

Age is not just ‘a’ number:

through the lens of systems physiology

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Abstract

Causal inference in the field of ageing has remained largely misunderstood for a long time. Contrary to popular contemporary belief, chronological time does not influence ageing process in individuals equally and uniformly. In fact, different organs within an individual may have differential rates of ageing - something that can now be precisely measured in a tissue-specific context. In the recent past, quantitative tools have been developed that can provide proxies of Biological ageing (BA), and capture differences between the Chronological ageing (CA) and BA; and thus, provide insight into the so-called accelerated-ageing. In this review, I compile, assess, and discuss several pleiotropic effects of ageing and the associated risks of age-related pathologies. In particular, the functional reduction of several critical complex components (organ systems) - namely the metabolism, immune system, and the (gut) microbiome is observed in ageing. Due to intrinsic network-like properties and emerging crosstalk amongst these components, salient hallmarks and diverse signatures of ageing are observed. More intriguingly, recent pieces of evidence have suggested that the ageing process is plastic, i.e. some aspects of ageing can be rescued and might be reversible - at least partially if not fully. By compiling such pieces of evidence, I submit that researching these aspects of ageing will further unite these seemingly different effects of ageing, into a fundamentally cohesive understanding of what it means to age biologically and physiologically. In this light, I opine for a greater consideration of age-related pathologies within a ‘geroscience’ framework, as an enabler for better global public health outcomes.

Keywords – metabolic deficits, epigenetics, immunosenescence, systems physiology, ageing pleiotropy

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1 Introduction

Ageing can be conceived as the gradual decline of strict regulatory processes as well as the appropriate signalling required at a molecular, cellular, physiological level. While epigenetics (within the landscape of underlying genetics) shaped by the environment seems to play a critical role in regulatory processes, emerging evidence increasingly suggests growing importance of the signalling in the gastrointestinal tract for health and homeostasis.

Ageing is an inevitable process of the eukaryotic kingdom, including humans. Ageing also happens to be the most significant risk factor for the majority of chronic diseases (Kennedy et al., 2014). By definition, ageing poses risks for age-related pathologies, such as Cardiovascular disease (CVD); metabolic diseases, e.g. type 2 Diabetes Mellitus (t2DM); neurodegenerative diseases, e.g., Alzheimer's disease (AD), cognitive decline; or other age-related functional declines such as frailty and sarcopenia, i.e., the condition of being weak and delicate and muscle loss in advanced ageing, respectively. In the recent decades, thanks to rigorous scientific endeavours on ageing, a rich body of literature has emerged in the field of geroscience and ageing (fig. 1).

While most of the early works were limited to animal and pre-clinical models, scientific research is now at an inflection point that has unique medical, commercial, and societal implications for arguably all biological research that impacts the human health-span, life-span, and longevity (Campisi et al., 2019). In the very recent past, tools have been developed that can quantify BA, as proxies of the true 'age', and hence can capture differences between the CA and BA. The difference between CA and BA is called accelerated-ageing; it quantifies the susceptibility to age-related pathologies. As accelerated-ageing drives, both risks of morbidity and all-cause mortality (Childs et al., 2015), developing truly accurate and precise techniques for quantification of biological ageing is of quintessential importance in geroscience research (Belsky et al., 2015; Jia et al., 2017; Levine et al., 2018). These progress enable researchers to quantify and capture ageing at a finer resolution, and thus explore insights related to the process of ageing.

1.1 A brief history of ageing research

Back in the year 1939, a prominent breakthrough in ageing research was the observation in mice and rats that suggested that Calorie restriction (CR) could increase life-span (McCay et al., 1939). After that, the concept of CR has been extended to nutrient restrictions (e.g., strict carbohydrate restriction via Ketogenic diet (KD)) (Bishop and Guarente, 2007); dietary restriction (e.g., elimination of potential allergens that may contribute to inflammatory pathogenesis, and disrupt the maintenance of immunological homeostasis) (Bishop and Guarente, 2007; Kirkwood, 2008); time-restricted eating protocols and protocols that mimic fasting or caloric restriction (Longo and Panda, 2016). Conspicuously, the impacts of CR have been replicated in several species, ranging from *S. cerevisiae*, *C. elegans*, and more recently in a primate model viz. rhesus monkeys (Mattison et al., 2017; Colman et al., 2014) and grey mouse lemurs (Pifferi et al., 2018).

Hence, CR was the first demonstration of plasticity (the ability to be modified) of the ageing process. And the effects of CR on longevity possibly extends across most (if not all) of the Eukarya

domain in the tree of life (Bonkowski and Sinclair, 2016; Rascón et al., 2012).

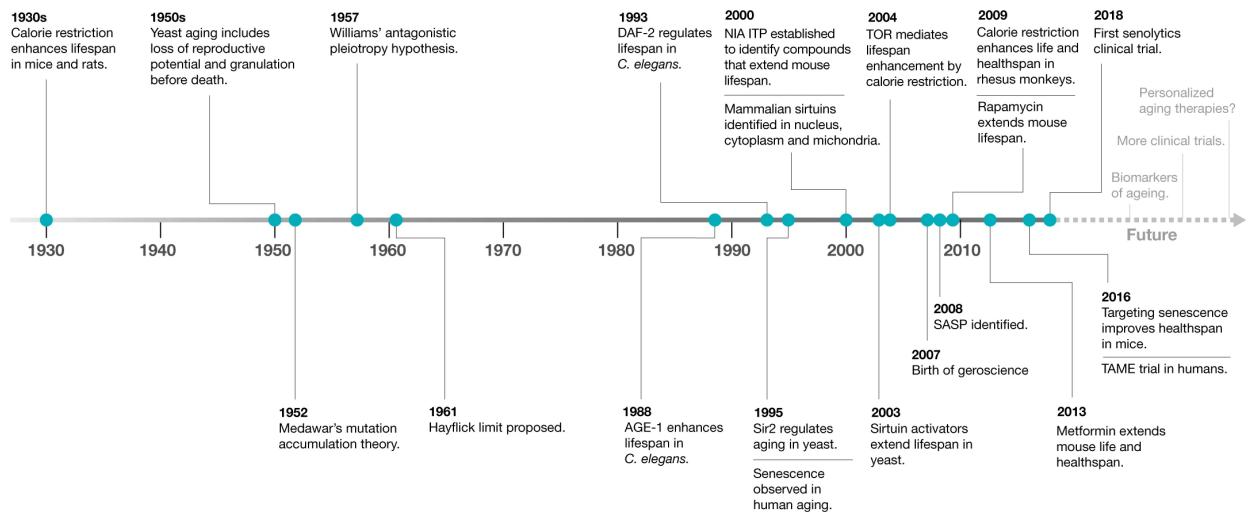


Figure 1: Timeline of scientific endeavours in the field ageing: The key discoveries in the field of ageing are highlighted here, starting from the 1930s (McCay et al., 1939), and leading up to 2019, wherein senolytics-based therapeutics were undergoing clinical trials. Notably, *SIR2* (Silent Information Regulator 2) was the first identified so-called longevity gene in yeasts (Anderson et al., 2003; Sinclair, 2005), wherein over-expression of the *SIR2* protein was sufficient to extend yeast lifespan. Subsequently, it was discovered that seven *SIR2* orthologs (now known as sirtuins) are encoded within the human genome. This figure is taken from Campisi et al. (2019).

Almost 60 years ago, the limited ability of human cell division was observed in cell culture (Hayflick and Moorhead, 1961), which is now formally recognized as cellular senescence (Campisi, 1998; Coppé et al., 2008). A subsequent breakthrough in the field was the evidence from Dimri et al. (1995) that the accumulation of senescent cells occurs in ageing. These so-called aged cells The contemporary delineation of salient features of cellular senescence, namely arrested cell proliferation, resistance to programmed cell death (apoptosis), and a complex Senescence-associated secretory phenotype (SASP) (Coppé et al., 2008, 2010), were steppingstones towards consensus and therapeutic targeting of senescence.

Later on, it was reported that mitochondrial dysfunction included a distinct state of secretory phenotype (Wiley et al., 2016). When it was demonstrated that clearance of senescent cells rescues age-related deficits and also partly rejuvenates functions, a new class of drugs known as senolytics emerged (Chang et al., 2016; Farr et al., 2017; Kirkland et al., 2017; Xu et al., 2018; Fuhrmann-Stroissnigg et al., 2017; Yosef et al., 2016).

The genetic variations underpinning longevity in experimental and pre-clinical models (fig. 1), namely the discovery of *SIR2* in yeasts, in the year 1995, provided a strong impetus to the field and sped up future scientific efforts. Soon after, it was in 2000 that the mammalian sirtuins - the orthologs of yeast *SIR2* - were identified. Sirtuins are a family of proteins that signal the cellular energy status and are involved in the metabolic regulation (Ye et al., 2017; Yamamoto et al., 2007), such as cell cycle, tumorigenesis, DNA repair, TNF secretion, and rRNA transcription amongst other functions. Importantly, these energy-sensing longevity genes are evolutionarily ancient, and their structure is highly conserved across all kingdoms of life (Ye et al., 2017). Such genes may be expected after all,

as the organization of the various complexities of life itself (which requires reduction of intracellular, molecular, or physiological entropy) is an energy-consuming process. It became increasingly evident that activation of sirtuins was dependent on Nicotinamide adenine dinucleotide (NAD) (Nogueiras et al., 2012; Haigis and Sinclair, 2010; Nogueiras et al., 2012; Bonkowski and Sinclair, 2016), which is a co-factor that is central to the metabolism of all living cells (Verdin, 2015).

Eukaryotes encode several sirtuins in their genomes, and mammals, in particular, possess seven sirtuins (SIRT 1-7). The different SIRTs are localized in different subcellular compartments, namely the nucleus localizes SIRT1, SIRT6, and SIRT7; cytoplasm localizes SIRT2; while SIRT3, SIRT4, and SIRT5 are compartmentalized in the mitochondria (Ye et al., 2017). Another cornerstone discovery was that the protein deacetylases activity of sirtuins was NAD dependent, and increasing pieces of evidence indicate that NAD levels decrease with ageing and during senescence (Campisi et al., 2019), with a parallel reduction in sirtuins activity in ageing (fig. 2).

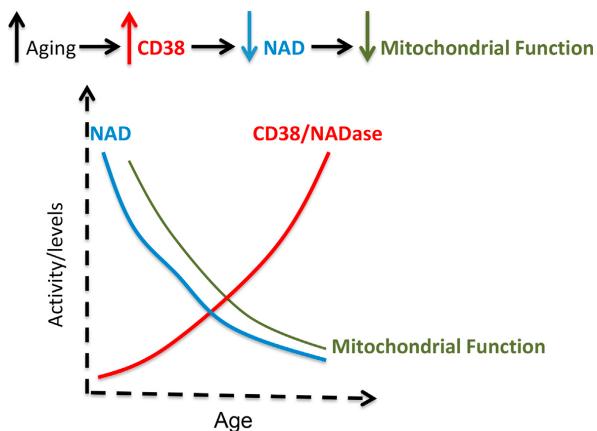


Figure 2: Ageing, CD38/NADase, and mitochondrial dysfunction: The role of NAD in ageing is increasingly believed to be central. Changes in NAD levels, its CD38 dependent degradation, and the downstream impact on mitochondria with ageing is represented here. This figure is taken from Camacho-Pereira et al. (2016).

To summarize, it is now well-understood that ageing in humans has several salient features. The salient features that will be discussed in this text are as follows:

- nine hallmarks of ageing have been observed and recognized so far (in humans) ([subsec. 2.1](#))
- integration of various hallmarks of ageing at a systemic level can be precisely measured by employing DNA based epigenetic clocks ([subsec. 2.2](#))
- deterioration of metabolism occurs with ageing ([subsec. 3.1](#))
- the role of mitochondrial health (including biogenesis) in metabolic homeostasis ([subsec. 3.2](#))
- deterioration of immunological components occurs and may further drive ageing ([subsec. 3.3](#))
- observation that various microbiomes, such as gut and skin, deteriorate in quality (i.e. loss of diversity and richness) with ageing ([subsec. 4.1](#))

2 Background

2.1 The hallmarks of ageing

Over several years, and in particular the past decade, numerous hallmarks of mammalian ageing have been identified. The seminal paper López-Otín et al. (2013) exhaustively identified, and compiled nine hallmarks of ageing in humans (fig. 3). López-Otín et al. add that the nine hallmarks are not isolated from one another, and rather extensively interact with one another in a network-like manner (fig. 5). In this work, I focus on the hallmarks of ageing, in particular, the metabolic component involving mitochondrial dysfunction, dysregulated nutrient sensing, epigenetic alterations, altered intercellular communication, and immunosenescence.

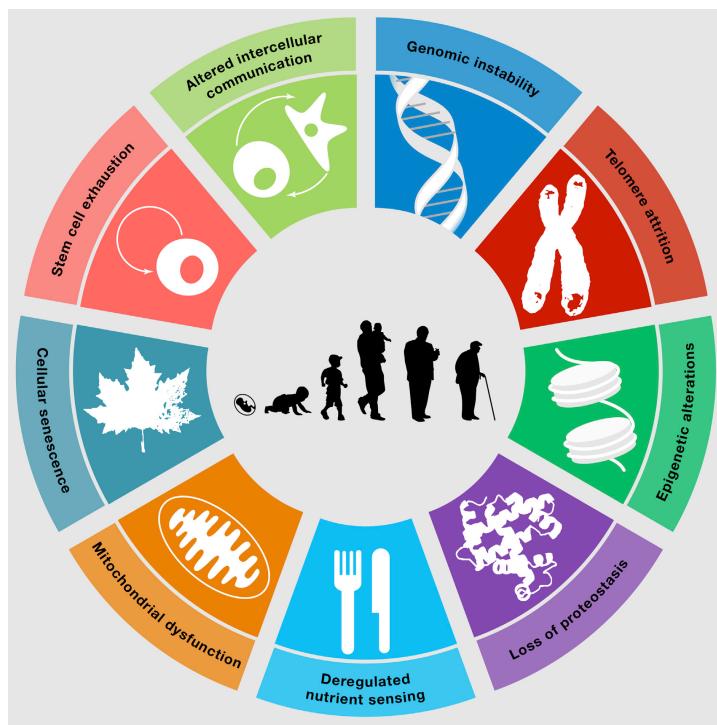


Figure 3: Hallmarks of ageing in humans: Genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, dysregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication are recognized as the nine hallmarks of ageing (López-Otín et al., 2013). This figure is taken from López-Otín et al. (2013).

Amongst the hallmarks of ageing, there are potential candidates as the *the primary causes of ageing and age-associated damage*; the hallmarks fall in either of three functional categories (fig. 4 - a, b, c). The hallmarks of ageing are categorized as the (purported) primary causes of damage (fig. 4, a), the compensatory (antagonistic) responses to damages (fig. 4, b), or the integrative hallmarks that are the outcomes of the preceding two hallmark categories (fig. 4, c). Thus, more often, the integrative hallmarks (fig. 4, c) are the observed characteristics (i.e. phenotype), and may be indicative of the underlying age-related functional deficits and decline. As a typical feature of biological systems, several factors can activate or repress, and hence influence ageing and the associated hallmarks (fig. 5).

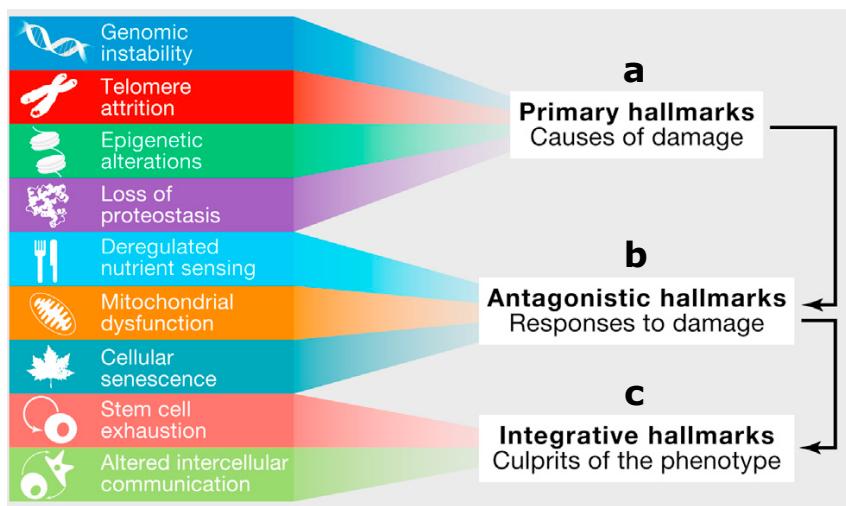


Figure 4: Functional interconnections amongst the ageing hallmarks: The nine hallmarks fall in either of three functional categories. The primary hallmarks (in the top, **a**) are debated as the primary drivers of cellular damage. The antagonistic hallmarks (the middle, **b**) encompass the compensatory responses to cellular damage, wherein they mitigate the damage (in youth), but when chronic or exacerbated deleterious impacts are observed. The hallmarks that integrate other hallmarks (bottom, **c**), that ultimately lead to functional decline and deficits associated with the ageing process. This figure is taken from López-Otín et al. (2013).

However, an important question that was raised in the past decade: are the consequences of ageing inevitable, or is ageing reversible? Can some of the effects be delayed or even rescued? Which specific effects of ageing has current science been able to delay or rescue? Recent papers on therapeutics that target ageing, and their potential mechanisms suggest that age-related deficits can be delayed, mitigated, and might be somewhat reversible (Verdin, 2015; Campisi, 2013; Lautrup et al., 2019). The rescue of ageing - as per current knowledge, understanding, and progress in the field - can occur at least partially with appropriate therapeutics or lifestyle changes.

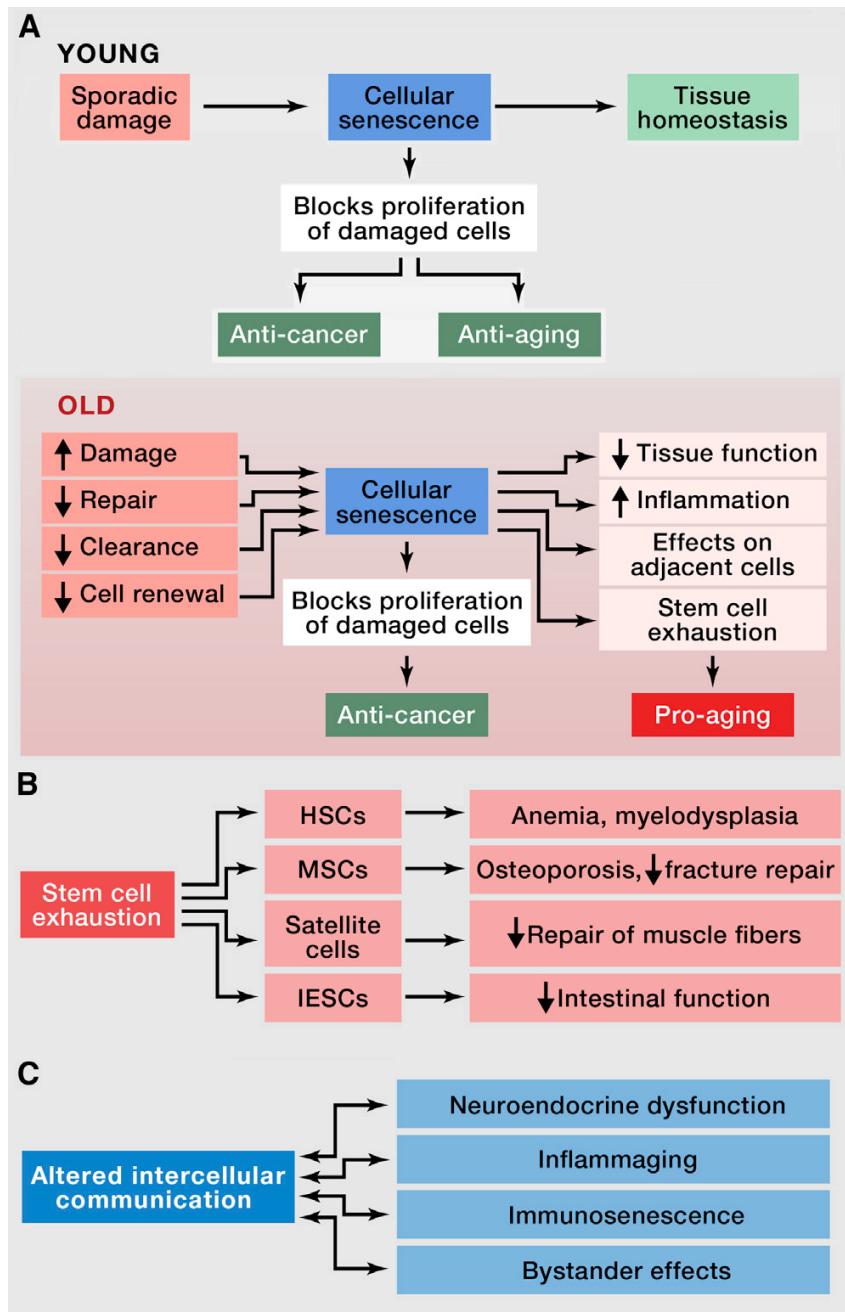


Figure 5: Cellular senescence, stem cell exhaustion, and altered intercellular communication: **A** In youth, cellular senescence of damaged cells mitigates the proliferation of damaged cells, hence contributing to systemic homeostasis while also preventing from cancer. **B** At the same time, exhaustion of stem cells (such as hematopoietic stem cells HSCs, mesenchymal stem cells- MSCs, intestinal epithelial stem cells- IESCs etc.) is exemplified with ageing. **C** Ultimately, intercellular communication is dampened with ageing, manifesting as the phenotypes observed in advanced age. This figure is taken from López-Otín et al. (2013).

While the hallmarks of ageing are excellent qualitative metrics to understand ageing, to gain more quantitative understanding of ageing it is essential to estimate the ‘true’ biological age. To this end, precise quantitative measurements based on individual epigenetic biomarkers, have been instrumental for the development of ‘ageing clocks’ that quantify BA.

2.2 The rise of epigenetic clocks

In the past decade, epigenetic modifications have emerged as one of the most powerful and robust biomarkers of tissue-specific ageing (Pyrkov et al., 2017; Levine et al., 2018). When methyl groups are added to specific parts of the Deoxyribonucleic acid (DNA) molecule, the process is known as DNAm. Time (and thus chronological age) evokes predictable hypo- and hypermethylation changes in various regions across the genome (Fraga and Esteller, 2007; Rakyan et al., 2010; Teschendorff et al., 2010; Jung and Pfeifer, 2015; Zheng et al., 2016); henceforth several DNAm based ageing clocks have been recently developed Levine et al. (2018). While DNAm based biomarkers of ageing were explored for prediction of CA (Bocklandt et al., 2011; Garagnani et al., 2012; Hannum et al., 2013; Horvath, 2013; Weidner et al., 2014; Lin et al., 2016), several different algorithms produce interesting estimations of biological age, and the inherent heterogeneity of these results are exciting, nonetheless.

Intriguingly, a blood and serum-based algorithm from Hannum et al. (2013) was a so-called first-generation ‘clock’ for estimating biological ageing. As the epigenetic based data-driven methods gained traction, this marked the beginning of rapid developments in estimations of ageing. Recently, a more comprehensive multi-tissue algorithm from Horvath et al. (2016) produces age estimates (DNAm age) that correlate very strongly with chronological age ($r \geq 0.90$), for full age range samples.

A current state-of-the-art DNAm based ageing clock drew inspirations from the work of Horvath et al. (2016). Further on, to better estimate, individual-level differences of BA rates, the prediction of CA was replaced with a surrogate ‘phenotypic age’ proxy, that could differentiate the morbidity and mortality (Levine et al., 2018) risk amongst individuals of the same CA. Levine et al. (2018) described (quantitative) biomarkers of biological ageing as individual-specific measures of ageing that can capture inter-individual differences in age-related functional deficits, the onset of disease related to ageing, and risk of mortality. Importantly, while the epigenetic age estimators show significant associations with age-related outcomes such as pieces of evidence from (Horvath et al., 2014; Marioni et al., 2015a,b; Horvath et al., 2015b; Horvath and Levine, 2015; Horvath et al., 2015a; Levine et al., 2015a,b, 2016; Chen et al., 2016; Quach et al., 2017; Dugué et al., 2018; Simpkin et al., 2017; Maierhofer et al., 2017) the effect sizes lie between small and moderate (fig. 6).

| | | Pooled WHI samples | | | |
|--------------------|--------------------------|------------------------------------|--------|---------------|-------|
| | | Adjusted for ethnicity and dataset | | | |
| | | n | μ | PhenoAgeAccel | |
| Diet | log2(Total energy) | 3700 | 10.58 | 0.01 | 0.40 |
| | Carbohydrate | 3700 | 48.64 | -0.02 | 0.23 |
| | Protein | 3700 | 16.39 | -0.01 | 0.45 |
| | Fat | 3700 | 35.05 | 0.03 | 0.12 |
| | log2(1+Red meat) | 3700 | 0.69 | 0.05 | 2E-3 |
| | log2(1+Poultry) | 3700 | 0.39 | -0.02 | 0.14 |
| | log2(1+Fish) | 3700 | 0.25 | -0.03 | 0.10 |
| | log2(1+Dairy) | 3700 | 1.21 | -0.01 | 0.76 |
| | log2(1+Whole grains) | 3700 | 1.00 | -0.02 | 0.19 |
| | log2(1+Nuts) | 3700 | 0.10 | -0.04 | 0.03 |
| Dietary biomarkers | log2(Fruits) | 3700 | 0.57 | -0.02 | 0.13 |
| | log2(Vegetables) | 3700 | 0.71 | -0.03 | 0.09 |
| | Retinol | 2267 | 0.58 | 0.03 | 0.17 |
| | Mean carotenoids | 2266 | 0.06 | -0.22 | 2E-27 |
| | Lycopene | 2267 | 0.36 | -0.11 | 3E-7 |
| | log2(alpha-Carotene) | 2267 | -4.21 | -0.19 | 5E-20 |
| | log2(beta-Carotene) | 2266 | -2.15 | -0.18 | 2E-17 |
| | log2(Lutein+Zeaxanthin) | 2267 | -2.38 | -0.17 | 2E-16 |
| | log2(beta-Cryptoxanthin) | 2267 | -3.76 | -0.17 | 2E-15 |
| Measurements | log2(alpha-Tocopherol) | 2267 | 3.87 | -0.03 | 0.19 |
| | log2(gamma-Tocopherol) | 2267 | 0.88 | 0.07 | 6E-4 |
| | log2(C-reactive protein) | 2809 | 1.57 | 0.18 | 5E-22 |
| | log2(Insulin) | 4042 | 5.78 | 0.15 | 2E-20 |
| | log2(Glucose) | 4144 | 6.57 | 0.10 | 2E-10 |
| | log2(Triglyceride) | 4148 | 7.02 | 0.09 | 5E-9 |
| | Total cholesterol | 4148 | 224.00 | -0.04 | 4E-3 |
| | LDL cholesterol | 4084 | 140.00 | -0.05 | 2E-3 |
| | HDL cholesterol | 4145 | 53.00 | -0.09 | 7E-9 |
| | log2(Creatinine) | 2748 | -0.43 | 0.01 | 0.52 |
| Socio-behavioral | Systolic blood pressure | 4177 | 129.00 | 0.08 | 1E-6 |
| | Diastolic blood pressure | 4178 | 76.00 | 0.02 | 0.12 |
| | log2(Waist / hip ratio) | 4037 | -0.28 | 0.15 | 5E-22 |
| | BMI | 4145 | 28.88 | 0.13 | 5E-16 |
| | Education | 4143 | 7.00 | -0.09 | 6E-9 |
| | Income | 4054 | 3.00 | -0.06 | 9E-5 |
| | log2(1+Exercise) | 3914 | 2.75 | -0.06 | 7E-5 |
| | Current smoker | 2321 | 0.00 | 0.10 | 3E-6 |
| | log2(1+Alcohol) | 3700 | 0.14 | -0.04 | 0.02 |

Figure 6: PhenoAge acceleration - lifestyle factors that influence DNA methylation based epigenetic clocks: Data from the Women's Health Initiative (WHI) (Hays et al., 2003; Levine et al., 2015a), was assessed by employing DNA methylation PhenoAge for quantification of age-acceleration. The significant factors (p ; $p \leq 0.05$) are highlighted in green. Nonetheless, most of the effect sizes (bicor; $-1 \leq \text{bicor} \leq 1$) lie between small to moderate. Intuitively, the bicor values that are highlighted in blue reduce age-acceleration (desirable for longevity), while those highlighted in red increase age-acceleration (undesirable for longevity); the darker colours indicate larger effect size. Interestingly, dietary biomarkers such as high levels of alpha-Carotene and beta-Carotene seem to have health protective effects. On the other hand, high levels of C-reactive protein, insulin, waist/hip ratio, and BMI have negative longevity and health outcomes. Notably, those highlighted in red are well documented in literature with poor (metabolic) health outcomes. This figure is taken from Levine et al. (2018)

3 Signatures of age-related ‘deficits’

3.1 Metabolic homeostasis: a predictor of all-cause mortality

From [Facchini et al. \(2001\)](#), a convincing proof emerged that baseline measurement of insulin resistance was indicative of future clinical outcomes (fig. 7). [Facchini et al. \(2001\)](#) investigated if insulin sensitivity (measured by Steady state plasma glucose (SSPG)) could be indicative of future metabolic outcomes. During this 1988-1995 study, 290 healthy volunteers were included, out of which follow-up data were collected for 208 (98 males, 110 females) subjects.

The baseline measurement of SSPG was grouped into three tertiles (three equally-sized ‘bins’ for the range of SSPG in the cohort). During follow up, the clinical outcomes ($n = 40$) were reported in 37 subjects, of which there were 12 who developed Hypertension (HT) outcomes; 9 had cancer, 7 had Coronary heart disease (CHD), and 5 had type 2 diabetes, 3 of which also had high blood pressure, and 4 had stroke ([Facchini et al., 2001](#)). Surprisingly, none of the outcomes was from subjects in the most insulin-sensitive tertile (fig. 7, first tertile). On the contrary, 25 subjects of the most insulin-resistant tertile (36% of this group) had a total of 28 clinical outcomes.

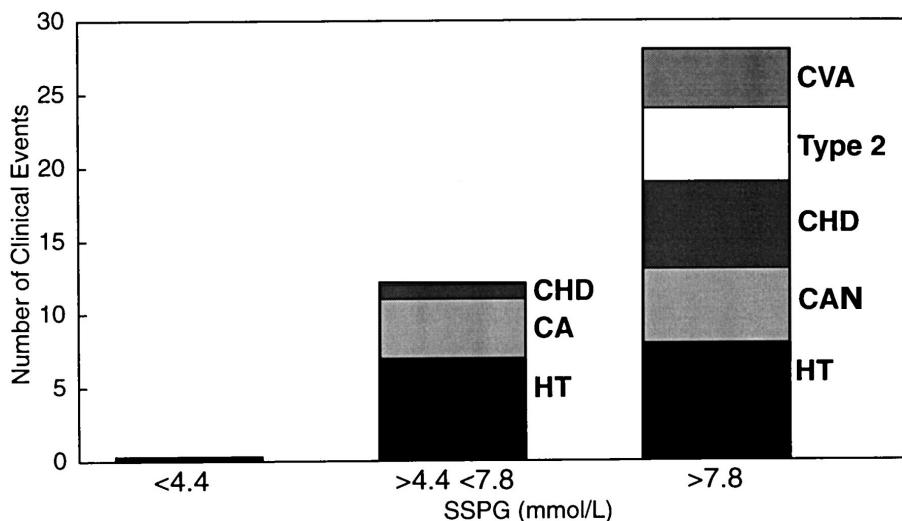


Figure 7: Tertiles of steady state plasma glucose: The subjects in the most insulin sensitive tertile ($\text{SSPG} < 4.4 \text{ mMol/L}$) had no events; in the intermediate tertile ($4.4 < \text{SSPG} < 7.8 \text{ mMol/L}$) 12 events were recorded; in the highest tertile ($7.8 \text{ mMol/L} < \text{SSPG}$, strongest insulin resistance) 28 events were observed. Legends: Cancer (CAN); type 2 diabetes (Type 2); HT, CHD, Cerebrovascular accident (CVA). This figure is taken from [Facchini et al. \(2001\)](#)

Intriguingly, the highest SSPG tertile subjects were older, had higher Body mass index (BMI), total cholesterol, including low-density lipoprotein (LDL) cholesterol, plasma triglycerides, and diastolic blood pressure. No significant sex-bias was observed; either the tertiles lacked the resolution or the biomarkers of future metabolic outcomes were not considered. For the middle SSPG tertile, 12 subjects had clinical outcomes. Moreover, the differential risk of developing age-related pathologies was statistically significant in the three tertiles ($p = 0.002$) ([Facchini et al., 2001](#)). Deaths were also confined to the most insulin resistant tertiles ([Facchini et al., 2001](#)).

Note that these studies are observational, and hence are not well-controlled. Typical of observational or correlational studies, causality is difficult to establish. It could be equally plausible that so-called healthy (lowest tertile) subjects had a better insulin sensitivity and not the other way around. In light of the ongoing global public health crisis (from Covid-19), it is becoming increasingly evident that the two may indeed be equivalent, although a formal proof has not been established yet.

Thus, age-related pathologies were observed in approximately one of three healthy subjects, of those falling into the most insulin-resistant tertile. In stark contrast, none of the subjects in the most insulin-sensitive tertile developed clinical events, when followed up to an average of six years. Hence, this study suggested that the metabolic deficits (specifically insulin resistance) is a powerful predictor of accelerated-ageing and age-related clinical outcomes. Various other studies show similar results on the relationship between health (in homeostasis) and insulin sensitivity.

3.2 Mitochondria: link between metabolic diseases and cancer

Mitochondria Adenosine triphosphate (ATP) production involves a series of complex metabolic reactions, that catabolize nutrients (i.e., glucose, fatty acids, and amino acids) via the Tricarboxylic acid (TCA) cycle and Oxidative Phosphorylation (OxPhos) pathways. The human brain consumes almost 20% of total energy production, and considering it is only 2% of body weight, the brain is a highly energy-intensive organ (relative to its density). Hence it is not surprising that mitochondrial dysfunction leads to a myriad of neurological manifestations ([Sun et al., 2016](#); [Mukherjee et al., 2016](#); [Lautrup et al., 2019](#)). Additionally, neuroinflammation from ageing or arising from other chronic conditions also remains a substantial challenge in global public health.

In the adult human brain, mitochondria catabolize glucose via the glycolysis, TCA cycle, and the OxPhos pathways for producing energy in the form of cellular ATP in neurons. Inadequate energy production, from mitochondrial dysfunction, often results in neurological conditions e.g., cognitive decline, epilepsy; myopathic symptoms e.g. Amyotrophic lateral sclerosis (ALS); or neurodegenerative disorders e.g., AD, and Parkinson's diseases ([Lautrup et al., 2019](#); [Verdin, 2015](#)). Intriguingly, mitochondrial deficits can also manifest as neurodevelopmental disorders e.g., autism spectrum disorder and are also associated with diseases under the neuroinflammation umbrella e.g., anxiety, and Major depressive disorder (MDD) ([Mukherjee et al., 2016](#)).

The morphology of mitochondria is highly dynamic - cellular mitochondria maintain highly connected network-like characteristics ([Wang et al., 2015](#); [Glancy et al., 2015](#)), involving sustained fission and fusion cycles ([Chan, 2006](#)). Disruption in either the fusion or fission process results in deficits of mitochondrial bioenergetics ([Twig et al., 2008](#)). E.g. mutations in the OxPhos pathway have been implicated in mitochondrial deficits of ATP production, and several mitochondrial disorders. Notably, deficits in ATP production are said to be one of the most common causes of metabolic disorders in both adults and children. E.g. deficits in ATP production, stemming from mitochondrial defects can affect multiple organ systems (especially those with high energy demands), such as the brain, heart, and skeletal muscles ([Mukherjee et al., 2016](#)). Multiple studies provide extensive evidence of mitochondrial dysfunction in both neurodevelopmental and neurodegenerative disorders ([Frye and Rossignol, 2011](#); [Anitha et al., 2013](#); [Calkins et al., 2011](#); [DiMauro and Schon, 2008](#); [Lin and Beal, 2006](#)).

Age-dependent hypermethylation of PcG genes as a signature of cancer

A striking hallmark of cancer is the age-dependent DNA methylation of genes that would usually be suppressed in stem cells ([Teschendorff et al., 2010](#)). A class of proteins known as the Polycomb group Polycomb group (PcG) is involved in repression of genes that are necessary for the differentiation of stem cells. Recently, it was shown that the promoters of PcG target genes were 12-fold more likely to be methylated in cancer, as compared to non-PcG target genes. [Teschendorff et al. \(2010\)](#) identified a specific subset of 69 cytosine and guanine base-pair, separated by only one phosphate group, wherein the cytosine is 5' to the guanine base (CpG)s (related to PcG target genes) strongly associated with carcinomas, that undergo hypermethylation in ageing. Importantly, successful validation of the CpG hypermethylation profile in their study was performed to demonstrate the robustness of their findings. Upon comparison of healthy and various solid cancer tissues and a population of bone marrow mesenchymal stem/stromal cells, significant differences were observed ($p < 10^{-5}$). [Teschendorff et al. \(2010\)](#) report that the age-dependent methylation of the promoters of PcG target genes (PcGTs) is found in preneoplastic (neoplasm is a new, abnormal, and potentially cancerous tissue growth) conditions. PcGTs could drive changes to the gene expression that is associated with carcinogenesis.

Defects in cellular respiration and metabolic deficits implicated in cancer

The Warburg effect was the first to propose that all cancers originate from respiratory insufficiency. Despite various remote causes of cancer, a commonality wherein all causes of cancer merge is the irreversible damage to cellular respiration ([Warburg et al., 1927](#); [Warburg, 1956](#)). A compelling case from [Seyfried et al. \(2014\)](#) envisaged that “ major hallmarks of cancer, including genomic instability, can be linked directly or indirectly to the respiratory dysfunction and the compensatory fermentation of the tumour cell”. Metabolic disturbances are implicated in cancer and tumour formation ([Seyfried and Shelton, 2010](#)), in particular in the energy production through respiration and fermentation pathway. [Seyfried and Shelton \(2010\)](#) have conjectured and argued that the genomic instability that is observed in tumour cells, including all other hallmarks of cancer, may be downstream of initial disturbances in the energy metabolism of cells. Even further, the dysfunction in the metabolism of tumour cells may be linked to abnormalities in the structure and function of the mitochondria ([Seyfried et al., 2014](#)). The perspective that somatic mutations can arise as the effects rather than the cause of tumorigenesis is supported by experimental pieces of evidence such as nuclear transfer experiments (fig. 8).

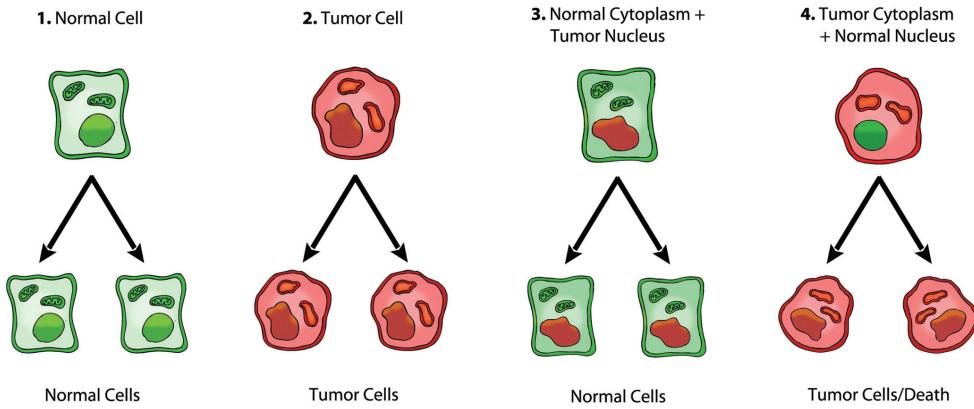


Figure 8: Dominant role of mitochondrial in tumorigenesis Cancer cells and its cytoplasmic compartments are shown in red, while for the counterpart normal cells (under homeostasis) are depicted in green. For both the cell types, their nucleus and cytoplasm (including the mitochondria) were swapped with one another, creating cybrids. Interestingly, the cells turn cancerous (or dies) only when a normal cell nucleus is delivered into a tumour cell cytoplasm, but were never healthy. On the contrary, the delivery of tumour cell nucleus into a normal cellular cytoplasm engenders normal cells, despite the presence of tumour-associated mutations. These results suggest that nuclear DNA damages alone do not lead to cancer, and importantly, healthy mitochondria and associated cytoplasm may have tumour suppressing capabilities. This figure is taken from Seyfried et al. (2014)

While the integrity of the nuclear genome largely depends on the mitochondrial respiration efficiency and functioning (Seyfried, 2012c), the above lines of reasoning raise questions on the role of somatic mutations as drivers of cancer and tumorigenesis (Seyfried et al., 2014). Several lines of evidence indicate that a persistent retrograde response or mitochondrial stress response leads to deficits in DNA repair, and the upregulation of fermentation pathways (Kaipparettu et al., 2013; Guha and Avadhani, 2013; Singh et al., 2005; Nargund et al., 2012; Jazwinski, 2005; Al Mamun et al., 2012).

Additionally, the metabolic waste product of fermentation can disrupt the (morphogenetic field of the) tumour microenvironment, hence contributing to inflammation, angiogenesis, and cancer progression (Bissell and Hines, 2011; Gatenby and Gillies, 2007; Husain et al., 2013). On the other hand, mitochondrial homeostasis is crucial for stable levels of intracellular calcium, which is needed for chromosomal integrity and cell division fidelity (Seyfried et al., 2014). E.g. abnormalities in calcium homeostasis could manifest aneuploidy during cell division (Seyfried, 2012c). In essence, with generalizations and simplifications, an abnormal genomic landscape observed in tumour cells might be considered a downstream (Seyfried et al., 2014), and protracted oncogene-driven fermentation (Seyfried, 2012b,a).

Some of the cancer-related mitochondrial defects could be rescued, albeit it is unknown if it can be salvaged partially or fully. The introduction of mutations in Mitochondrial DNA (mtDNA) could reverse the anti-tumorigenic effect of healthy mitochondria Petros et al. (2005), which had demonstrated that cancer could be best defined as a mitochondrial disease (Wallace, 2005, 2012). Recent developments have further been proposed that cancer progression may be managed by adopting a transition from fermentable metabolites, namely glucose and glutamine, to respiratory metabolites, primarily ketone bodies via low-carbohydrate high-fat KD (Seyfried et al., 2014). Several other works such as Miller et al. (2018); Hyde et al. (2017); Paoli et al. (2013); Chung and Park (2017); Nebeling and Lerner (1995) also recommend KD for the same, although clinical trials are yet to transpire, and would be awaited.

Impairment in mitochondrial biogenesis and homeostasis

In the context of regulators of mitochondrial biogenesis and oxidative metabolism, the transcriptional coactivators of the Peroxisome proliferator-activated receptor (PPAR) γ coactivator (PGC)-1 family play a central role (Handschin and Spiegelman, 2006). Several studies have demonstrated that PGC-1 α and PGC-1 β are potent regulators of mitochondrial functioning, biogenesis, and respiration (Liu and Lin, 2011; Lin et al., 2002). The PGC-1 α and β both are expressed in tissues of high energy requirements such as the brain, kidneys, and heart (Puigserver et al., 1998; Lin et al., 2002) - while the PGC-related coactivator is expressed ubiquitously (Andersson and Scarpulla, 2001). Notably, PGC-1 significantly increases the transcriptional activity of PPAR γ (Puigserver et al., 1998). As PGC-1 interacts with the nuclear receptor protein PPAR γ , this PPAR-PGC-1 α axis enables interaction with multiple transcription factors. Intriguingly, agonists that target the PPAR-PGC-1 α axis are widely prescribed for the treatment of diabetes also rescue (mitochondrial) deficits in neurological diseases (Garrido-Gil et al., 2012; Sanchis-Gomar et al., 2014; Corona and Duchen, 2016) and recently in animal model of neurodegenerative diseases (Seok et al., 2019; D'Angelo et al., 2019).

Investigations exemplify the role of mitochondria in the determination of cell fates, such as on the functional impact of nonsense mtDNA mutations in the *COXI* and *ND5* genes in a colorectal tumour cell line (Srivastava et al., 2007). While the colorectal tumour cell line displayed efficient oxidative phosphorylation (OxPhos), upon mitochondrial transfer from these cells to an osteosarcoma nuclear background (osteosarcoma cybrids), the respiration rate significantly declined. The result from Srivastava et al. (2007) suggests that the colorectal tumour nuclear background prevented the expression of the mtDNA mutations in the cybrid phenotype. Srivastava et al. (2007) reported significant increase in steady-state levels of PGC-1 α and PGC-1 β transcription coactivators. Concomitantly, this was in tandem with the increase of steady-state levels of mitochondrial proteins. Interestingly, adenoviral-mediated overexpression of PGC-1 α and PGC-1 β in the cybrids, also lead to improved mitochondrial respiration. The result indicates that the upregulation of PGC-1 α/β coactivators may partially recover some deficits in the OxPhos pathways in cells with mtDNA nonsense mutations, hence improving respiration in mitochondria.

3.3 The immunological signatures of ageing

Inflammageing is often defined as the chronic (putatively low-grade) inflammation during ageing and related deteriorating consequences in the immune functioning. In particular, inflammageing occurs in the absence of an overt infection or other immunological challenge (Sanada et al., 2018) and poses a substantial risk factor of morbidity and mortality in the elderly. Chronic inflammation can cause low-grade and persistent inflammation, which may lead to tissue degeneration (or sarcopenia as seen in ageing) (Straub and Schradin, 2016). Inflammatory cytokines such as Tumour necrosis factor (TNF)- α , Interleukin (IL)-1 β , IL-6, which are upregulated in several tissues of the elderly, and are known to dampen the anabolic signalling cascade, including insulin and erythropoietin signalling, contributing to the development of sarcopenia (Beyer et al., 2012). (Sanada et al., 2018) have argued several mechanisms that contribute to inflammageing, namely accumulation of cellular or immunoglobulin debris, compromise of the gut mucosa and microbiota dysbiosis, cellular senescence and immunosenescence,

and increased coagulation and fibrinolysis activity in the elderly.

Briefly, the accumulation of cellular debris and compromise of gut mucosa are relevant in the context of this text. Insufficient clearing of cellular debris, along with immunoglobulin accumulation in ageing, can trigger and sustain chronic activation of the innate immune system, thereby leading to persistent inflammation (Sanada et al., 2018). The immune system also displays the fundamental hallmarks of ageing (fig. 3) such as cellular senescence (in particular exhaustion of T cells) and decreased (and altered) intercellular communication, especially mediation of signals between the innate and adaptive immunity via macrophages. Altered communication of immune cells has been implicated as one of the reasons driving chronic low-grade inflammation in the elderly (Tu and Rao, 2016; Leonardi et al., 2018; Sanada et al., 2018; Dalle et al., 2017). Note that overall immune responses do not necessarily undergo an age-related decline. Importantly, the innate arm of the immune system is primarily spared (Ottaviani et al., 2008), while the ability to manifest adaptive immune response is upregulated in the elderly (Franceschi et al., 2000b). Hence, by combining (Ottaviani et al., 2008) and (Franceschi et al., 2000a), Franceschi et al. (2000b) proposed a simplifying hypothesis, wherein hyperstimulation of both innate and adaptive immunity may occur in the elderly. However, as increased inflammatory status becomes pervasive during ageing, owing to the exposure to a variety of stressors (including the continuous exposure to a wide range of antigens, super-antigens, or latent viral loads), chronic inflammation could be addressed for longevity outcomes.

Cellular and subcellular secretory phenotypes

Cellular (and subcellular) senescence have an important role in ageing, partly mediated by secretory molecules that contribute to accelerated-ageing. As mentioned in the introduction, the SASP is said to have numerous biological activities, but all are highly dependent on the existing physiological context. The roles of SASP include stimulation of angiogenesis, alteration of the proliferative capacity of neighbouring cells, contributing to chronic inflammation, stimulation of epithelial-to-mesenchymal transition, and changes to the tissue repair process e.g., via alterations to stem cell renewal (Campisi, 2013). Mitochondria is another cellular organelle that has been implicated as a complex determinant of ageing. The consequences of age-related failure in mitochondrial quality control and corresponding mitochondrial dysfunction include the secretion of Damage-associated molecular pattern (DAMP)s. The role of cell-free circulation mtDNA and mitochondrial DAMPs has been the subject of investigation in ageing, chronic inflammation, inflammaging, and degenerative diseases (Dall’Olio et al., 2013; Zhang et al., 2010). Hence, it is not surprising that interventions that selectively target and destroy senescent cells, namely ‘senolytic therapies’ (Farr et al., 2017; Collins et al., 2018; Lehmann et al., 2017; Roos et al., 2016), can offer improved therapeutic opportunities, thus blocking (or at least mitigating by blunting) some of the effects of multi-source inflammaging (Sanada et al., 2018).

Reduction and remodelling of immunological space in ageing

In the seminal paper, Franceschi et al. (1995) described that ageing is associated with complex remodelling of various immune parameters, rather than a unidirectional decline in immune function. A widely accepted paradigm is that the T cell compartment progressively deteriorates with age, and is strongly

associated with the thymus involution (or the shrinkage of the thymus) (Bodey et al., 1997). A gradual reduction in the production of nascent naïve T cells by the thymus, ultimately culminates in a complete stall in production of naïve T cells population, in aged individuals (Tu and Rao, 2016). An inevitable consequence of the thymic involution leaves the adaptive compartment of the immune system access to either the mature memory T cells or the pre-existing naïve T cell population present in the lymph nodes or other tissues wherein they reside. Another characteristic of the immune system is the gradual and progressive decline of naïve T cells (CD95⁻; CD stands for cluster of differentiation - a protocol for immunophenotyping of cells based on their cell surface molecules), and where progressive ageing most profoundly correlates with an increase in levels of CD8⁺ sub-population (Tu and Rao, 2016). Additionally, both CD4⁺ T cells and CD19⁺ B cells decrease with ageing, whereas the exhausted naïve T cells (CD95⁻) are replaced with clonal expansion of CD28⁺ (Tu and Rao, 2016).

Investigations on immunosenescence have been revealed the following major attributes of the impacts of ageing on functional immunology: a global reduction of the so-called ‘immunological space’ (Franceschi et al., 2000a), i.e., expansion of memory T cells, is often accompanied with the decrease (and ultimately, the exhaustion) of naïve cells. Additionally, the gradual decline of naïve T cell repertoire with ageing is also well reported. Alongside the reduction in lymphocyte count, profound remodelling of circulating lymphocyte subsets can be seen:

1. a reduction of the absolute number of total T lymphocytes (CD3⁺) including both helper (CD4⁺) and cytotoxic (CD8⁺) cells
2. a marked decrease of B-cells (CD19⁺)
3. an increase of activated T cells (CD3⁺HLA-DR⁺) as well as of cells with NK and NK-like cells, i.e., T lymphocytes expressing NK markers (Mariani et al., 1999; Sansoni et al., 1993)
4. while the count of NK cells increases with age, their cytotoxic capacity seems well preserved (Rothstein, 1993).

Cytokine signalling network

A significant impact of ageing on the immune system is the up-regulation of the inflammatory responses, such as progressive age-dependent increase of type 1 (IL-2, IFN- γ , TNF- α) and type 2 (IL-4, IL-6, IL-10) positive CD8⁺ T cells (Tu and Rao, 2016; Childs et al., 2015). In particular, type 1 cytokine-positive cells increase significantly with age, in all CD8⁺ subsets particularly among effector/cytotoxic and the memory cell (Alberti et al., 2006). Consequently, accumulation of differentiated T cells may further contribute to a reduction in immunological space. By depletion of the naïve T cell repertoire, opportunities for the appearance of several age-related Immune-mediated inflammatory disease (IMID)s may arise, and thereby contribute to impaired immune defence against new antigenic challenges.

Chronic antigenic stimulation via latent pathogens

Ageing is associated with reduced vaccination efficacy and response, higher systemic inflammation, increased oxidative stress, tissue damage. Moreover, ageing impacts various microbiomes, including

the gut microbiota, and changes in the physiology of Gastrointestinal tract (GI) tract, microbiome, and associated immune system are observed concomitantly (Rampelli et al., 2013; Tang et al., 2019). E.g. expansion of CD8⁺ CD28 T cells with ageing upregulates pro-inflammatory cytokines, which can be maintained by viral infections such as Cytomegalovirus (CMV) and Epstein barr virus (EBV) (Tu and Rao, 2016). Persistent viral load of latent CMV and EBV pathogens, maybe a major driving force in the development of a (chronic) pro-inflammatory state during ageing.

Chronic autoreactive stimulation

Notably, an ‘autoreactive or autoimmune’ process can fuel the onset or progression of chronic diseases, which could alter the ageing process locally or systemically. Hence, inflammageing is touted as a major target for longevity strategies in geroscience (Franceschi et al., 2017). Cellular damage, as well as infectious and non-infectious agents, activate inflammatory cells and trigger the most common inflammatory signalling such as the Nuclear factor (NF)-κB, Mitogen-activated protein kinase (MAPK), and Janus kinase signal transducer and activator of transcription (JAK-STAT) pathways (Chen et al., 2018).

A vital cell type implicated in inflammageing is the macrophage; macrophages reside in several tissues and organs (microbiomes, including the gut microbiota, are some of the vital components). Several works argue that the primary source of inflammatory stimuli in the elderly is presented by self-antigens (Agrawal et al., 2017), which are either misplaced or altered molecules stemming from damaged or quiescent cells and cellular debris. Antigens, including auto-antigens, upon recognition by the innate immunity in a macrophage-mediated manner, can initiate a cascade of immune response (Chen et al., 2018; Firestein and McInnes, 2017).

When the immune responses - for any cause whatsoever - becomes unresolvable, it may manifest as chronic inflammation. Evidence links that for cellular disposal of waste products by various proteasome-mediated mechanisms such as autophagy or mitophagy progressively declines with age (Franceschi et al., 2017), thus creating potential opportunities for chronic auto-antigenic presentation. The progressive exhaustion of T cell subtypes with ageing ultimately leads to weak defence against physiological stressors. Specialist defences of T cell against new antigen challenges (like from viral, microbial, or neoplastic) reduces with ageing, partly because of the drop in thymic function. Alternatively, lifelong chronic antigenic stimulation (such as but not limited to, from CMV or EBV), poses increasing challenges to impaired immunity in ageing. Hence, ‘autoreactive or autoimmune’ processes may contribute to the inflammageing process, either locally or systemically (Franceschi et al., 2017). Consequently, inflammageing could be a potent therapeutic target for longevity outcomes.

All the above-mentioned factors, considered either individually or cumulatively, pose detriments to longevity and pose risks for developing IMIDs. In the light of such pieces of evidence, studies on centenarians have been pivotal for understanding age-related chronic low-grade inflammation, and highlight the apparent paradox on inflammation.

The centenarian paradox: balancing inflammation

Centenarians embody delay in major age-related pathologies; research involving centenarians are at the forefront of longevity and healthy ageing studies (Franceschi et al., 1995). Centenarians often display increased plasma levels of inflammatory markers such as cytokines, coagulation factors, and acute-phase proteins, namely, IL-6, IL-18, C reactive protein (CRP), serum amyloid A, fibrinogen, Von Willebrand factor, and resistin (Franceschi et al., 2007). Concomitantly, the levels of anti-inflammatory molecules, such as Transforming growth factor (TGF)- β 1, and cortisol, adiponectin, is accompanied by the abundance of pro-inflammatory molecules. Thus, low levels of inflammatory compounds by themselves may not be deleterious. Instead, acceleration of ageing and age-related pathologies might occur when the balance is disrupted, through a myriad of pathophysiology aetiologies. Conversely, health and longevity may ultimately arise from an intricate balance between pro- and anti-inflammatory responses and immunological homeostasis (Franceschi et al., 2007). Taken together, a timeline of the milestones in the development of the theory of inflammageing (Franceschi et al., 2017) (fig. 9), was founded upon on several earlier works (Franceschi et al., 2000b, 2007; Biagi et al., 2010; Franceschi and Campisi, 2014; Kennedy et al., 2014; Ottaviani et al., 2008; Vitale et al., 2013; Ostan et al., 2015).

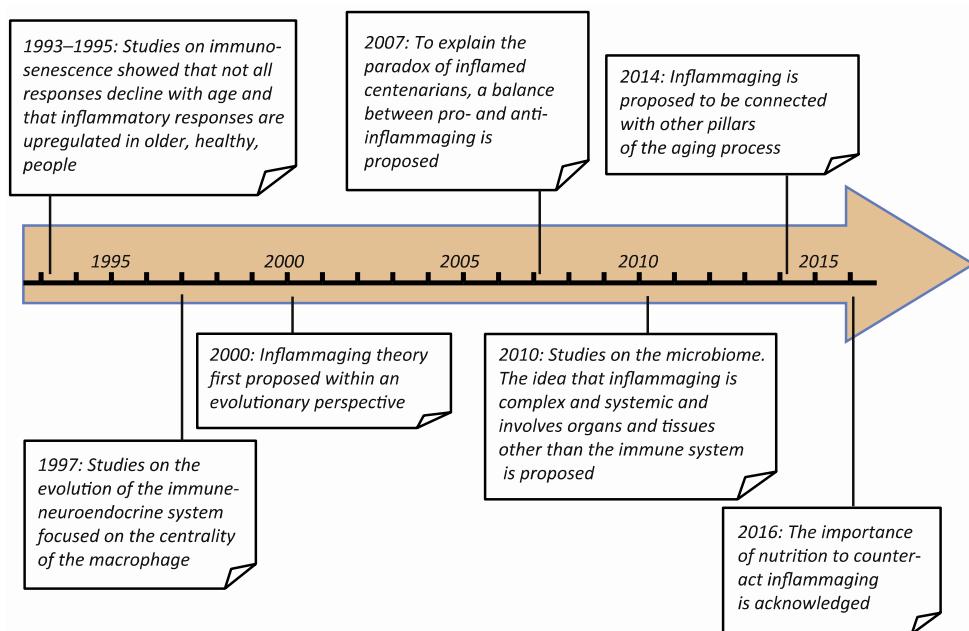


Figure 9: Milestones in the ‘evolution’ of the theory of inflammageing An intriguing observation is that only in 2016, the importance of nutrition was considered a viable therapeutic for addressing inflammageing. These described milestones have successfully led to an integrative understanding of inflammageing and how it relates to ageing and age-related diseases. Thus, the recently emerged field of geroscience adequately equips researchers to tackle one of the fundamental contributors to age-related diseases, namely biological ageing. This figure is taken from (Franceschi et al., 2017).

4 Outlook

In light of the arguments in the previous text, prof David A. Sinclair's book **Lifespan: Why We Age - and Why We Don't Have To** had been a driving motivation for this review and diving deeper into reviewing the literature on ageing research. The book is a powerful and comprehensive memoir of prof Sinclair's research on ageing, and it proposes a compelling hypothesis on the **Information Theory of Ageing**. This popular science book from an eminent scientist in the ageing field has several convincing pieces of evidence that support the **Information Theory of Ageing**.

As proposed theory embodies the integration of the hallmarks of ageing (fig. 5, fig. 4), **Information Theory of Ageing** may perhaps be universal. The theory postulates that it is the information processing - at a molecular, cellular, and physiological level - that gets altered in ageing.

In support of the overarching theory, two striking *bioRxiv* preprints, have come to light. I would like to draw attention to these:

1. 'DNA Break-Induced Epigenetic Drift as a Cause of Mammalian ageing' ([Hayano et al., 2019](#))
2. 'Erosion of the Epigenetic Landscape and Loss of Cellular Identity as a Cause of ageing in Mammals' ([Yang et al., 2019](#))

These *bioRxiv* preprints are conditionally accepted in SSRN (preprint archive from Cell publishing), the abstracts are now online while they await peer-review. From these online abstracts, the centrality of intracellular NAD levels seems to be evident to the ageing process. Additionally, senolytics based therapeutics for targeting age-related outcomes, aim to target the cells that lose their cellular identity, and hence may support the second preprint ([Yang et al., 2019](#)). Another convincing aspect of the Sinclair lab hypothesis argues that DNA damage can account for only a small fraction of mutations, as seen in 'aged' cells. Since the DNA is not modified significantly, from childhood to late adulthood, it begs a critical and open question: at molecular and cellular levels, what components undergo alteration or remodelling in ageing?

Importance of biological age estimates

Despite consensus on the causality of ageing, the ageing process in humans is characterized by an accelerated decline in (healthy) bodily functions and homeostasis, as described in this text. [Fransquet et al. \(2019\)](#)'s meta-analysis of the association between measured DNAm age with longevity, (age-related) morbidity status, and mortality risk; the authors found some evidence between increased DNAm age and increased risk of disease, although the association deemed inconsistent. While the relationship between DNAm age and longevity was inconclusive ([Fransquet et al., 2019](#)) for the large part. Nonetheless, [Fransquet et al. \(2019\)](#)'s demonstrated that each 5-year increment in DNAm age was correlated to between 8 and 15% increased risk of mortality, holds some merit in its own accord.

Proxies of ageing estimates, and why they lack consistency

While commendable progress on the precise quantification of the ageing process (consistent in a tissue-specific context) has been made in this past decade, several earlier works were pivotal for recent breakthroughs. Before 2017, algorithms for estimating BA had employed CA as a reference, and hence by definition, may have excluded CpGs whose DNA profiles do not display time-dependent changes (Levine et al., 2018).

A recent breakthrough that improved ageing estimates stems from the works of Horvath et al. (2016); Horvath and Raj (2018); Levine et al. (2015a,b); phenotypic ageing measures derived from clinical biomarkers that can robustly predict the variation in risk of all-cause mortality, cause-specific mortality, cognitive performance deficits, and physical functioning amongst individuals of the same CA (Levine et al., 2018). Following the above developments, Levine et al. (2018) further refined the existing algorithm from Horvath et al. (2016), and lead to the development of the state-of-the-art epigenetic clock, known as PhenoAge (Levine et al., 2018). By capturing the DNA patterns in CpGs that account for differences in risk and physiological status among subjects of the same CA, the state-of-the-art algorithm hence incorporates deviations between BA and CA (Levine et al., 2018).

CA remains arguably the most potent risk factor of all-cause mortality and age-related pathologies. albeit BA is strongly correlated with CA (obviously), the BA does not necessarily correspond to CA. For individuals of the same CA, the (quantitative) difference in susceptibility to age-related outcomes, is termed as accelerated-ageing (Levine et al., 2018). As different proxies of BA have been published in the recent past, which are reflective of specific aspects of the underlying biological ageing process, these may not be quantifying ‘a’ single ageing attribute. Instead, it may be more conceivable that the many estimates (hence many proxies) of ageing but individual heterogeneity in how one age estimate relates to the other is still a significant confounder that perplexes researchers.

4.1 Signatures of the ageing microbiome

Because the epithelium and mucosal environmental niches are vital barriers from the outside world, microbiome such as gut and skin are extremely important and play an essential role in maintaining homeostasis. A microbiome is typically characterized by its richness (total counts of microbial taxa) and diversity (how the abundance profiles vary for different microbes).

It is now well recognized that the barriers within microbiomes deteriorate with age (Sanada et al., 2018). Decreased diversity is one major hallmark of ageing in the (gut) microbiome (Kinross and Nicholson, 2012; Claesson et al., 2011), and chronic low-grade inflammation (Ohtsubo and Marth, 2006) (which is alternatively referred to as inflammageing). E.g. periodontal disease (possibly involving oral microbiome, although) is proven to cause low-grade inflammation (Ohtsubo and Marth, 2006). Aged individuals display a decline in the balance of pro- and anti-inflammatory microbes (Sanada et al., 2018; Toward et al., 2012), with pro-inflammatory microbiota eventually prevailing in ageing. Members of Clostridium cluster XIVa, Bifidobacterium spp., and F. prausnitzii diminish with ageing, tilting the balance favouring pro-inflammatory cytokine signalling (Toward et al., 2012). Concomitantly, Toward et al. (2012) provided supporting evidence that the level of Bifidobacterium is inversely correlated

with serum levels of inflammatory cytokines, such as TNF- α and IL-1 β . Pathogenic microbes such as Streptococcus spp., Staphylococcus spp., Enterococcus spp. and Enterobacter spp. are associated with an increased inflammatory response with ageing ([Toward et al., 2012](#)). Additionally, loss in gut microbiota diversity with ageing may increase vulnerability towards attack from infectious agents, and subsequent pathobiont colonization may occur ([Sanada et al., 2018](#)).

4.2 Future work

Given the niche perspective described in the text, I would thereby further extend the same and hypothesize that the gut-microbiome dynamics may be exploitable as a proxy for measuring accelerated-ageing (deviations of BA and CA) vis-à-vis in health and homeostasis. This concept is not itself novel, as recent papers from the massive public undertaking, namely the American gut project for citizen-science microbiome research ([McDonald et al., 2018](#)), came out with a new paper. Their paper was titled “Human Skin, Oral, and Gut Microbiomes Predict Chronological Age” ([Huang et al., 2020](#)), whose verbatim title is compelling in and of itself.

Based on the findings so far, conceivable hypotheses for future work could focus on the gut microbiome and general gut health. Because the gut is at a confluence of several complex systems - namely metabolism, neuroendocrine, immune system - so it interacts extensively at both local players and systemic levels. For example, it could be worthwhile for estimating the rate turnover of gut-microbial adaptations and signalling, whether it occurs in a short time frame (hours or days, in response to food, environment, or stress), would be interesting to explore in nutrition-first efforts in (precision) bio-medicine. With quantitative approaches on measuring dynamics of the microbiome perturbations in health and diseases, and mapping the functional profile associated with changes, insights may be achieved on how the microbiome is involved in the ageing process. E.g. apart from the general loss of diversity of microbiota, it is currently unknown what specific functional features of the microbiomes change with ageing (or inflammageing). Nonetheless, it is now recognized that loss of diversity and richness might precede the onset of frailty and sarcopenia ([Verdi et al., 2018](#); [Jackson et al., 2016](#); [Gabriela and Riscuta, 2018](#)) seen in advanced age.

Some open questions on the ageing microbiome that require further investigations:

- For the microbial taxa that decline with ageing, which of those play functional role(s) on physiological signalling under homeostasis or ‘disease’?
- Is it be possible to delay, rescue, or prevent frailty by appropriate therapeutic or lifestyle intervention that minimize the loss in microbiota diversity and richness?
- With ageing, pro-inflammatory microbes increase in abundance and with a reduction in overall diversity of microbial population. How does this association differ in centenarian vis-à-vis non-centenarians?

Some open broader problems that impede further quantitative understanding of ageing:

1. In a molecular and cellular consistent framework, what does the process of ageing exactly entail physiologically?
2. To what extent can ageing be reversed?
3. Which components of biological ageing are amenable (as some are more than the others)?

4.3 Conclusion

This review is my attempt to submit pieces of evidence on integrative aspects of biological ageing. Apart from the established nine hallmarks of ageing, several salient signatures often go hand in hand in (the systems physiology) of ageing. In trying to engender a comprehensive framework of the ageing physiology - susceptibility to dysregulated glucose response (i.e. insulin resistance), deficits in mitochondrial homeostasis, chronic inflammation (i.e. inflammageing) - I have stitched together diverse signatures of ageing. However, the literature is only recently beginning to explore the role of (gut) microbiota and general gut homeostasis in health and disease. A lot more research is required to investigate, for example, what the decrease in diversity and richness of the gut microbiota with ageing functionally entails at a molecular, cellular, and physiological level. Nonetheless, further research on long-term longitudinal studies that aim to mitigate some of the systemic effects from dietary and lifestyle approaches are much needed for global public health.

5 Layman's summary

Ageing is one of the principal risk factors for many chronic diseases (Fransquet et al., 2019). However, considerable heterogeneity exists in the variation in the individual rate of ageing, as well as differences in their susceptibility to morbidity and mortality (Fransquet et al., 2019). Albeit a large and significant proportion of chronic diseases is age-related, ageing is not yet recognized as a disease by the World Health Organization (WHO).

Ageing of the human body is known to accelerate the progression of existing pathologies, particularly those that involve metabolism, and immune system. While scientific research has attempted to find the causal factor(s) for ageing and chronic age-related pathologies, a cause-effect relationship has not been established yet. Nevertheless, a longstanding and prevailing hypothesis in the past was that life-span was primarily determined from underlying genetics. Based on the pieces of evidence of restoring health and longevity in animal models, such as by rescuing female fertility in aged mice (Bertoldo et al., 2020), the hypothesis might turn out to be untrue. In the popular science book *Lifespan: Why We Age - and Why We Don't Have To*, prof David A Sinclair, one of the prominent researchers in the field of longevity, argues through an increasingly compelling **Information Theory of Ageing** that the informational process that reads the genetic codes (namely DNA and Ribonucleic acid (RNA)) known as epigenetics, holds greater importance as compared to the underlying genetics of ageing.

Future research could consider integrative aspects of biological ageing, as described extensively (sec. 3), and their association with chronic IMIDs, as well as other age-related pathologies. As global public health may greatly benefit from successfully targeting ageing as the primary cause of many chronic diseases in the elderly, a paramount change in perspective is much needed for improvements in how public health is viewed. Moving the focus from targeting symptomatic relief for chronic diseases, to attempting to modulate potentially upstream causes of diseases via nutrition, exercise, and other lifestyle interventions such as meditation, or intermittent heat and cold exposures could be a vital first step in bio-medicine. This could concomitantly improve overall well-being in ageing populations across the globe, may reduce global health expenditure, as well as reduce morbidity (diseased conditions) and 'premature' mortality.

List of abbreviations

| | |
|-----------------|---|
| TCA | Tricarboxylic acid |
| OxPhos | Oxidative Phosphorylation |
| ATP | Adenosine triphosphate |
| ALS | Amyotrophic lateral sclerosis |
| MDD | Major depressive disorder |
| mtDNA | Mitochondrial DNA |
| PPAR | Peroxisome proliferator-activated receptor |
| PGC | PPAR γ coactivator |
| KD | Ketogenic diet |
| SSPG | Steady State plasma Glucose |
| t2DM | type 2 Diabetes Mellitus |
| HT | Hypertension |
| CVD | Cardiovascular disease |
| CHD | Coronary heart disease |
| CVA | Cerebrovascular accident |
| CR | Calorie restriction |
| CA | Chronological ageing |
| BA | Biological ageing |
| NAD | Nicotinamide adenine dinucleotide |
| AD | Alzheimer's disease |
| JAK-STAT | Janus kinase signal transducer and activator of transcription |
| NF | Nuclear factor |
| MAPK | Mitogen-activated protein kinase |
| DNAm | DNA methylation |
| DNA | Deoxyribonucleic acid |
| RNA | Ribonucleic acid |
| HSC | Hematopoietic stem cell |
| MSC | Mesenchymal stem cell |
| IESC | Intestinal epithelial stem cell |
| IMID | Immune-mediated inflammatory disease |
| mTOR | Mammalian (or mechanistic) target of rapamycin |
| IGF-1 | Insulin-like growth factor 1 |
| SSPG | Steady state plasma glucose |
| BMI | Body mass index |
| TNF | Tumour necrosis factor |
| IL | Interleukin |
| PcG | Polycomb group |
| SASP | Senescence-associated secretory phenotype |

| | |
|-------------|---|
| CMV | Cytomegalovirus |
| EBV | Epstein barr virus |
| DAMP | Damage-associated molecular pattern |
| CRP | C reactive protein |
| GI | Gastrointestinal tract |
| TGF | Transforming growth factor |
| WHO | World Health Organization |
| CpG | cytosine and guanine base-pair, separated by only one phosphate group, wherein the cytosine is 5' to the guanine base |

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