

1 **TITLE**

2 Mediterranean diet and the hallmarks of ageing

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4 **RUNNING TITLE**

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51 **ABSTRACT**

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

67 INTRODUCTION

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

78

79 The ageing trajectory is plastic, and may be modulated by dietary and other lifestyle factors
80 [3,4]. One dietary approach that has attracted particular attention in this regard is the
81 Mediterranean dietary pattern (MedDiet), which was characteristic of countries such as Greece,
82 Italy, and Spain in the 20th century before globalisation of food production, processing and
83 distribution [5]. The MedDiet is rich in plant-based foods such as fruits, vegetables, olive oil,
84 legumes, grains, nuts and seeds, and also comprises a high intake of fish, and a moderate intake
85 of red wine around mealtimes. Conversely, red meat, high-fat dairy products, and highly
86 processed foods are consumed infrequently [5,6]. This dietary pattern contains an abundance
87 of bioactive compounds, including a range of vitamins and minerals, polyphenols, fibre, nitrate,
88 and monounsaturated and polyunsaturated fatty acids [7–9], many of which have been shown,
89 individually or when combined, to elicit beneficial health effects [10–14]. Indeed, higher
90 adherence to the MedDiet has been associated with reduced risk of several age-related chronic
91 diseases, including cardiovascular disease (CVD) [15], type II diabetes [16], neurodegenerative

92 diseases [17], and cancer [18]. In addition, epidemiological evidence has demonstrated
93 increased longevity with higher adherence to the MedDiet [19,20], whilst a meta-analysis of
94 over 1.5 million participants reported a 10% decrease in overall mortality for a 2-point increase
95 in MedDiet adherence score on a 9-point scale [21]. In this review, using the López-Otín et al.
96 [1] *Hallmarks of Ageing Model* as a framework, we discuss the potential mechanisms through
97 which the MedDiet may modulate the ageing process. The findings of this review are likely to
98 be of relevance to researchers and nutritional practitioners, by advancing understanding of
99 potential mechanistic pathways through which the MedDiet may influence health, and by
100 providing information which could help inform the design of future randomised controlled
101 trials (RCTs) and nutritional guidelines. We also identify limitations to the current body of
102 evidence, which may serve as inspiration for future research in this area.

103

104 **METHODS**

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

114

115 **GENOMIC INSTABILITY**

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

126

127 Reactive oxygen species (ROS) and/or reactive nitrogen species (RNS), by-products of the
128 cell's oxidative metabolism, generate oxidative stress resulting in damage to biological
129 macromolecules, including DNA. Of the four DNA bases, guanine oxidized metabolites, such as
130 8-oxo-2'-deoxyguanosine (8-OHdG), are well established markers of oxidative stress and have
131 mutagenic potential [27–29]. Both the MedDiet as a whole, and a moderate consumption of
132 wine (the predominant source of alcohol in this dietary pattern), have been demonstrated to
133 decrease levels of oxidative stress reflected by lower concentration of 8-OHdG in DNA from
134 peripheral blood leukocytes [30]. Likewise, sofrito, a cooked tomato sauce used frequently in
135 Mediterranean cooking [31], olive oil [32,33], and nuts [34] have been shown to protect against
136 oxidative DNA damage in various tissues. DNA double strand breaks in peripheral blood cells
137 are significantly lower following a diet with a high virgin olive oil intake compared with
138 sunflower oil [35]. Among participants in the European Investigation into Cancer and Nutrition
139 (EPIC) study resident in Florence (Italy), higher adherence to MedDiet was associated with
140 lower levels of the deoxyguanosine adduct pyrimido [1,2- α] purin-10(3H)-one

141 (M₁dG), which results from the interaction between lipid peroxides and DNA [36]. Thus, the
142 MedDiet as a whole, and several key constituents of this dietary pattern, have the potential to
143 ameliorate genomic instability.

144

145 **TELOMERE ATTRITION**

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

157

158 Although not a universal finding [42], several studies have reported a positive association
159 between the MedDiet and both leukocyte telomere length and telomerase activity [43–46]. In
160 an observational study of 217 older (mean age=77.9±2.7 years) residents of Campania
161 (Southern Italy), Boccardi and colleagues [43] reported that individuals with high MedDiet
162 adherence had longer telomeres and higher telomerase activity compared with those with
163 medium or low MedDiet adherence. Similarly, in an analysis of 4676 disease-free women
164 from the Nurses' Health Study, Crous-Bou et al. [44] observed longer telomeres in those with
165 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 $\gamma 2$ (*PPAR* $\gamma 2$) 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-

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

192

193 **EPIGENETIC EFFECTS**

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

208

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

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

222

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

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

250

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

262

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

265 **PROTEOSTASIS**

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

271

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

289

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

294

295 **NUTRIENT SENSING PATHWAYS**

296 Kirkwood's disposal soma theory of ageing argues that, because organisms have limited access
297 to resources, they age due to an evolutionary trade-off between resources required for growth,
298 reproduction and for cellular maintenance [75,76]. Therefore, the signalling systems involved
299 in detecting and interpreting the availability of the key cellular resources i.e. energy and
300 nutrients – known collectively as nutrient sensing systems - play critical roles in regulating
301 physiological decision-making and the processes that support growth, reproduction, and ageing
302 [77]. The increased risk of non-communicable diseases with age has been attributed, at least in
303 part, to the deregulation of several nutrient sensing pathways including insulin/insulin-like
304 growth factor-1 (IIS), mTOR, AMPK and sirtuins [1,78]. The IIS pathway is responsible for
305 glucose homeostasis and was the first nutrient-sensing pathway implicated in this response
306 [79]. Downregulation of the IIS pathway activates Forkhead Box O (FOXO) proteins which
307 promote longevity via increased insulin sensitivity [80], cell cycle arrest [81], suppression of
308 inflammation, enhanced mitochondrial biogenesis and a metabolic shift from glucose to lipid
309 oxidation [82]. This subsequently reduces risk of age-dependant diseases such as cancer,
310 neurodegenerative diseases and diabetes [83]. The mTOR kinase pathway, which is responsible
311 for detecting high amino acid concentrations, comprises two complexes, mTORC1 and
312 mTORC2. Genetic downregulation of mTORC1 activity in yeast, worms, flies and mice
313 promotes healthy ageing [84]. In contrast to the IIS and mTOR pathways which detect nutrient
314 abundance, AMPK and sirtuins detect nutrient scarcity. Upregulation of AMPK and sirtuins

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

317

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

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

344

345 **MITOCHONDRIAL DYSFUNCTION**

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

354

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

365 mitochondrial function, oxidative stress and neuronal inflammation [106]. Fish oil rich in
366 omega-3 polyunsaturated fatty acids also has a protective effect on mitochondrial function
367 during ageing. Notably, administration of fish oil for 21 days restored docosahexaenoic acid
368 (DHA) concentration, mitochondrial respiration, and ATP production in the brains of older
369 mice (24 months old) to levels similar to those in their younger counterparts (3 months old)
370 [107]. Two studies from the group of Lanza demonstrated that omega-3 polyunsaturated fatty
371 acids can restore mitochondrial oxidative capacity in old mouse and human skeletal muscle
372 [108,109]. The MedDiet includes moderate consumption of red wine that is rich in bioactive
373 phenols such as flavanols, flavonols, anthocyanins, and resveratrol [110]. Quercetin, an
374 abundant flavonol in red wine, promotes AMPK phosphorylation and induces overexpression
375 of AMPK/SIRT1 signaling pathway genes in an osteoarthritis rat model. This activation
376 enhanced mitochondrial membrane potential, oxygen consumption, and ATP generation,
377 whilst simultaneously decreasing ROS production [111]. Resveratrol, another flavonol,
378 improves mitochondrial function [112] and may have potential applications in the treatment of
379 age-related diseases [113].

380

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

385

386 **CELLULAR SENESENCE**

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

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

403

404 There is a growing body of evidence that adherence to the MedDiet, or consumption of its
405 constituents, can reduce oxidative DNA damage and augment DNA repair [125]. The MedDiet
406 is rich in many compounds with putative senolytic activity [123] including the antioxidant
407 vitamin E which can prevent oxidative stress [126]. Tocotrienols are a member of the vitamin
408 E family and have been demonstrated to exert senolytic properties in healthy tissues [127].
409 Constituent foods of the MedDiet, such as nuts [128] and several different vegetables
410 [129,130], reduce DNA damage and so may inhibit the accumulation of senescent cells.

411

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

415 **STEM CELL EXHAUSTION**

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

429

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

440 [137]. Additionally, in a crossover trial involving 20 older participants (>65 years), the
441 consumption of a MedDiet was associated with an increased number of circulating endothelial
442 progenitors cells [138]. Likewise, a 12-week MedDiet intervention, with and without exercise,
443 significantly increased endothelial progenitor cells [139]. These effects may contribute
444 towards the improved endothelial function reported with MedDiet interventions [140]

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

454

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

457

458 **ALTERED INTERCELLULAR COMMUNICATION**

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

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

477

478 Several sub-studies from the PREDIMED trial have shown beneficial effects of the MedDiet
479 on inflammatory biomarkers, including IL-6, VCAM-1, sICAM-1, CRP, and TNF- α [26,154–
480 156]. One PREDIMED sub-study [157] there was no association between overall MedDiet
481 adherence and inflammatory biomarkers, there were links between higher intake of fruit,
482 cereals, nuts and olive oil and lower circulating concentration of inflammatory markers. The
483 beneficial effects of the MedDiet on inflammation have also been substantiated by other
484 investigations [158,159]. Mechanistically, these effects have been linked with a
485 downregulation of the NF- κ B pathway [160], altered methylation of genes linked with
486 inflammation [26], and indirectly via reduced obesity (and therefore decreased adipose tissue
487 derived inflammation) [158,161]. In addition, there is growing evidence that several plant
488 secondary metabolites (including polyphenols) can modulate GJIC [144] and contribute to the
489 protective effects of the MedDiet against age-related diseases.

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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].

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

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520 N/A

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522 **Conflict of interest**

523 All authors declare no conflict of interest.

524

525 **Author contributions**

526 This study was conceived by MS, and designed by OMS, MS and JCM. OMS, AWA, FS, GS,
527 CMR, JL, JM, AG, NR, LL, ES, BCMS, AMM, MS, and JCM drafted and critically revised
528 the manuscript, with OMS taking a lead role. NR created the schematic. All authors approved
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530

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544 **REFERENCES**

- 545 1 López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The Hallmarks of
546 Aging. *Cell*. 2013; 153: 1194–1217.
- 547 2 Niccoli T, Partridge L. Ageing as a Risk Factor for Disease. *Curr Biol*. 2012; 22: R741–
548 R752.
- 549 3 Mathers JC. Impact of nutrition on the ageing process. *Br J Nutr*. 2015; 113: S18–S22.
- 550 4 Stevenson EJ, Shannon OM, Minihane AM, Adamson A, Burns A, Hill T et al. NuBrain:
551 UK consortium for optimal nutrition for healthy brain ageing. *Nutr Bull*. 2020; 45: 223–
552 229.
- 553 5 Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S et al.
554 Mediterranean diet pyramid today. Science and cultural updates. *Public Health Nutr*.
555 2011; 14: 2274–2284.
- 556 6 Shannon OM, Stephan BCM, Granic A, Lentjes M, Hayat S, Mulligan A et al.
557 Mediterranean diet adherence and cognitive function in older UK adults: the European
558 Prospective Investigation into Cancer and Nutrition-Norfolk (EPIC-Norfolk) Study. *Am*
559 *J Clin Nutr*. 2019; 110:938-948.
- 560 7 Shannon OM, Stephan BCM, Minihane A-M, Mathers JC, Siervo M. Nitric oxide
561 boosting effects of the Mediterranean diet: A potential mechanism of action. *J Gerontol*
562 *A Biol Sci Med Sci*. 2018; 73:902-904.
- 563 8 Hernández JM, Rentero MPZ. Bioactive compounds contained in Mediterranean Diet
564 and their effects on neurodegenerative diseases. In: Shiomi N (ed). *Current Topics on*
565 *Superfoods*. (IntechOpen, London, 2018), pp 13-32.
- 566 9 Tosti V, Bertozzi B, Fontana L. Health benefits of the Mediterranean diet: Metabolic
567 and molecular mechanisms. *J Gerontol A Biol Sci Med Sci*. 2018; 73: 318–326.

- 568 10 Del Rio D, Costa LG, Lean MEJ, Crozier A. Polyphenols and health: What compounds
569 are involved? *Nutr Metab Cardiovasc Dis* 2010; 20: 1–6.
- 570 11 Buttriss JL, Stokes CS. Dietary fibre and health: An overview. *Nutr Bull*. 2008; 33: 186–
571 200.
- 572 12 Swanson D, Block R, Mousa SA. Omega-3 fatty acids EPA and DHA: Health benefits
573 throughout life. *Adv Nutr*. 2012; 3: 1–7.
- 574 13 Siervo M, Scialò F, Shannon OM, Stephan BCM, Ashor AW. Does dietary nitrate say
575 NO to cardiovascular ageing? Current evidence and implications for research. *Proc Nutr*
576 *Soc*. 2018; 77: 112–123.
- 577 14 Ashor AW, Shannon OM, Werner A-D, Scialo F, Gilliard CN, Cassel KS et al. Effects
578 of inorganic nitrate and vitamin C co-supplementation on blood pressure and vascular
579 function in younger and older healthy adults: A randomised double-blind crossover trial.
580 *Clin Nutr*. 2019; 39:708-717.
- 581 15 Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F et al. Primary
582 prevention of cardiovascular disease with a Mediterranean Diet supplemented with
583 extra-virgin olive oil or nuts. *N Engl J Med*. 2018; 378:e34.
- 584 16 Salas-Salvadó J, Bulló M, Babio N, Martínez-González MÁ, Ibarrola-Jurado N, Basora
585 J et al. Reduction in the incidence of type 2 diabetes with the Mediterranean diet: Results
586 of the PREDIMED-Reus nutrition intervention randomized trial. *Diabetes Care*. 2011;
587 34: 14–19.
- 588 17 Petersson SD, Philippou E. Mediterranean diet, cognitive function, and dementia: A
589 systematic review of the evidence. *Adv Nutr*. 2016; 7: 889–904.
- 590 18 Schwingshackl L, Schwedhelm C, Galbete C, Hoffmann G. Adherence to Mediterranean
591 diet and risk of cancer: An updated systematic review and meta-analysis. *Nutrients*.
592 2017; 9: 1063.

- 593 19 Lasheras C, Fernandez S, Patterson AM. Mediterranean diet and age with respect to
594 overall survival in institutionalized, nonsmoking elderly people. *Am J Clin Nutr.* 2000;
595 71: 987–992.
- 596 20 Trichopoulou A, Orfanos P, Norat T, Bueno-de-Mesquita B, Ocké MC, Peeters PH et
597 al. Modified Mediterranean diet and survival: EPIC-elderly prospective cohort study.
598 *BMJ.* 2005; 330: 991.
- 599 21 Soltani S, Jayedi A, Shab-Bidar S, Becerra-Tomás N, Salas-Salvadó J. Adherence to the
600 Mediterranean Diet in relation to all-cause mortality: A systematic review and dose-
601 response meta-analysis of prospective cohort studies. *Adv Nutr.* 2019; 10: 1029–1039.
- 602 22 Aguilera A, Gómez-González B. Genome instability: A mechanistic view of its causes
603 and consequences. *Nat Rev Genet.* 2008; 9: 204–217.
- 604 23 Corella D, Ordovas J, Sorli J, Asensio E, Ortega C, Carrasco P et al. Effect of the
605 Mediterranean diet on DNA methylation of selected genes in the PREDIMED-Valencia
606 intervention trial. *FASEB J.* 2015; 29: LB242.
- 607 24 Herrera-Marcos LV, Lou-Bonafonte JM, Arnal C, Navarro MA, Osada J.
608 Transcriptomics and the Mediterranean diet: A systematic review. *Nutrients.* 2017; 9:
609 472.
- 610 25 Ungvari Z, Bagi Z, Feher A, Recchia FA, Sonntag WE, Pearson K et al. Resveratrol
611 confers endothelial protection via activation of the antioxidant transcription factor Nrf2.
612 *Am J Physiol Heart Circ Physiol.* 2010; 299: H18-24.
- 613 26 Arpón A, Riezu-Boj JI, Milagro FI, Marti A, Razquin C, Martínez-González MA et al.
614 Adherence to Mediterranean diet is associated with methylation changes in
615 inflammation-related genes in peripheral blood cells. *J Physiol Biochem.* 2016; 73: 445–
616 455.

- 617 27 Kaneko K, Akuta T, Sawa T, Kim HW, Fujii S, Okamoto T et al. Mutagenicity of 8-
618 nitroguanosine, a product of nitrative nucleoside modification by reactive nitrogen
619 oxides, in mammalian cells. *Cancer Lett.* 2008; 262: 239–247.
- 620 28 Zhang Y, Yuan F, Wu X, Wang M, Rechkoblit O, Taylor J-S et al. Error-free and error-
621 prone lesion bypass by human DNA polymerase κ in vitro. *Nucleic Acids Res.* 2000;
622 28: 4138–4146.
- 623 29 Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA
624 damage and cancer incidence. *Mol Cell Biochem.* 2004; 266: 37–56.
- 625 30 Urquiaga I, Strobel P, Perez D, Martinez C, Cuevas A, Castillo O et al. Mediterranean
626 diet and red wine protect against oxidative damage in young volunteers. *Atherosclerosis.*
627 2010; 211: 694–699.
- 628 31 Vilahur G, Cubedo J, Padró T, Casaní L, Mendieta G, González A et al. Intake of cooked
629 tomato sauce preserves coronary endothelial function and improves apolipoprotein A-I
630 and apolipoprotein J protein profile in high-density lipoproteins. *Transl Res J Lab Clin*
631 *Med.* 2015; 166: 44–56.
- 632 32 Erol Ö, Arda N, Erdem G. Phenols of virgin olive oil protects nuclear DNA against
633 oxidative damage in HeLa cells. *Food Chem Toxicol.* 2012; 50: 3475–3479.
- 634 33 Rangel-Zuñiga OA, Haro C, Tormos C, Perez-Martinez P, Delgado-Lista J, Marin C et
635 al. Frying oils with high natural or added antioxidants content, which protect against
636 postprandial oxidative stress, also protect against DNA oxidation damage. *Eur J Nutr.*
637 2017; 56: 1597–1607.
- 638 34 Calcabrini C, De Bellis R, Mancini U, Cucchiari L, Stocchi V, Potenza L. Protective
639 effect of *Juglans regia* L. walnut extract against oxidative DNA damage. *Plant Foods*
640 *Hum Nutr.* 2017; 72: 192–197.

- 641 35 Quiles JL, Ochoa JJ, Ramirez-Tortosa C, Battino M, Huertas JR, Martín Y et al. Dietary
642 fat type (virgin olive vs. sunflower oils) affects age-related changes in DNA double-
643 strand-breaks, antioxidant capacity and blood lipids in rats. *Exp Gerontol.* 2004; 39:
644 1189–1198.
- 645 36 Saieva C, Peluso M, Palli D, Cellai F, Ceroti M, Selvi V et al. Dietary and lifestyle
646 determinants of malondialdehyde DNA adducts in a representative sample of the
647 Florence City population. *Mutagenesis.* 2016; 31: 475–480.
- 648 37 von Zglinicki T. Oxidative stress shortens telomeres. *Trends Biochem Sci.* 2002; 27:
649 339–344.
- 650 38 Calado RT, Young NS. Telomere Diseases. *N Engl J Med.* 2009; 361: 2353–2365.
- 651 39 Boonekamp JJ, Simons MJP, Hemerik L, Verhulst S. Telomere length behaves as
652 biomarker of somatic redundancy rather than biological age. *Aging Cell.* 2013; 12: 330–
653 332.
- 654 40 Astuti Y, Wardhana A, Watkins J, Wulaningsih W, PILAR Research Network. Cigarette
655 smoking and telomere length: A systematic review of 84 studies and meta-analysis.
656 *Environ Res* 2017; 158: 480–489.
- 657 41 Arsenis NC, You T, Ogawa EF, Tinsley GM, Zuo L. Physical activity and telomere
658 length: Impact of aging and potential mechanisms of action. *Oncotarget.* 2017; 8:
659 45008–45019.
- 660 42 Meinilä J, Perälä M-M, Kautiainen H, Männistö S, Kanerva N, Shivappa N et al. Healthy
661 diets and telomere length and attrition during a 10-year follow-up. *Eur J Clin Nutr.* 2019;
662 73: 1352–1360.
- 663 43 Boccardi V, Esposito A, Rizzo MR, Marfella R, Barbieri M, Paolisso G. Mediterranean
664 diet, telomere maintenance and health status among elderly. *PLoS ONE.* 2013; 8:
665 e62781.

- 666 44 Crous-Bou M, Fung TT, Prescott J, Julin B, Du M, Sun Q et al. Mediterranean diet and
667 telomere length in Nurses' Health Study: Population based cohort study. *BMJ*. 2014;
668 349: g6674.
- 669 45 Gu Y, Honig LS, Schupf N, Lee JH, Luchsinger JA, Stern Y et al. Mediterranean diet
670 and leukocyte telomere length in a multi-ethnic elderly population. *Age (Dordr)*. 2015;
671 37: 24.
- 672 46 García-Calzón S, Martínez-González MA, Razquin C, Corella D, Salas-Salvadó J,
673 Martínez JA et al. Pro12Ala polymorphism of the PPAR γ 2 gene interacts with a
674 Mediterranean diet to prevent telomere shortening in the PREDIMED-NAVARRA
675 randomized trial. Clinical perspective. *Circ Genomic Precis Med*. 2015; 8: 91–99.
- 676 47 Gomez-Delgado F, Delgado-Lista J, Lopez-Moreno J, Rangel-Zuñiga OA, Alcalá-Díaz
677 JF, Leon-Acuña A et al. Telomerase RNA component genetic variants interact with the
678 Mediterranean diet modifying the inflammatory status and its relationship with aging:
679 CORDIOPREV study. *J Gerontol A Biol Sci Med Sci*. 2018; 73: 327–332.
- 680 48 García-Calzón S, Zalba G, Ruiz-Canela M, Shivappa N, Hébert JR, Martínez JA et al.
681 Dietary inflammatory index and telomere length in subjects with a high cardiovascular
682 disease risk from the PREDIMED-NAVARRA study: cross-sectional and longitudinal
683 analyses over 5 y. *Am J Clin Nutr*. 2015; 102: 897–904.
- 684 49 García-Calzón S, Martínez-González MA, Razquin C, Arós F, Lapetra J, Martínez JA et
685 al. Mediterranean diet and telomere length in high cardiovascular risk subjects from the
686 PREDIMED-NAVARRA study. *Clin Nutr*. 2016; 35: 1399–1405.
- 687 50 Cora' D, Re A, Caselle M, Bussolino F. MicroRNA-mediated regulatory circuits:
688 outlook and perspectives. *Phys Biol*. 2017; 14: 045001.
- 689 51 Pal S, Tyler JK. Epigenetics and aging. *Sci Adv*. 2016; 2: e1600584.

- 690 52 Mathers JC, Strathdee G, Relton CL. Induction of epigenetic alterations by dietary and
691 other environmental factors. *Adv Genet.* 2010; 71: 3–39.
- 692 53 Mathers JC. Session 2: Personalised nutrition. Epigenomics: a basis for understanding
693 individual differences? *Proc Nutr Soc.* 2008; 67: 390–394.
- 694 54 Park JH, Yoo Y, Park YJ. Epigenetics: Linking nutrition to molecular mechanisms in
695 aging. *Prev Nutr Food Sci.* 2017; 22: 81–89.
- 696 55 Lisanti S, Omar WAW, Tomaszewski B, Prins SD, Jacobs G, Koppen G et al.
697 Comparison of methods for quantification of global DNA methylation in human cells
698 and tissues. *PLoS ONE.* 2013; 8: e79044.
- 699 56 Muka T, Koromani F, Portilla E, O’Connor A, Bramer WM, Troup J et al. The role of
700 epigenetic modifications in cardiovascular disease: A systematic review. *Int J Cardiol.*
701 2016; 212: 174–183.
- 702 57 Cardelli M. The epigenetic alterations of endogenous retroelements in aging. *Mech*
703 *Ageing Dev.* 2018; 174: 30–46.
- 704 58 Agodi A, Barchitta M, Quattrocchi A, Maugeri A, Canto C, Marchese AE et al. Low
705 fruit consumption and folate deficiency are associated with LINE-1 hypomethylation in
706 women of a cancer-free population. *Genes Nutr.* 2015; 10: 480.
- 707 59 Marques-Rocha JL, Milagro FI, Mansego ML, Zulet MA, Bressan J, Martínez JA.
708 Expression of inflammation-related miRNAs in white blood cells from subjects with
709 metabolic syndrome after 8 wk of following a Mediterranean diet-based weight loss
710 program. *Nutrition.* 2016; 32: 48–55.
- 711 60 Tang B, Lei B, Qi G, Liang X, Tang F, Yuan S et al. MicroRNA-155-3p promotes
712 hepatocellular carcinoma formation by suppressing FBXW7 expression. *J Exp Clin*
713 *Cancer Res.* 2016; 35: 93.

714 61 Spolverini A, Fuchs G, Bublik DR, Oren M. let-7b and let-7c microRNAs promote
715 histone H2B ubiquitylation and inhibit cell migration by targeting multiple components
716 of the H2B deubiquitylation machinery. *Oncogene*. 2017; 36: 5819–5828.

717 62 Ma J, Rebholz CM, Braun KVE, Reynolds LM, Aslibekyan S, Xia R et al. Whole blood
718 DNA methylation signatures of diet are associated with cardiovascular disease risk
719 factors and all-cause mortality. *Circ Genomic Precis Med*. 2020; e-pub ahead of print 11
720 June 2020; doi:10.1161/CIRCGEN.119.002766.

721 63 Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol*. 2013;
722 14: R115.

723 64 Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory
724 of ageing. *Nat Rev Genet*. 2018; 19: 371–384.

725 65 Horvath S, Erhart W, Brosch M, Ammerpohl O, von Schönfels W, Ahrens M et al.
726 Obesity accelerates epigenetic aging of human liver. *Proc Natl Acad Sci U S A*. 2014;
727 111: 15538–15543.

728 66 Quach A, Levine ME, Tanaka T, Lu AT, Chen BH, Ferrucci L et al. Epigenetic clock
729 analysis of diet, exercise, education, and lifestyle factors. *Aging*. 2017; 9: 419–446.

730 67 Gensous N, Garagnani P, Santoro A, Giuliani C, Ostan R, Fabbri C et al. One-year
731 Mediterranean diet promotes epigenetic rejuvenation with country- and sex-specific
732 effects: A pilot study from the NU-AGE project. *GeroScience*. 2020; 42: 687–701.

733 68 Labbadia J, Morimoto RI. The biology of proteostasis in aging and disease. *Annu Rev*
734 *Biochem*. 2015; 84: 435–464.

735 69 Yerbury JJ, Ooi L, Dillin A, Saunders DN, Hatters DM, Beart PM et al. Walking the
736 tightrope: proteostasis and neurodegenerative disease. *J Neurochem*. 2016; 137: 489–
737 505.

738 70 Henning RH, Brundel BJJM. Proteostasis in cardiac health and disease. *Nat Rev Cardiol.*
739 2017; 14: 637–653.

740 71 Román GC, Jackson RE, Reis J, Román AN, Toledo JB, Toledo E. Extra-virgin olive
741 oil for potential prevention of Alzheimer disease. *Rev Neurol.* 2019; 175: 705–723.

742 72 Fernández del Río L, Gutiérrez-Casado E, Varela-López A, Villalba JM. Olive Oil and
743 the Hallmarks of Aging. *Molecules.* 2016; 21: 163.

744 73 Rigacci S, Miceli C, Nediani C, Berti A, Cascella R, Pantano D et al. Oleuropein
745 aglycone induces autophagy via the AMPK/mTOR signalling pathway: A mechanistic
746 insight. *Oncotarget.* 2015; 6: 35344–35357.

747 74 Abuznait AH, Qosa H, Busnena BA, El Sayed KA, Kaddoumi A. Olive-oil-derived
748 oleocanthal enhances β -amyloid clearance as a potential neuroprotective mechanism
749 against Alzheimer’s disease: In vitro and in vivo studies. *ACS Chem Neurosci.* 2013; 4:
750 973–982.

751 75 Kirkwood TBL. Evolution of ageing. *Nature.* 1977; 270: 301–304.

752 76 Kirkwood TB, Holliday R. The evolution of ageing and longevity. *Proc R Soc Lond B*
753 *Biol Sci.* 1979; 205: 531–546.

754 77 Templeman NM, Murphy CT. Regulation of reproduction and longevity by nutrient-
755 sensing pathways. *J Cell Biol.* 2018; 217: 93–106.

756 78 de Lucia C, Murphy T, Steves CJ, Dobson RJB, Proitsi P, Thuret S. Lifestyle mediates
757 the role of nutrient-sensing pathways in cognitive aging: cellular and epidemiological
758 evidence. *Commun Biol.* 2020; 3: 1–17.

759 79 Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. A *C. elegans* mutant that lives
760 twice as long as wild type. *Nature.* 1993; 366: 461–464.

761 80 Puig O, Tjian R. Transcriptional feedback control of insulin receptor by
762 dFOXO/FOXO1. *Genes Dev.* 2005; 19: 2435–2446.

763 81 van der Horst A, Burgering BMT. Stressing the role of FoxO proteins in lifespan and
764 disease. *Nat Rev Mol Cell Biol.* 2007; 8: 440–450.

765 82 van Heemst D. Insulin, IGF-1 and longevity. *Aging Dis.* 2010; 1: 147–157.

766 83 Calnan DR, Brunet A. The FoxO code. *Oncogene.* 2008; 27: 2276–2288.

767 84 Johnson SC, Rabinovitch PS, Kaeberlein M. mTOR is a key modulator of ageing and
768 age-related disease. *Nature.* 2013; 493: 338–345.

769 85 Alers S, Löffler AS, Wesselborg S, Stork B. Role of AMPK-mTOR-Ulk1/2 in the
770 regulation of autophagy: cross talk, shortcuts, and feedbacks. *Mol Cell Biol.* 2012; 32:
771 2–11.

772 86 Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control
773 of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature.* 2005;
774 434: 113–118.

775 87 Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM et al.
776 Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science.* 2009;
777 325: 201–204.

778 88 Vasto S, Buscemi S, Barera A, Carlo MD, Accardi G, Caruso C. Mediterranean diet and
779 healthy ageing: A Sicilian perspective. *Gerontology.* 2014; 60: 508–518.

780 89 Fontana L, Adelaye RM, Rastelli AL, Miles KM, Ciamporcero E, Longo VD et al.
781 Dietary protein restriction inhibits tumor growth in human xenograft models.
782 *Oncotarget.* 2013; 4: 2451–2461.

783 90 Kazemi A, Speakman JR, Soltani S, Djafarian K. Effect of calorie restriction or protein
784 intake on circulating levels of insulin like growth factor I in humans: A systematic
785 review and meta-analysis. *Clin Nutr.* 2020. 39:1705-1716.

786 91 Runchey SS, Pollak MN, Valsta LM, Coronado GD, Schwarz Y, Breymeyer KL et al.
787 Glycemic load effect on fasting and post-prandial serum glucose, insulin, IGF-1 and

788 IGFBP-3 in a randomized, controlled feeding study. *Eur J Clin Nutr.* 2012; 66: 1146–
789 1152.

790 92 Di Bona D, Accardi G, Virruso C, Candore G, Caruso C. Association between genetic
791 variations in the insulin/insulin-like growth factor (Igf-1) signaling pathway and
792 longevity: a systematic review and meta-analysis. *Curr Vasc Pharmacol.* 2014; 12: 674–
793 681.

794 93 de Lucia C, Murphy T, Thuret S. Emerging molecular pathways governing dietary
795 regulation of neural stem cells during aging. *Front Physiol.* 2017; 8: 17.

796 94 Menendez JA, Joven J, Aragonès G, Barraji3n-Catal3n E, Beltr3n-Deb3n R, Borr3s-
797 Linares I et al. Xenohormetic and anti-aging activity of secoiridoid polyphenols present
798 in extra virgin olive oil. *Cell Cycle.* 2013; 12: 555–578.

799 95 Jung CH, Ro S-H, Cao J, Otto NM, Kim D-H. mTOR regulation of autophagy. *FEBS*
800 *Lett.* 2010; 584: 1287–1295.

801 96 Lee IH, Cao L, Mostoslavsky R, Lombard DB, Liu J, Bruns NE et al. A role for the
802 NAD-dependent deacetylase Sirt1 in the regulation of autophagy. *Proc Natl Acad Sci.*
803 2008; 105: 3374–3379.

804 97 Russo MA, Sansone L, Polletta L, Runci A, Rashid MM, De Santis E et al. Sirtuins and
805 resveratrol-derived compounds: a model for understanding the beneficial effects of the
806 Mediterranean diet. *Endocr Metab Immune Disord Drug Targets.* 2014; 14: 300–308.

807 98 McBride HM, Neuspiel M, Wasiak S. Mitochondria: More than just a powerhouse. *Curr*
808 *Biol CB.* 2006; 16: R551-560.

809 99 Hekimi S, Lapointe J, Wen Y. Taking a ‘good’ look at free radicals in the aging process.
810 *Trends Cell Biol.* 2011; 21: 569–576.

- 811 100 Park J-S, Davis RL, Sue CM. Mitochondrial dysfunction in Parkinson's disease: New
812 mechanistic insights and therapeutic perspectives. *Curr Neurol Neurosci Rep.* 2018; 18:
813 21.
- 814 101 Birnbaum JH, Wanner D, Gietl AF, Saake A, Kündig TM, Hock C et al. Oxidative stress
815 and altered mitochondrial protein expression in the absence of amyloid- β and tau
816 pathology in iPSC-derived neurons from sporadic Alzheimer's disease patients. *Stem*
817 *Cell Res.* 2018; 27: 121–130.
- 818 102 Varela-Lopez A, Pérez-López MP, Ramirez-Tortosa CL, Battino M, Granados-Principal
819 S, Ramirez-Tortosa MDC et al. Gene pathways associated with mitochondrial function,
820 oxidative stress and telomere length are differentially expressed in the liver of rats fed
821 lifelong on virgin olive, sunflower or fish oils. *J Nutr Biochem.* 2018; 52: 36–44.
- 822 103 Amel N, Wafa T, Samia D, Yousra B, Issam C, Cheraif I et al. Extra virgin olive oil
823 modulates brain docosahexaenoic acid level and oxidative damage caused by 2,4-
824 Dichlorophenoxyacetic acid in rats. *J Food Sci Technol.* 2016; 53: 1454–1464.
- 825 104 Schaffer S, Podstawa M, Visioli F, Bogani P, Müller WE, Eckert GP. Hydroxytyrosol-
826 rich olive mill wastewater extract protects brain cells in vitro and ex vivo. *J Agric Food*
827 *Chem.* 2007; 55: 5043–5049.
- 828 105 Sun W, Wang X, Hou C, Yang L, Li H, Guo J et al. Oleuropein improves mitochondrial
829 function to attenuate oxidative stress by activating the Nrf2 pathway in the hypothalamic
830 paraventricular nucleus of spontaneously hypertensive rats. *Neuropharmacology.* 2017;
831 113: 556–566.
- 832 106 Peng Y, Hou C, Yang Z, Li C, Jia L, Liu J et al. Hydroxytyrosol mildly improve
833 cognitive function independent of APP processing in APP/PS1 mice. *Mol Nutr Food*
834 *Res.* 2016; 60: 2331–2342.

- 835 107 Afshordel S, Hagl S, Werner D, Röhner N, Kögel D, Bazan NG et al. Omega-3
836 polyunsaturated fatty acids improve mitochondrial dysfunction in brain aging--impact
837 of Bcl-2 and NPD-1 like metabolites. *Prostaglandins Leukot Essent Fatty Acids*. 2015;
838 92: 23–31.
- 839 108 Johnson ML, Lalia AZ, Dasari S, Pallauf M, Fitch M, Hellerstein MK et al.
840 Eicosapentaenoic acid but not docosahexaenoic acid restores skeletal muscle
841 mitochondrial oxidative capacity in old mice. *Aging Cell*. 2015; 14: 734–743.
- 842 109 Lalia AZ, Dasari S, Robinson MM, Abid H, Morse DM, Klaus KA et al. Influence of
843 omega-3 fatty acids on skeletal muscle protein metabolism and mitochondrial
844 bioenergetics in older adults. *Aging*. 2017; 9: 1096–1129.
- 845 110 Markoski MM, Garavaglia J, Oliveira A, Olivaes J, Marcadenti A. Molecular Properties
846 of Red Wine Compounds and Cardiometabolic Benefits. *Nutr Metab Insights*. 2016; 9:
847 51–57.
- 848 111 Qiu L, Luo Y, Chen X. Quercetin attenuates mitochondrial dysfunction and biogenesis
849 via upregulated AMPK/SIRT1 signaling pathway in OA rats. *Biomed Pharmacother*.
850 2018; 103: 1585–1591.
- 851 112 Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F et al.
852 Resveratrol improves mitochondrial function and protects against metabolic disease by
853 activating SIRT1 and PGC-1alpha. *Cell*. 2006; 127: 1109–1122.
- 854 113 Markus MA, Morris BJ. Resveratrol in prevention and treatment of common clinical
855 conditions of aging. *Clin Interv Aging*. 2008; 3: 331–339.
- 856 114 Putti R, Sica R, Migliaccio V, Lionetti L. Diet impact on mitochondrial bioenergetics
857 and dynamics. *Front Physiol*. 2015; 6: 109.

858 115 von Zglinicki T, Wan T, Miwa S. Senescence in post-mitotic cells: A driver of aging?
859 Antioxid Redox Signal. 2020; e-pub ahead of print 27 April 2020:
860 doi:10.1089/ars.2020.8048.

861 116 Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. *Cell*. 2007;
862 130: 223–233.

863 117 Tchkonja T, Zhu Y, van Deursen J, Campisi J, Kirkland JL. Cellular senescence and the
864 senescent secretory phenotype: therapeutic opportunities. *J Clin Invest* 2013; 123: 966–
865 972.

866 118 Song S, Lam EW-F, Tchkonja T, Kirkland JL, Sun Y. Senescent cells: Emerging targets
867 for human aging and age-related diseases. *Trends Biochem Sci* 2020; 45: 578–592.

868 119 Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J et al. Naturally
869 occurring p16 Ink4a -positive cells shorten healthy lifespan. *Nature* 2016; 530: 184–189.

870 120 Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weivoda MM et al. Senolytics
871 improve physical function and increase lifespan in old age. *Nat Med* 2018; 24: 1246–
872 1256.

873 121 Kleemann R, Verschuren L, Morrison M, Zadelaar S, van Erk MJ, Wielinga PY et al.
874 Anti-inflammatory, anti-proliferative and anti-atherosclerotic effects of quercetin in
875 human in vitro and in vivo models. *Atherosclerosis* 2011; 218: 44–52.

876 122 Medina-Remón A, Tresserra-Rimbau A, Pons A, Tur JA, Martorell M, Ros E et al.
877 Effects of total dietary polyphenols on plasma nitric oxide and blood pressure in a high
878 cardiovascular risk cohort. The PREDIMED randomized trial. *Nutr Metab Cardiovasc*
879 *Dis* 2015; 25: 60–67.

880 123 Gurău F, Baldoni S, Prattichizzo F, Espinosa E, Amenta F, Procopio AD et al. Anti-
881 senescence compounds: A potential nutraceutical approach to healthy aging. *Ageing Res*
882 *Rev* 2018; 46: 14–31.

- 883 124 Hickson LJ, Langhi Prata LGP, Bobart SA, Evans TK, Giorgadze N, Hashmi SK et al.
884 Senolytics decrease senescent cells in humans: Preliminary report from a clinical trial of
885 Dasatinib plus Quercetin in individuals with diabetic kidney disease. *EBioMedicine*
886 2019; 47: 446–456.
- 887 125 Del Bo' C, Marino M, Martini D, Tucci M, Ciappellano S, Riso P et al. Overview of
888 human intervention studies evaluating the impact of the Mediterranean diet on markers
889 of DNA damage. *Nutrients* 2019; 11. doi:10.3390/nu11020391.
- 890 126 Corina A, Rangel-Zúñiga OA, Jiménez-Lucena R, Alcalá-Díaz JF, Quintana-Navarro G,
891 Yubero-Serrano EM et al. Low intake of vitamin E accelerates cellular aging in patients
892 with established cardiovascular disease: The CORDIOPREV study. *J Gerontol Ser A*
893 2019; 74: 770–777.
- 894 127 Durani LW, Jaafar F, Tan JK, Tajul Arifin K, Mohd Yusof YA, Wan Ngah WZ et al.
895 Targeting genes in insulin-associated signalling pathway, DNA damage, cell
896 proliferation and cell differentiation pathways by tocotrienol-rich fraction in preventing
897 cellular senescence of human diploid fibroblasts. *Clin Ter* 2015; 166: e365-373.
- 898 128 López-Uriarte P, Nogués R, Saez G, Bulló M, Romeu M, Masana L et al. Effect of nut
899 consumption on oxidative stress and the endothelial function in metabolic syndrome.
900 *Clin Nutr* 2010; 29: 373–380.
- 901 129 Riso P, Martini D, Visioli F, Martinetti A, Porrini M. Effect of broccoli intake on
902 markers related to oxidative stress and cancer risk in healthy smokers and nonsmokers.
903 *Nutr Cancer* 2009; 61: 232–237.
- 904 130 Moser B, Szekeres T, Bieglmayer C, Wagner K-H, Mišik M, Kundi M et al. Impact of
905 spinach consumption on DNA stability in peripheral lymphocytes and on biochemical
906 blood parameters: results of a human intervention trial. *Eur J Nutr* 2011; 50: 587–594.

- 907 131 Mihaylova MM, Sabatini DM, Yilmaz ÖH. Dietary and metabolic control of stem cell
908 function in physiology and cancer. *Cell Stem Cell* 2014; 14: 292–305.
- 909 132 Gruver A, Hudson L, Sempowski G. Immunosenescence of ageing. *J Pathol* 2007; 211:
910 144–156.
- 911 133 Fulop T, Larbi A, Dupuis G, Le Page A, Frost EH, Cohen AA et al. Immunosenescence
912 and inflamm-aging as two sides of the same coin: Friends or foes? *Front Immunol* 2018;
913 8: 1960.
- 914 134 Ungvari Z, Kaley G, de Cabo R, Sonntag WE, Csiszar A. Mechanisms of vascular aging:
915 New perspectives. *J Gerontol A Biol Sci Med Sci* 2010; 65: 1028–1041.
- 916 135 Marin C, Delgado-Lista J, Ramirez R, Carracedo J, Caballero J, Perez-Martinez P et al.
917 Mediterranean diet reduces senescence-associated stress in endothelial cells. *AGE* 2012;
918 34: 1309–1316.
- 919 136 Cesari F, Marcucci R, Gori AM, Burgisser C, Francini S, Roberts AT et al. Adherence
920 to lifestyle modifications after a cardiac rehabilitation program and endothelial
921 progenitor cells. A six-month follow-up study. *Thromb Haemost* 2014; 112: 196–204.
- 922 137 Cesari F, Sofi F, Molino Lova R, Vannetti F, Pasquini G, Cecchi F et al. Aging process,
923 adherence to Mediterranean diet and nutritional status in a large cohort of nonagenarians:
924 Effects on endothelial progenitor cells. *Nutr Metab Cardiovasc Dis NMCD* 2018; 28:
925 84–90.
- 926 138 Marin C, Ramirez R, Delgado-Lista J, Yubero-Serrano EM, Perez-Martinez P,
927 Carracedo J et al. Mediterranean diet reduces endothelial damage and improves the
928 regenerative capacity of endothelium. *Am J Clin Nutr* 2011; 93: 267–274.
- 929 139 Fernández JM, Rosado-Álvarez D, Da Silva Grigoletto ME, Rangel-Zúñiga OA,
930 Landaeta-Díaz LL, Caballero-Villarraso J et al. Moderate-to-high-intensity training and

931 a hypocaloric Mediterranean diet enhance endothelial progenitor cells and fitness in
932 subjects with the metabolic syndrome. *Clin Sci Lond Engl* 1979 2012; 123: 361–373.

933 140 Shannon OM, Mendes I, Köchl C, Mazidi M, Ashor AW, Rubele S et al. Mediterranean
934 diet increases endothelial function in adults: A systematic review and meta-analysis of
935 randomized controlled trials. *J Nutr* 2020; 150: 1151-1159.

936 141 Liu H, Huang H, Li B, Wu D, Wang F, Zheng X hua et al. Olive oil in the prevention
937 and treatment of osteoporosis after artificial menopause. *Clin Interv Aging* 2014; 9:
938 2087–2095.

939 142 Fernández-Real JM, Bulló M, Moreno-Navarrete JM, Ricart W, Ros E, Estruch R et al.
940 A Mediterranean diet enriched with olive oil is associated with higher serum total
941 osteocalcin levels in elderly men at high cardiovascular risk. *J Clin Endocrinol Metab*
942 2012; 97: 3792–3798.

943 143 Mittelbrunn M, Sánchez-Madrid F. Intercellular communication: diverse structures for
944 exchange of genetic information. *Nat Rev Mol Cell Biol* 2012; 13: 328–335.

945 144 Leone A, Longo C, Trosko JE. The chemopreventive role of dietary phytochemicals
946 through gap junctional intercellular communication. *Phytochem Rev* 2012; 11: 285–307.

947 145 Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E et al. Inflamm-
948 aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci* 2000;
949 908: 244–254.

950 146 Cohen HJ, Pieper CF, Harris T, Rao KM, Currie MS. The association of plasma IL-6
951 levels with functional disability in community-dwelling elderly. *J Gerontol A Biol Sci*
952 *Med Sci* 1997; 52: M201-208.

953 147 Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential
954 contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci* 2014; 69 Suppl
955 1: S4-9.

- 956 148 Rosano C, Marsland AL, Gianaros PJ. Maintaining brain health by monitoring
957 inflammatory processes: a mechanism to promote successful aging. *Aging Dis* 2012; 3:
958 16–33.
- 959 149 Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger WH et al.
960 Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the
961 elderly. *Am J Med* 1999; 106: 506–512.
- 962 150 Michaud M, Balardy L, Moulis G, Gaudin C, Peyrot C, Vellas B et al. Proinflammatory
963 cytokines, aging, and age-related diseases. *J Am Med Dir Assoc* 2013; 14: 877–882.
- 964 151 Szarc vel Szic K, Declerck K, Vidaković M, Vanden Berghe W. From inflammaging to
965 healthy aging by dietary lifestyle choices: is epigenetics the key to personalized
966 nutrition? *Clin Epigenetics* 2015; 7: 33.
- 967 152 Xia S, Zhang X, Zheng S, Khanabdali R, Kalionis B, Wu J et al. An update on inflamm-
968 aging: mechanisms, prevention, and treatment. *J Immunol Res* 2016; 2016: 8426874.
- 969 153 Fransen F, van Beek AA, Borghuis T, Aidy SE, Hugenholtz F, van der Gaast – de Jongh
970 C et al. Aged gut microbiota contributes to systemical inflammaging after transfer to
971 germ-free mice. *Front Immunol* 2017; 8: 1385.
- 972 154 Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, Ruiz-Gutiérrez V,
973 Covas MI et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: A
974 randomized trial. *Ann Intern Med* 2006; 145: 1–11.
- 975 155 Mena M-P, Sacanella E, Vazquez-Agell M, Morales M, Fitó M, Escoda R et al.
976 Inhibition of circulating immune cell activation: a molecular antiinflammatory effect of
977 the Mediterranean diet. *Am J Clin Nutr* 2009; 89: 248–256.
- 978 156 Casas R, Sacanella E, Urpí-Sardà M, Chiva-Blanch G, Ros E, Martínez-González M-A
979 et al. The effects of the Mediterranean diet on biomarkers of vascular wall inflammation

980 and plaque vulnerability in subjects with high risk for cardiovascular disease. A
981 randomized trial. *PloS One* 2014; 9: e100084.

982 157 Salas-Salvadó J, Garcia-Arellano A, Estruch R, Marquez-Sandoval F, Corella D, Fiol M
983 et al. Components of the Mediterranean-type food pattern and serum inflammatory
984 markers among patients at high risk for cardiovascular disease. *Eur J Clin Nutr* 2008;
985 62: 651–659.

986 158 Richard C, Royer M-M, Couture P, Cianflone K, Rezvani R, Desroches S et al. Effect
987 of the Mediterranean diet on plasma adipokine concentrations in men with metabolic
988 syndrome. *Metabolism* 2013; 62: 1803–1810.

989 159 Sureda A, Bibiloni MDM, Julibert A, Bouzas C, Argelich E, Llupart I et al. Adherence
990 to the Mediterranean diet and inflammatory markers. *Nutrients* 2018; 10: 62.

991 160 Perez-Martinez P, Lopez-Miranda J, Blanco-Colio L, Bellido C, Jimenez Y, Moreno JA
992 et al. The chronic intake of a Mediterranean diet enriched in virgin olive oil, decreases
993 nuclear transcription factor kappaB activation in peripheral blood mononuclear cells
994 from healthy men. *Atherosclerosis* 2007; 194: e141-146.

995 161 Park Y-M, Zhang J, Steck SE, Fung TT, Hazlett LJ, Han K et al. Obesity mediates the
996 association between Mediterranean diet consumption and insulin resistance and
997 inflammation in US adults. *J Nutr* 2017; 147: 563–571.

998 162 Ordovas JM, Ferguson LR, Tai ES, Mathers JC. Personalised nutrition and health. *BMJ*
999 2018; 361: k2173.

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1006 **Figure Legends**

1007 **Figure 1.** A schematic representation of the mechanisms through which the Mediterranean
1008 diet, or key components within this dietary pattern, may influence the nine hallmark features
1009 of ageing.

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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 ₂ O 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 PPAR γ 2 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 Fe ²⁺ 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 gmentation.
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 <i>in vivo</i> .
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 <i>ex vivo</i> H ₂ O 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.

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