are well established markers of oxidative stress and have mutagenic potential [27–29]. Both the MedDiet as a whole, and a moderate consumption of wine (the predominant source of alcohol in this dietary pattern), have been demonstrated to decrease levels of oxidative stress reflected by lower concentration of 8-OHdG in DNA from peripheral blood leukocytes [30]. Likewise, sofrito, a cooked tomato sauce used frequently in Mediterranean cooking [31], olive oil [32,33], and nuts [34] have been shown to protect against oxidative DNA damage in various tissues. DNA double strand breaks in peripheral blood cells are significantly lower following a diet with a high virgin olive oil intake compared with sunflower oil [35]. Among participants in the European Investigation into Cancer and Nutrition (EPIC) study resident in Florence (Italy), higher adherence to MedDiet was associated with lower levels of the deoxyguanosine adduct pyrimido [1,2-α] purin-10(3H)-one

(M $_1$ dG,).which results from the interaction between lipid peroxides and DNA [36]. Thus, the MedDiet as a whole, and several key constituents of this dietary pattern, have the potential to ameliorate genomic instability.

TELOMERE ATTRITION

Telomeres are regions of repeated nucleotide sequences (TTAGGG in humans) bound to the protein complex shelterin, which are situated at both ends of each chromosome and protect against chromosome degradation and inter-chromosomal fusion. A small portion of telomeric DNA (50-100 base pairs in human fibroblasts) is lost during cell division due to the End Replication Problem (ERP), and hence telomeres become gradually shorter with increasing age (i.e., telomere attrition). Telomeres also shorten due to oxidative stress [37]. Shorter telomeres are associated with increased risk of cancer and CVD [38], and have been linked with increased mortality, particularly at younger ages [39]. Smoking, which increases risk of cardiometabolic disease and cancer and reduces life expectancy, accelerates telomere attrition [40]. Conversely, some, but not all, studies suggest that higher levels of physical activity protect against telomere shortening [41].

Although not a universal finding [42], several studies have reported a positive association between the MedDiet and both leukocyte telomere length and telomerase activity [43–46]. In an observational study of 217 older (mean age=77.9±2.7 years) residents of Campania (Southern Italy), Boccardi and colleagues [43] reported that individuals with high MedDiet adherence had longer telomeres and higher telomerase activity compared with those with medium or low MedDiet adherence. Similarly, in an analysis of 4676 disease-free women from the Nurses' Health Study, Crous-Bou et al. [44] observed longer telomeres in those with greater adherence to the MedDiet. Higher plasma concentrations of monounsaturated fatty

166 acids, which are the main fatty acids in olive oil, a core component of the MedDiet, were
167 associated with greater leukocyte telomere length in those carrying the CC version of the
168 telomerase gene (*TERC*) rs12696304 single nucleotide polymorphism [47]. The mechanisms
169 through which a MedDiet may influence telomere length are poorly understood, but may be
170 related to lower levels of inflammation and oxidative stress with this dietary pattern [43,48].
171
172 In the Prevención con Dieta Mediterránea (PREDIMED) Study of middle-aged people at
173 higher CVD risk, 5 years intervention with a MedDiet supplemented with additional nuts was
174 associated with greater risk of telomere shortening when compared with the low-fat control
175 diet [49]. However, intervention with the MedDiet plus additional extra virgin olive oil did not
176 influence telomere length when compared with the low-fat control diet [49]. There is no
177 obvious explanation for these apparently conflicting findings but they could be related to the
178 participant cohort studied, given some evidence that factors such as ethnicity, genetics, and sex
179 may moderate the effects of a MedDiet on telomere lengths. Notably, Gu et al. [45] observed
180 a positive association between MedDiet adherence and telomere length in non-Hispanic white
181 participants, but not in African American or Hispanic individuals. Additionally, in a secondary
182 analysis of data from the PREDIMED-Navarra trial, Garcia-Calzon et al. [46] found an
183 association between the MedDiet and telomere attrition only in participants carrying the Ala
184 allele of the peroxisome proliferator-activated receptor γ2 (*PPARγ2*) gene. In another analysis
185 of the same cohort, higher adherence to the MedDiet at baseline was linked with higher
186 telomere length only in female participants [49].
187
188 Overall, the available evidence suggests that higher adherence to a MedDiet pattern may reduce
189 telomere attrition, although these beneficial effects may be confined to specific population sub-

groups. More research is needed to understand exactly how, and for whom, this dietary pattern confers beneficial effects on telomere length.

EPIGENETIC EFFECTS

Epigenetics describes heritable changes to the genome that occur in the absence of alternations in the DNA sequence. A consortium of marks and molecules including DNA methylation, histone modifications and the enzymes and other proteins that enable the reading, writing and erasing of these marks, constitutes the complex epigenetic machinery that regulates access to the genome. In addition, patterns of expression of non-coding RNAs (ncRNA) ranging in size from microRNA (miRNA; typically 22 nucleotides (nt)) to long non-coding RNAs (>200nt) [50] contribute to the epigenetic mechanisms that modulate gene expression and, consequently, cellular and tissue functions. Aberrant patterns of epigenetic marks and molecules are observed in many diseases and, given the importance of molecular damage in driving the ageing process, it is unsurprising that epigenetic factors are a hallmark of ageing [1]. Cells in young healthy individuals maintain a compact chromatin structure and good epigenetic regulation of all biological processes. In contrast, over time, cells in older individual accrue damage from multiple insults to the chromatin landscape, DNA accessibility and ncRNA that, eventually, compromises genomic integrity and alters cell function [51].

The exposome, including diet, modifies epigenetic 'signatures' which represents a key mechanism through which the organism senses its environment and responds through altered gene expression [52]. This process has been described as the 4Rs of nutritional epigenetics which comprise (1) receiving the signal from the exposome; (2) recording that signal as an altered epigenetic mark; (3) remembering those marks across multiple cell generations; and, eventually (4) revealing the consequences of the original exposure as altered phenotype [53].

There is growing evidence that alterations in epigenetic processes are a central, unifying mechanism through which nutrition influences the ageing trajectory and the risk of all common age-related diseases [54]. This has led to the suggestion that it may be possible to develop an "epigenetic diet" that could not only reduce the risk of a specific non-communicable diseases but also enhance healthy ageing by reducing the risk of the multiple diseases and disorders that characterise the ageing phenotype [51]. To this end, the MedDiet might constitute a palatable and readily-available "epigenetic diet".

Methylation of long interspersed elements (LINE-1) is a useful index of global (whole genome) DNA methylation [55]. In addition, LINE-1 hypomethylation occurs during ageing and is associated with an increased risk of several cancers and CVD [56], possibly as a consequence of greater genomic instability [57]. In a study of young (mean age 30 years), cancer-free women, participants with low MedDiet adherence, and in particular fruit intake below the median, were 3.7 times more likely to show LINE-1 hypomethylation in blood leukocytes than women whose consumption was above the median [58]. A similar effect was seen in those women with lower folate intake [58]. Since folate is an important methyl donor, it is possible that this nutrient mediated the effect of lower fruit intake but that hypothesis could not be tested in this observational study. Using a sub-set of participants in the PREDIMED Study, Arpon and colleagues [26] observed that the methylation status of eight genes related to inflammation and immunocompetence, including *EEF2, COL18A1, IL4I1, LEPR, PLAGL1, IFRD1, MAPKAPK2* and *PPARGC1B,* measured in peripheral blood mononuclear cells correlated with adherence to the MedDiet. In a study of middle-aged men and women who participated in an 8-weeks weight loss programme based on the MedDiet, expression of the miRNA miR-155-3p decreased, whereas that of Let-7b increased, in white blood cells [59]. Since higher miR-155-3p expression is associated with carcinogenesis [60], the reduced

expression of this miRNA may be a mechanism through which the MedDiet (and/or weight loss) lower cancer risk. In addition, changes in expression of Let-7b, miR-125b, miR-130a, miR132-3p and miR-422b correlated with changes in the quality of the diet as assessed by the Healthy Eating Index [59]. Recent studies show that Let-7b is a regulator of histone H2B ubiquitination which may be a mechanism though which this miRNA exerts its anti-tumour effects [61]. A recent epigenome-wide analysis of methylation at >400,000 CpG sites in leucoctye-derived DNA from 6,662 individuals of European ancestry revealed 30 CpG sites at which methylation status was associated with diet quality, measured using the MedDiet Score and/ or Alternative Healthy Eating Index [62]. Of these, methylation status at 12 CpG sites was associated significantly with all-cause mortality [62].

The pattern of DNA methylation has been used to calculate a so-called epigenetic clock (DNAm age) that attempts to measure biological age [63]. At the molecular level, DNAm age is hypothesised to reflect the consequences of a constellation of innate ageing processes that contribute towards a gradual loss of cell and tissue function [64]. DNAm age is increased by obesity [65] and is influenced by lifestyle factors, including diet [66]. Very recently, a pilot study conducted within the NU-AGE project, used Horvath's Clock to estimate DNAm age using data for methylation at 353 CpG sites in genomic DNA from whole blood before and after intervention with a MedDiet for 1 year in older (65-79 years) Italian and Polish participants [67]. The authors suggested that MedDiet intervention may promote epigenetic rejuvenation in older people but that the effect is dependent on several individual-specific factors [67].

Overall, adherence to the MedDiet is associated with changes in epigenetic marks and molecules but available data from intervention studies are limited.

PROTEOSTASIS

Cellular protein homeostasis, or proteostasis, is maintained by the proteostasis network (PN), a multi-compartmental system that coordinates protein synthesis, folding, disaggregation, and degradation [1]. Maintenance of proteostasis is associated with healthy ageing. Loss of proteostasis leads to loss of stability, failed autophagy, and accumulation of misfolded proteins. Thermal, oxidative, and osmotic stressors cause misfolding of proteins [68].

Age-related diseases are associated with the dysregulation of protein maintenance. Failure of proteostasis is thus associated with an increased incidence of age-related diseases such as neurodegenerative diseases [69] and CVDs [70]. Alzheimer's and Parkinson's diseases, have been associated with accumulation of unfolded, misfolded, or aggregated proteins which provides strong evidence that protein homeostasis is disrupted in these disease states. The modulation of proteostasis capacity is one of the mechanism through which the MedDiet may prevent neurodegeneration. For example, olive oil, a central component of the MedDiet, may mitigate the effects of adverse vascular factors and have potential for prevention of late-onset Alzheimer's disease [71]. Whilst the mechanisms responsible for this apparent protection are not well established, polyphenols, such as those found in olives and in olive oil, are involved in regulation of cell proteostasis through activation of the protein deacetylase SIRT1 and enhanced autophagy [72]. Additionally, evidence from *in vivo* and *in vitro* studies indicates a potential for the phenolic components of extra virgin olive oil such as oleuropein [73] and oleocanthal [74] in reducing amyloid aggregation. Activation of autophagy appears to be one of the important mechanisms through which polyphenols induce beneficial effects against neurodegeneration. Oleuropein enhances autophagy by an mTOR- and adenosine monophosphate–activated protein kinase (AMPK)–dependent mechanism [73].

Collectively, these results suggest a potential protective effect of the MedDiet on proteostatis and, consequently, reduced the risk of age-related cognitive decline. The effect of the MedDiet and its components on other age-related health outcomes, particularly those involving dysregulated proteostasis, requires further exploration.

NUTRIENT SENSING PATHWAYS

Kirkwood's disposal soma theory of ageing argues that, because organisms have limited access to resources, they age due to an evolutionary trade-off between resources required for growth, reproduction and for cellular maintenance [75,76]. Therefore, the signalling systems involved in detecting and interpreting the availability of the key cellular resources i.e. energy and nutrients – known collectively as nutrient sensing systems - play critical roles in regulating physiological decision-making and the processes that support growth, reproduction, and ageing [77]. The increased risk of non-communicable diseases with age has been attributed, at least in part, to the deregulation of several nutrient sensing pathways including insulin/insulin-like growth factor-1 (IIS), mTOR, AMPK and sirtuins [1,78]. The IIS pathway is responsible for glucose homeostasis and was the first nutrient-sensing pathway implicated in this response [79]. Downregulation of the IIS pathway activates Forkhead Box O (FOXO) proteins which promote longevity via increased insulin sensitivity [80], cell cycle arrest [81], suppression of inflammation, enhanced mitochondrial biogenesis and a metabolic shift from glucose to lipid oxidation [82]. This subsequently reduces risk of age-dependant diseases such as cancer, neurodegenerative diseases and diabetes [83]. The mTOR kinase pathway, which is responsible for detecting high amino acid concentrations, comprises two complexes, mTORC1 and mTORC2. Genetic downregulation of mTORC1 activity in yeast, worms, flies and mice promotes healthy ageing [84]. In contrast to the IIS and mTOR pathways which detect nutrient abundance, AMPK and sirtuins detect nutrient scarcity. Upregulation of AMPK and sirtuins

promotes longevity via the deactivation of mTORC1 [85] and activation of PGC-1α [86], respectively.

Beneficial effects of dietary energy (calorie) restriction on nutrient sensing pathways and healthy ageing are well established in animal models [87]. However the sustainability of similar, severe dietary strategies in humans is questioned. Alternatively, the use of more moderate dietary interventions to modulate nutrient sensing pathways and promote healthy ageing has received increasing attention [78]. Specifically, a MedDiet characterised by low-moderate protein intake, low glycaemic index (GI) and polyphenol-rich foods may provide an appropriate surrogate [88]. In this regard, lower dietary protein intake attenuates circulating insulin-like growth factor-1 (IGF-1) concentrations, a moderator of both IIS and mTOR pathways [89,90]. In addition, intervention for 28 days with a low glycaemic load (GL) diet in healthy young adults reduced fasting concentrations of IGF-1 and of IGF-1/IGFBP-3 compared with the high-GL diet [91]. The subsequent downregulation of the IIS pathway activates the FOXO transcription factor FOXO3A, which induces transcription of homeostatic genes and attenuates the mitogenic effects of RAS [92,93]. Further, low IGF-1 concentrations facilitate the inhibition of mTOR activity, as evidenced by down-regulation of phosphorylated mTOR and p70-S6K, which subsequently reduce cell proliferation [89]. It has also been demonstrated that the polyphenol rich nature of MedDiet components such as olive oil activates AMPK pathways [94]. The age-protective effects of this derive, in part, from the stimulation of autophagy by inhibiting the mTOR complex [95]. Autophagy is also stimulated by the sirtuin, SIRT1 which acts to upregulate AMPK in a positive feedback loop via the acylation and activation of LKB1 [96]. Further, the interaction of AMPK and sirtuins induced by a diet rich in polyphenols also results in the deacylation and inactivation of NFkB, which is likely important for suppression of immune response and inflammation [97].

Overall, key features of the MedDiet including the moderate protein intake, low GI and abundance of polyphenol-rich foods may contribute towards healthy ageing via positive effects on nutrient sensing pathways, although direct evidence for this dietary pattern as a whole is presently lacking.

MITOCHONDRIAL DYSFUNCTION

Mitochondria are dynamic organelles, commonly known as the "powerhouse of the cell", which produce most of the adenosine triphosphate (ATP) available to the cell. They are also the hub for multiple signaling cascades that can drive the cell fate towards survival or death by apoptosis [98]. In addition, mitochondria are the main producers of ROS that, when they exceed the antioxidant capacity of the cell, have been suggested to be a key cause of ageing and age-related diseases such as Parkinson's and Alzheimer's disease [99–101]. Indeed, ageing is characterized by an accumulation of dysfunctional mitochondria, decreased ATP production, and increased ROS generation [1].

The beneficial effects of the MedDiet on mitochondrial function, are related, at least in part, to the high content of antioxidants and bioactive polyphenols derived from foods such as red wine, olive oil, fruits and vegetables [8]. Recently Varela-Lopez et al. demonstrated that olive oil had a beneficial impact on mitochondrial structure, function, as well as oxidative stress level in older rats [102]. In another study, extra virgin olive oil was protective in rats exposed to the herbicide 2,4-dichlorophenoxyacetic acid which uncouples mitochondrial respiration and induces ROS production and neurodegeneration [103]. Hydroxytyrosol and oleuropein, two phenolic constituents of extra virgin olive oil, reduce oxidative stress and improve mitochondrial function [104,105]. It has been suggested that hydroxytryosol crosses the blood-brain barrier and may delay the development of Alzheimer's disease by improving

mitochondrial function, oxidative stress and neuronal inflammation [106]. Fish oil rich in omega-3 polyunsaturated fatty acids also has a protective effect on mitochondrial function during ageing. Notably, administration of fish oil for 21 days restored docosahexaenoic acid (DHA) concentration, mitochondrial respiration, and ATP production in the brains of older mice (24 months old) to levels similar to those in their younger counterparts (3 months old) [107]. Two studies from the group of Lanza demonstrated that omega-3 polyunsaturated fatty acids can restore mitochondrial oxidative capacity in old mouse and human skeletal muscle [108,109]. The MedDiet includes moderate consumption of red wine that is rich in bioactive phenols such as flavanols, flavonols, anthocyanins, and resveratrol [110]. Quercetin, an abundant flavonol in red wine, promotes AMPK phosphorylation and induces overexpression of AMPK/SIRT1 signaling pathway genes in an osteoarthritis rat model. This activation enhanced mitochondrial membrane potential, oxygen consumption, and ATP generation, whilst simultaneously decreasing ROS production [111]. Resveratrol, another flavonol, improves mitochondrial function [112] and may have potential applications in the treatment of age-related diseases [113].

Overall, it is apparent that several individual components of the MedDiet such as olive oil, fish oil and red wine can positively influence mitochondrial bioenergetics and function [114]. However, few studies have evaluated the synergistic effect of MedDiet components on mitochondrial function during ageing and in age-related diseases.

CELLULAR SENESCENCE

Cellular senescence is an irreversible growth arrest in response to unrepairable DNA damage. However, recent studies have shown that senescence is a cellular stress and damage response that involves not only cell cycle arrest but also the senescence-associated secretory phenotype

and other hallmarks of ageing including senescence-associated mitochondrial dysfunction, autophagy/mitophagy dysfunction, altered nutrient and stress signaling, and epigenetic reprogramming [1,115]. Cellular senescence is also often associated with the accumulation of non-telomeric DNA damage as well as a activation of the *INK4a/ARF* locus [116]. With age, the number of senescent cells increases, enhancing the likelihood of age-related diseases [117]. Killing of senescent cells using genetic or pharmacological means increases the healthspan of experimental animals [118] and evidence has shown that treatment with senolytics (including dietary components) reduces the prevalence of senescent cells *in vivo* and delays, or prevents, organismal ageing [119,120]. Polyphenols such as quercetin, which are readily available in the MedDiet [121,122], have "anti-senescence effects", possibly via their antioxidant and anti-inflammatory properties, or via modulation of the gut microbiota [121,123]. In a recent clinical trial, treatment with Dasatinib plus quercetin in individuals with diabetic kidney disease reduced the numbers of senescent cells in human adipose tissue [124].

There is a growing body of evidence that adherence to the MedDiet, or consumption of its constituents, can reduce oxidative DNA damage and augment DNA repair [125]. The MedDiet is rich in many compounds with putative senolytic activity [123] including the antioxidant vitamin E which can prevent oxidative stress [126]. Tocotrienols are a member of the vitamin E family and have been demonstrated to exert senolytic properties in healthy tissues [127]. Constituent foods of the MedDiet, such as nuts [128] and several different vegetables [129,130], reduce DNA damage and so may inhibit the accumulation of senescent cells.

Therefore, the MedDiet as a whole, and many of its constituents, are likely to have senolytic effects, and further studies, particularly RCTs exploring the effects of the entire MedDiet on prevalence of senescent cells in target tissues, are warranted.

STEM CELL EXHAUSTION

Human tissues are maintained by adult stem cells that possess two defining characteristics. The first is the capacity to self-renew and generate more stem cells that persist for life. The second is the ability to differentiate into downstream progenitor cells that engender the cellular diversity inherent to tissues [131]. Stem cells are found throughout the body, and their functional decline due to various intrinsic and extrinsic causes contributes to the fall in the regenerative potential of tissues, which contributes to ageing and the risk of numerous age-related diseases. For example, the decline in the regenerative potential of the haemopoietic tissue with age may result in diminished production of adaptive immune cells (immunosenescence), which is associated with an increase in the risk of anaemia and myeloid malignancies [132]. Immunosenescence is often accompanied by subclinical accumulation of proinflammatory factors and inflammaging which drives the development of age-related disease [133]. Dietary and other lifestyle factors that reduce, delay, or attenuate the rate of stem cell exhaustion may therefore play a role in facilitating healthy ageing.

The first stage of atherosclerosis is endothelial dysfunction, which occurs at sites where the endothelial cell layer is exposed to injury or stress. The vascular endothelium deteriorates progressively during ageing [13,134]. Considerable evidence indicates that an imbalance between the magnitude of vascular injury and the capacity for repair plays an important role in age-related impaired endothelial function [135]. Previous studies have reported a favourable role of bone marrow-derived endothelial progenitor cells and circulating progenitor cells (CPCs) in vascular homeostasis [136]. Endothelial progenitor cells are key players in restoring injured endothelial cells, integrating into the endothelial cell layer or secreting angiogenic growth factors [136]. In an observational study involving 421 very old individuals, higher adherence to the MedDiet was associated with significantly higher endothelial progenitor cells

[137]. Additionally, in a crossover trial involving 20 older participants (>65 years), the consumption of a MedDiet was associated with an increased number of circulating endothelial progenitors cells [138]. Likewise, a 12-week MedDiet intervention, with and without exercise, significantly increased endothelial progenitor cells [139]. These effects may contribute towards the improved endothelial function reported with MedDiet interventions [140]

Olive oil enhances osteoblastogenesis and adipogenesis in mesenchymal stem cells, reducing the risk of osteoporosis in rats and humans [141]. Additionally, in a sub-study of the PREDIMED trial, which involved 127 older participants (mean age 68 years), intervention with a MedDiet enriched with extra virgin olive oil for two years significantly increased serum osteocalcin, suggesting protective effects on bone [142]. Moreover, *in vitro* studies have shown that active ingredients of olive oil (including the polyphenols oleuropein, apigenin 7-glucoside and luteolin 7-glucoside) enhance hematopoietic stem cell survival and differentiation potential [72].

Therefore, the MedDiet as a whole and key components such as olive oil, may help mitigate against stem cell exhaustion with attendant beneficial effects on the ageing process.

ALTERED INTERCELLULAR COMMUNICATION

Intercellular communication (or cell-to-cell communication) is essential for coordination of cell functions within tissues, organs and the whole body and involves soluble factors including cytokines, chemokines, growth factors and neurotransmitters that are recognised by specific cell-surface receptors [143]. For example, gap-junction intercellular communication (GJIC) contributes to intracellular signalling by facilitating the intercellular exchange of ions and regulatory molecules associated with key cell proliferation, differentiation and apoptosis, and

so plays an important role in maintaining tissue homeostasis [144]. Ageing is associated with significant alterations in the communication between cells via endocrine, neuroendocrine, and neuronal routes [1]. Inflammation is one of the most important and widely studied intercellular communication processes which is altered during ageing. Indeed, one key feature of ageing is the presence of low-grade, chronic, systemic inflammation - termed 'inflammaging' [145] – which is associated with, and is predictive of, frailty [146], type II diabetes [147], neurodegenerative diseases [148], and an increased risk of mortality [149,150]. Inflammaging may have numerous different causes, including increased production of reactive oxygen species (ROS), enhanced secretion of pro-inflammatory cytokines and adipokines, increased activation of the NK- κB pathway, and changes in the gut microbiome and intestinal permeability [151–153]. The MedDiet has been proposed as a potential nutritional strategy to slow down or decrease inflammaging.

Several sub-studies from the PREDIMED trial have shown beneficial effects of the MedDiet on inflammatory biomarkers, including IL-6, VCAM-1, sICAM-1, CRP, and TNF-a [26,154–156]. One PREDIMED sub-study [157] there was no association between overall MedDiet adherence and inflammatory biomarkers, there were links between higher intake of fruit, cereals, nuts and olive oil and lower circulating concentration of inflammatory markers. The beneficial effects of the MedDiet on inflammation have also been substantiated by other investigations [158,159]. Mechanistically, these effects have been linked with a downregulation of the NF-kB pathway [160], altered methylation of genes linked with inflammation [26], and indirectly via reduced obesity (and therefore decreased adipose tissue derived inflammation) [158,161]. In addition, there is growing evidence that several plant secondary metabolites (including polyphenols) can modulate GJIC [144] and contribute to the protective effects of the MedDiet against age-related diseases.

Therefore, current evidence supports the hypothesis that the MedDiet is an anti-inflammatory dietary pattern with potential to protect against inflammaging through a range of different mechanisms and, consequently, to influence positively the ageing phenotype.

CONCLUDING REMARKS

The MedDiet is associated with reduced risk of numerous age-related diseases and with increased longevity [21]. As demonstrated in this review, the MedDiet positively influences the hallmark features of ageing, which may contribute towards the beneficial effects of this dietary pattern on human health. Nevertheless, numerous questions remain unanswered. Although numerous studies have investigated the impact of individual MedDiet components (foods or their bioactive constituents), relatively few investigations have focussed on the effects of the MedDiet as a whole on the hallmarks of ageing. Moreover, much of the evidence has been derived from observational studies, which do not allow the inference of causal relationships, and may be subject to issues such as reverse causality and residual confounding. Although some RCTs exploring health effects of the MedDiet are available, many are sub-studies from the PREDIMED trial, which investigated a MedDiet supplemented with additional extra-virgin olive oil or nuts. It is possible that different effects may emerge from studies implementing the MedDiet in other settings without the emphasis on these two components. Further research, particularly RCTs, exploring the effects of a MedDiet on the hallmarks of ageing are therefore warranted. Such research should explore heterogeneity in response and the impact of participant characteristics including age, sex and genotype on MedDiet-ageing relationships. The findings could be valuable in developing stratified, or personalised, nutrition recommendations and interventions [162].

In summary, there is growing evidence that the MedDiet, and its individual components, may have positive effects on all of the hallmarks of ageing and, by doing so, positively affect the human health span. Further research is warranted to better understand the mechanisms of action of the MedDiet on the ageing process.

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Conflict of interest

All authors declare no conflict of interest.

Author contributions

This study was conceived by MS, and designed by OMS, MS and JCM. OMS, AWA, FS, GS, CMR, JL, JM, AG, NR, LL, ES, BCMS, AMM, MS, and JCM drafted and critically revised the manuscript, with OMS taking a lead role. NR created the schematic. All authors approved the final version of the manuscript prior to submission.

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Figure Legends

Figure 1. A schematic representation of the mechanisms through which the Mediterranean diet, or key components within this dietary pattern, may influence the nine hallmark features of ageing.

1030 **Table 1.** A summary of research exploring effects of the MedDiet and its components on the Hallmarks of Ageing

Authors	Study design	Sample size (male)	Type of intervention or exposure	Summary of key findings
			Genomic instability	
Urquiaga et al. [30]	RCT	42	MedDiet MedDiet + wine Western diet	Both MedDiet and wine decreased 8-OHdG in DNA from peripheral blood leukocytes and plasma nitrotyrosine compared with a western diet and MedDiet without wine.
Vilahur et al. [31]	Animal model	15 pigs	Cooked tomato sauce (sofrito)	Sofrito attenuated diet-induced endothelial dysfunction. Effects were associated with increased eNOS transcription and activation, lower vascular DNA oxidative damage and enhanced HDL functionality.
Erol et al. [32]	Cell model	-	Olive oil phenolic extract	Pre-treatment of HeLa cells with olive oil phenolic extract reduced H_2O_2-induced nuclear DNA damage.
Rangel-Zuñiga et al. [33]	RCT	20 (7)	Breakfast cooked with olive oil and other oils	Post-prandial levels of 8-OHdG were lower after breakfast cooked with olive oil or mixed oil with added olive oil antioxidants versus sunflower oil or mixed oil with added dimethylpolysiloxane.
Calcabrini et al. [34]	Cell model	-	Walnut extract	Walnut extract protected against oxidative DNA damage as demonstrated via plasmid DNA cleavage and fast Halo assay.
Quiles et al. [35]	Animal model	112 Wistar rats	Lifelong dietary inclusion of olive oil or sunflower oil	Ageing increased plasma cholesterol, triglycerides, phospholipids, total lipids, polyunsaturated fatty acids and DNA double-strand breaks. These parameters were all lower in rats fed olive oil. Ageing diminished total antioxidant capacity with both diets, but to a lesser degree with the olive oil diet.
Saieva et al. [36]	Observational	313	Adherence to a 9-point MedDiet score	Higher adherence to MedDiet was associated with lower levels of M1dG, a biomarker of lipid peroxidation and oxidative stress.
			Telomere attrition	
Meinilä et al. [42]	Observational	1046 (456)	Adherence to a 9-point MedDiet score	Higher MedDiet score at baseline was associated with significantly faster telomere shortening during follow up.
Boccardi et al. [43]	Observational	217 (115)	Adherence to a 9-point MedDiet score	Higher MedDiet adherence was associated with longer telomere lengths and greater telomerase activity.

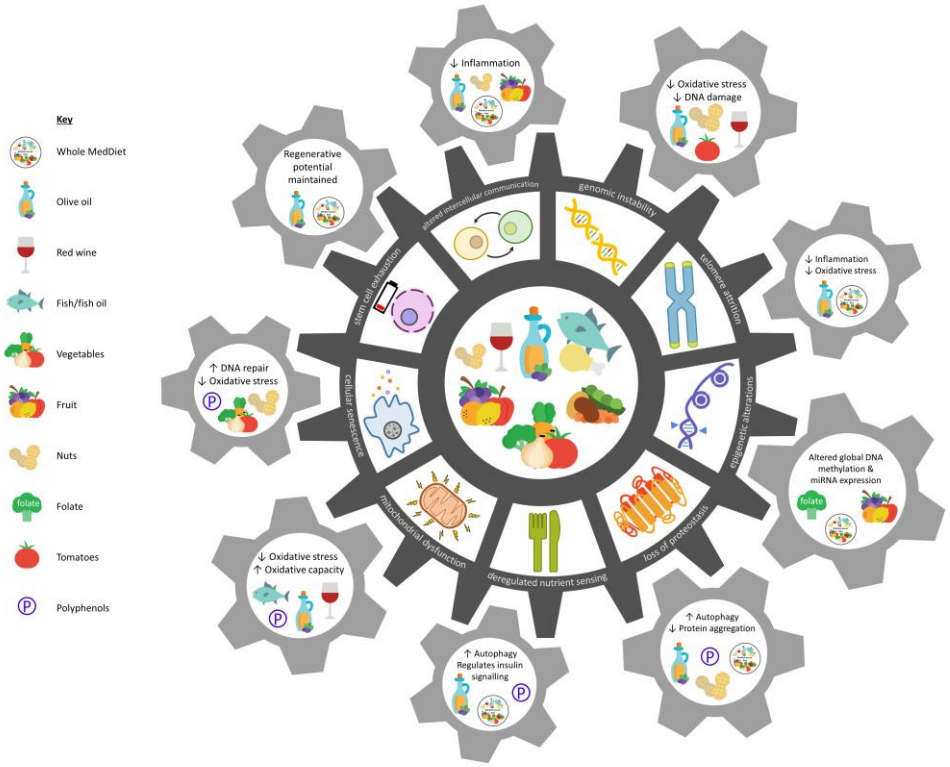
Crous-Bou et al [44]	Observational	4676 (0)	Adherence to a 9-point MedDiet score	Higher MedDiet adherence was associated with longer telomere lengths
Gu et al. [45]	Observational	1743 (552)	Adherence to a 9-point MedDiet score	Higher MedDiet adherence was associated with longer telomere lengths in non-Hispanic white but not African American or Hispanic individuals. Higher intake of vegetables and lower intake of cereal, meat and dairy was associated with longer telomere lengths.
García-Calzón et al. [46]	RCT	521 (236)	MedDiet + nuts MedDiet + olive oil Low fat diet	MedDiet reduced telomere attrition in participants carrying the Ala allele of the PPARγ2 gene
Gomez-Delgado et al [47]	RCT	1002 (-)	MedDiet intervention	Higher mono-unsaturated fatty acid levels were associated with greater telomere lengths in CC allele carriers of the TERC rs12696304 SNP.
García-Calzón et al. [49]	RCT	520 (235)	MedDiet + nuts MedDiet + olive oil Low fat diet	Higher baseline MedDiet adherence was associated with longer telomeres in females and shorter telomeres in males. MedDiet + nuts intervention was associated with shorter telomere lengths. No difference was found for MedDiet + olive oil versus control.
Epigenetic effects				
Arpón et al. [26]	RCT	36 (18)	Adherence to a 14-point MedDiet score	MedDiet adherence was significantly associated with methylation status of eight genes related to inflammation and immunocompetence (EEF2, COL18A1, IL4I1, LEPR, PLAGL1, IFRD1, MAPKAPK2 and PPARGC1B)
Agodi et al. [58]	Observational	177 (0)	Adherence to a 9-point MedDiet score	Participants with low Mediterranean diet adherence, and in particular fruit and folate intake, were more likely to show LINE-1 hypomethylation in blood leukocytes.
Marques-Rocha et al. [59]	RCT	40 (20)	Hypocaloric MedDiet intervention	MedDiet decreased expression of the miRNA miR-155-3p and increased expression of Let-7b in white blood cells.
Ma et al. [62]	Observational	6662 (-)	Adherence to a 9-point MedDiet score or the Alternative Healthy Eating Index	Diet quality was associated with differential DNA methylation levels of 30 CpGs in peripheral leukocytes. Methylation status at 12 of these CpG sites was associated significantly with all-cause mortality.
Gensous et al. [67]	RCT	120 (51)	MedDiet intervention	One year MedDiet intervention promotes epigenetic rejuvenation, as assessed by Hovarth's clock, with effects differing by country, sex, and individual characteristics.

Proteostasis				
Rigacci et al. [73]	Animal model	-	Olive oil-derived oleuropein aglycone	Oleuropein aglycone triggered autophagy via the AMPK/mTOR signalling pathway.
Abuznait et al. [74]	Animal model	-	Olive oil-derived oleocanthal	Oleocanthal enhanced clearance of β-amyloid from the brain via up-regulation of P-glycoprotein and LDL lipoprotein receptor related protein-1.
Nutrient sensing pathways				
Fontana et al. [89]	Animal model	-	Lower protein diet Higher protein diet	Reduced protein intake inhibited tumour growth in human xenograft prostate and breast cancer models.
Runchey et al. [91]	RCT	80 (40)	Lower glycaemic load diet Higher glycaemic load diet	28-days of a low glycaemic load diet reduced fasting concentrations of IGF-1 (4%), and post-prandial glucose (-43%) and insulin responses (-27%) compared with a high glycaemic load diet.
Mitochondrial dysfunction				
Varela-Lopez et al. [102]	Animal model	72 Wistar rats	Lifelong dietary inclusion of olive oil, sunflower oil, or fish oil	Lifelong olive oil consumption beneficially impacted age-related alterations in mitochondrial structure, function, and oxidative stress.
Amel et al. [103]	Animal model	10 Wistar rats	Extra virgin olive oil	Extra virgin olive oil protected against 2,4-dichlorophenoxyacetic acid-induced brain damage by increasing brain docosahexaenoic acid (DHA) composition and reducing oxidative stress.
Schaffer et al. [104]	Animal model	NMRI mice	Hydroxytyrosol-rich olive mill wastewater	12 days feeding with olive mill wastewater reduced basal and stress-induced lipid peroxidation. Incubation of cells with hydroxytyrosol significantly attenuated the cytotoxic effect of Fe2+ and sodium nitroprusside.
Sun et al. [105]	Animal model	Wistar-Kyoto rats	Oleuropein supplementation	Eight weeks Oleuropein supplementation reduced blood pressure and oxidative stress, and improved mitochondrial function through the activation of the Nrf2-mediated signalling pathway.
Peng et al. [106]	Animal model	APP/PS1 mice	Hydroxytyrosol supplementation	Six months hydroxytyrosol supplementation mitigated neuronal impairment by reducing mitochondrial oxidative stress, neuronal inflammation, and apoptosis.

Afshordel et al. [107]	Animal model	18 NMRI-mice	Omega-3 supplementation	Twenty one days mega-3 supplementation restored mitochondrial oxidative capacity in the brains of older mice.
Johnson et al. [108]	Animal model	72 C57BL6 mice	Eicosapentaenoic acid Docosahexaenoic acid	Ten weeks eicosapentaenoic acid supplementation restored mitochondrial oxidative capacity in the skeletal muscle of older mice.
Lalia et al. [109]	RCT	24 (13)	Omega-3 supplementation	Four months omega-3 supplementation decreased mitochondrial oxidant emissions, increase post-absorptive muscle protein synthesis, and augmented anabolic responses to exercise in older adults.
Qiu et al. [111]	Animal model	Sprague–Dawley rats	Quercetin supplementation	Seven days of quercetin supplementation decreased mitochondrial ROS production and enhanced itochondrial membrane potential, oxygen consumption, and ATP gmeneration.
Lagouge et al. [112]	Animal model	C57Bl/6J and KKAy mice	Resveratrol supplementation	Fifteen weeks resveratrol supplementation enhanced running time to exhaustion - effects that were associated with an induction of genes for oxidative phosphorylation and mitochondrial biogenesis.
Cellular senescence				
Kleeman et al. [121]	Animal model	CRP and ApoE*3Leiden transgenic mice	Quercetin supplementation	Quercetin supplementation decreased expression of CRP and cardiovascular risk factors (SAA, fibrinogen) in mice *in vivo*.
Medina-Remón et al. [122]	RCT	200 (87)	MedDiet + nuts MedDiet + olive oil Low fat diet	Both MedDiet interventions decreased systolic and diastolic BP. Effects were associated with an increase in total polyphenol excretion and plasma nitric oxide biomarkers.
Hickson et al. [124]	RCT	9 (7)	Dasatinib + quercetin	11 days treatment with Dasatinib + quercetin decreased senescent cell burden in patients with diabetic kidney disease.
Corina et al. [126]	Observational	962 (-)	Estimated vitamin E intake	Lower levels of vitamin E were associated with shorter telomere lengths and higher glutathione peroxidase.
Durani et al. [127]	Cell model	-	Tocotrienols	Tocotrienols modulated the expression of multiple genes involved in senescence-associated signalling pathways in human diploid fibroblasts (e.g. SOD1, SOD2, CAT, GPX1, CCS-1, FOXO3A, TP53, MAPK14).

López-Uriarte et al. [128]	RCT	50 (28)	Mixed nut consumption	12 weeks of nut supplementation (30g/d) had no effect on plasma antioxidant capacity, oxidized LDL, conjugated diene formation nor urine 8-isoprostanes but reduced DNA damage assessed by yield of urine 8-oxo-dG.
Riso et al. [129]	RCT	20 (20)	Broccoli consumption	10 days broccoli intake (200g/d) reduced *ex vivo* H_2O_2-induced strand breaks in smokers and non-smokers. Oxidized purines also decreased significantly in smokers.
Moser et al. [130]	RCT	8 (4)	Spinach consumption	16 days spinach consumption (225 g/d) decreased ROS sensitivity and reduced DNA migration attributable to the formation of oxidatively damaged DNA bases
Stem cell exhaustion				
Cesari et al. [137]	Observational	421 (115)	Adherence to a 55-point MedDiet score	Higher adherence to the MedDiet in nonagenarians, as well as consumption of olive oil, fruit and vegetables, was associated with higher levels of endothelial progenitor and circulating progenitor cells
Marin et al. [138]	RCT	20 (10)	MedDiet Low fat diet Saturated fat diet	4 weeks intervention with a MedDiet resulted in lower total microparticle, activated endothelial microparticles, and apoptotic endothelial microparticles concentrations and higher endothelial progenitor cell numbers than low and saturated fat diets.
Fernandez et al. [139]	RCT	45 (13)	MedDiet MedDiet + exercise	12 weeks intervention with a MedDiet plus exercise led to greater increases in endothelial progenitor cell numbers than a MedDiet alone
Liu et al. [141]	RCT	120 (0) Sprague Dawley rats	Extra virgin olive oil	12 weeks extra virgin olive oil consumption significantly increased bone mineral density and decreased circulating concentrations of phosphatase, alkaline phosphatase, IL-6, malonyldialdehyde, and nitrate in an animal model of osteoporosis.
Fernández-Real et al. [142]	RCT	127 (127)	MedDiet + nuts MedDiet + olive oil Low fat diet	2 years intervention with a MedDiet + olive oil, but not MedDiet + nuts or a low fat diet, increased total osteocalcin, procollagen I N-terminal propeptide, and homeostasis model assessment-β-cell function.
Altered intercellular communication				
Estruch et al. [154]	RCT	772 (339)	MedDiet + nuts MedDiet + olive oil Low fat diet	3 months intervention with both MedDiets reduced plasma glucose concentrations, systolic BP, and the total cholesterol/ HDL ratio versus low fat diet. MedDiet + olive oil also reduced CRP concentrations.
Mena et al. [155]	RCT	106 (60)	MedDiet + nuts MedDiet + olive oil Low fat diet	3 months intervention with both MedDiets decreased monocyte expression of CD49d and CD40, and reduced serum IL-6, VCAM-1, sICAM-1. MedDiet + olive oil also reduced CRP. Conversely, low fat diet increased IL-6, VCAM-1 and sICAM-1.

Casas et al. [155]	RCT	164 (77)	MedDiet + nuts MedDiet + olive oil Low fat diet	12 month intervention with both MedDiets reduced systolic and diastolic BP, LDL cholesterol, P-selectin, IL-6 and CRP concentrations versus low fat diet. MedDiet + nuts also decreased monocyte expression of CD40, whilst MedDiet + olive oil decreased sICAM-1.
Salas-Salvadó et al. [157]	Observational	772 (339)	Adherence to a 14-point MedDiet score	MedDiet consumption as a whole was not associated with lower inflammatory markers. Consumption of fruits and cereals was associated with lower IL-6 concentrations. Higher intake of nuts was associated with lower ICAM-1, whilst higher intake of olive oil was associated with lower VCAM-1.
Richard et al. [158]	RCT	26 (26)	MedDiet MedDiet + weight loss North American diet	MedDiet alone did not influence plasma leptin, plasminogen activator inhibitor-1, resistin, visfatin, acylation stimulating protein and adiponectin concentrations. MedDiet + weight loss reduced plasma leptin and increased plasma adiponectin concentrations.
Sureda et al. [159]	Observational	598 (219)	Percentage adherence to the MedDiet	In adult but not adolescent males, higher MedDiet adherence was associated with higher adiponectin and lower levels of leptin, TNF-α, PAI-1 and CRP in adults. In females, higher MedDiet adherence was associated with lower CRP in both adults and adolescents, plus lower leptin concentration in adolescents, PAI-1 in adults.
Perez-Martinez et al. [160]	RCT	16 (16)	MedDiet Low fat diet +alpha-linolenic acid Western diet	4 weeks intervention with MedDiet and low fat diet + alpha linoleic acid were associated with lower NF-kappaB activation in mononuclear cells compared with a Western diet.
Park et al. [161]	Observational	4700 (2543)	Adherence to a 50-point MedDiet score	Waist circumference, and to a lesser degree BMI, mediated beneficial associations between MedDiet adherence and insulin resistance, glucose intolerance, and inflammatory markers.


Key
Whole MedDiet
Olive oil
Red wine
Fish/fish oil
Vegetables
Fruit
Nuts
Folate
Tomatoes
Polyphenols
↓ Inflammation
↓ Oxidative stress
↓ DNA damage
↓ Inflammation
↓ Oxidative stress
Altered global DNA methylation & miRNA expression
folate
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↑ Autophagy
Regulates insulin signalling
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cellular senescence
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