Inflammaging: a new immune–metabolic viewpoint for age-related diseases

'Inflammaging: a new immune–metabolic viewpoint for age-related diseases

Claudio Franceschi[1#], Paolo Garagnani[2,3,4,5#], Paolo Parini[3], Cristina Giuliani[6,7*] and Aurelia Santoro[2,7]

1. IRCCS, Institute of Neurological Sciences of Bologna, Bologna, Italy.
2. Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy
3. Clinical Chemistry, Department of Laboratory Medicine, Karolinska Institutet at Huddinge University Hospital, Stockholm, Sweden
4. Applied Biomedical Research Center (CRBA), S. Orsola-Malpighi Polyclinic, Bologna, Italy.
5. CNR Institute of Molecular Genetics, Unit of Bologna, Bologna , Italy
6. Laboratory of Molecular Anthropology and Centre for Genome Biology Department of Biological, Geological and Environmental Sciences (BiGeA), University of Bologna, Bologna, Italy
7. Interdepartmental Centre 'L. Galvani' (CIG), University of Bologna, Bologna, Italy.

# these authors contributed equally to this work

* e-mail: *cristina.giuliani2@unibo.it*

Abstract

Ageing and age-related diseases share some basic pillars that largely converge on inflammation. During ageing chronic, sterile, low-grade inflammation — called inflammaging — develops, which contributes to the pathogenesis of age-related diseases. From an evolutionary perspective, a variety of stimuli sustain inflammaging, including pathogens (non-self), endogenous cell debris and misplaced molecules (self) and nutrients and gut microbiota (quasi-self). A limited number of receptors, whose degeneracy allows them to recognize many signals and to activate the innate immune responses, sense these stimuli. In this situation, metaflammation (the metabolic inflammation accompanying metabolic diseases) is thought to be the form of chronic inflammation that is driven by nutrient excess or overnutrition; metaflammation is characterised by the same mechanisms underpinning inflammaging. The gut microbiota has a central role in both metaflammation and in inflammaging due to its ability to release inflammatory products, to contribute to circadian rhythms and to crosstalk with other organs. We argue that chronic diseases are not only the result of ageing and inflammaging, but they also accelerate the ageing process and can be considered a manifestation of accelerated ageing. Finally, we propose the use of new biomarkers (DNA methylation, glycomics, metabolomics and lipidomics) that are capable of assessing biological versus chronological age in metabolic diseases.

[H1] Introduction

The new geroscience field **[G]** offers a new perspective to address the increasing incidence of chronic age-related diseases, including metabolic disorders such as the metabolic syndrome, obesity, type 2 diabetes mellitus (T2DM) and cardiovascular diseases. The increasing incidence of these pathologies is a reflection of the increasing ageing population observed worldwide . The basic assumption of geroscience is that the mechanisms driving ageing and age-related diseases largely overlap, with seven common 'pillars' (mechanisms of ageing and major area of research) having been identified (**FIG. 1**)[1]. The great novelty is that experimental data emerged suggesting that these pillars are relatively few (adaptation to stress, epigenetics, inflammation, macromolecular damage, metabolism, proteostasis, stem cells and regeneration). Even more importantly, these pillars do not operate separately but are interconnected, influencing and modulating each other, thus constituting an integrated network [1,2]. Accordingly, the new geroscience approach proposes to counteract all major age-related diseases together, and not individually, by focusing on the basic ageing mechanisms underlying these diseases**.** This new approach should change the conventional views that often separate ageing and age-related diseases. Indeed, despite ageing being a major risk factor for age-related diseases, researchers often neglect the ageing process when they are investigating the mechanisms underpinning these diseases. Conversely, the contribution of age-related diseases in accelerating ageing is also underestimated.

Interestingly, the tightly networked ageing pillars converge on inflammation as impairment of any one pillar fuels inflammation, which subsequently affects all the other pillars. This chronic, sterile (occurring in the absence of infection and primarily driven by endogenous signals), low-grade inflammation that occurs during ageing is called inflammaging. A major characteristic of inflammaging is the chronic activation of the innate immune system[3] where macrophage plays a central role. Ilya Metchnikoff was the first to describe the macrophage in invertebrates and to define its pivotal role in the ingestion of foreign material (a primitive type of nutrition) and subsequent mounting of *'*physiological*'* inflammation, which is a basic mechanism to cope with and neutralize a large variety of stressors [4]. Several cellular and molecular mechanisms are involved in inflammaging, including cellular senescence, mitochondrial dysfunction, defective autophagy and mitophagy, activation of the inflammasome **[G]**, dysregulation of ubiquitin-proteasome system, activation of the DNA damage response and dysbiosis (changes in the composition of the host microbiota) [5].

This Review will focus on the similarities and differences between inflammaging and metaflammation[6] (the metabolic inflammation driven by nutrient excess or overnutrition that is present in metabolic diseases such as obesity and T2DM). This comparative immune–metabolic analysis presents a new perspective on the relationship between basic cellular and molecular mechanisms underpinning the ageing process and the chronic age-related metabolic diseases. Moreover, this Review will discuss the possible use of new biomarkers, which have been developed within the framework of geroscience, capable of distinguishing between biological and chronological age in metabolic diseases.

[H1] Immune–metabolic inflammaging

[H2] Evolutionary insight

The most challenging events for an organism's survival are nutrient deprivation and infection by pathogens; therefore, competition for food and the response to infectious diseases are the main factors that determine the ecological dynamics of populations and species and influence evolution [7–9]**.** Accordingly, food sources**,** metabolism, endocrine responses, innate immune responses and inflammation co-evolved, and the macrophage is a master cell at the interface between metabolism and immunity [10]. Several lines of evidence suggest that the immune, metabolic and endocrine responses co-evolved. Firstly, macrophages and adipocytes demonstrate remarkable functional overlap, as both cell types secrete cytokines and can be activated by bacterial products, such as lipopolysaccharide;[11] furthermore, pre-adipocytes can transdifferentiate into macrophages [12,13]. Secondly, the fat body in *Drosophila melanogaster* constitutes the functional unit that activates both metabolic and immune responses, suggesting that they evolved from a common ancestral structure [14,15]. A third consideration is that nutrition was inevitably linked to the activation of the immune response, as food and water were heavily contaminated with microbial stimuli for the majority of human evolution. A forth line of evidence is the activation of the innate immune response when food is ingested. This activation is called postprandial inflammation and it is part of the adaptive response to meals as inflammatory markers increase after ingestion of food through several molecular mechanisms [14,16]**.** Lastly, during infection a change in leptin synthesis and a reduction in food intake occur, reducing the probability of ingesting other pathogens and activating energy-requiring mechanisms (such as the digestive processes) and reducing the probability that epitopes from nutrients will compete for receptors crucial for pathogen sensing [17]. Moreover, chronic infection and inflammation are linked to insulin resistance, which reduces the intracellular

levels of glucose (glucose is required by most pathogens for replication) and optimizes energy allocation to the brain (the human brain is particularly large and has a high metabolic cost) to protect the brain during stress stimuli, such as starvation and infections [18–20]. Insulin resistance and storage of fats as adipose tissue might have favoured human survival because under stressful conditions, such as starvation or infections, peripheral cells (such as muscle cells) shift to use fats as their energy substrates and insulin resistance results in glucose only being used as a substrate for crucial organs, such as the brain, in which it is the preferred substrate [21].

The co-evolution of the immune and metabolic systems is also supported by data on low-grade chronic infections in ageing. Here we will focus on data from studies about cytomegalovirus infection[22], which is an example of a common chronic infection associated with accelerated immunosenescence **[G]** and age-related diseases according to several immunological, clinical and epidemiological studies[23,24] . Human cytomegalovirus persists as a lifelong infection alternating processes of reactivation and latency and cytomegalovirus infection can simultanenously alter lipid and glucose metabolism [25,26]. In the context of T2DM (a common age-related disease) an observation that could be predicted with an understanding of evolution is that cytomegalovirus seropositivity in the very elderly is associated with increased diagnoses of T2DM , leading to the hypothesis that cytomegalovirus seropositivity might facilitate the onset of T2DM in the long term [27]. Cytomegalovirus accelerates immunosenescence by promoting a pro-inflammatory environment [28]. This pro-inflammatory state has deleterious effects on pancreatic β-cells, which could lead to an insufficient response to insulin resistance, resulting in the onset of T2DM [29]. Cytomegalovirus resides in pancreatic β-cells without apparently causing cytopathic effects, but it can influence insulin production directly after repeated reactivations, thus impairing insulin metabolism and potentially leading to T2DM [30,31]. The systemic inflammation resulting from the activation and reactivation **[G]** of the cytomegalovirus, might be a biological process contributing to glucose regulation in the elderly. However the association between CMV infection and glucose metabolism in the elderly need to be further investigated since other study reported no association between them [24]. Contrasting data regarding CMV infection and glucose metabolism could derive from the profound differences in the prevalence of CMV infection in different populations, likely resulting in different adaptive mechanisms and phenotypes. Moreover experimental data adressing the effect that infections have on inflammaging and age-related diseases, including metabolic diseases, remains incomplete.

[H2] Degeneracy of damage sensors

Biological degeneracy is defined as 'the ability of structurally different elements to perform the same function', and it is recognized as one of the most prominent characteristics of biological complexity [32] . Degeneracy (also termed 'promiscuity') refers to the many structures–one function paradigm, where in a system composed of degenerate elements, if one fails other elements can take over its function [32]. Edelman and Gally[33] provided a list of examples of degeneracy at different levels of biological organization, such as the genetic code, the protein folding process, metabolism, immune responses and connectivity in neural networks. Experimental evidence shows that the same sensors such as pattern recognition receptors including Toll-like receptors (TLR) [34], NOD-like receptors and cyclic GMP–AMP synthase (cGAS), aryl hydrocarbon receptor (AHR) and taste receptors (described in **BOX 1**), among others, have overlapping characteristics. These receptors, present in the nucleus, cytosol and plasma membrane, are able to recognize self molecules (termed damage-associated molecular patterns (DAMPs) such as cell debris and misplaced or altered molecules), non-self viral and bacterial products (pathogen-associated molecular patterns (PAMPs)) and nutritional and metabolic products from the gut microbiota (which could be considered as quasi-self) [6]. Pattern recognition receptors are evolutionarily conserved from insects to vertebrates, and their primitive function was probably to provide anti-microbial immunity and to regulate autophagy [34]. During evolution, this array of receptors was optimized to increase inflammation and insulin resistance as a first response to nutrient deprivation (as autophagy is a cellular strategy to survive a decrease in nutrients by consuming intracellular constituents) serving at the same time as a strategy to combat pathogens. Thus, a situation shaped by evolution emerges, where a variety of different molecular motifs and stimuli (including nutritional stimuli) converge on a small number of sensors that are capable of triggering the innate immune response, causing inflammation and concomintantly an adaptive metabolic response to environmental (external or internal) stimuli [10,35,36] . A study published in 2017 shows that the activation of STING- dependent type I interferon response reduces the reactivity of microglia (tissue-resident macrophages in the central nervous system) and neuroinflammation, suggesting that the levels of activation of damage sensors are critical to have beneficial (low inflammation) or detrimental (excessive inflammation) effects [37] . Within this scenario inflammaging is the unpredicted consequence of the evolution-driven degeneracy of damage sensors that can be depicted as bow tie architecture [32] (**FIG. 2A).** "Bow tie" is a recent concept that tries to grasp the operational and functional architecture of complex systems. This kind of architecture has been observed in the structural organization of organisms throughout the biological scale, including in metabolic networks [38]. The main characteristic of bow

tie architecture is its ability to converge a wide range of inputs (fan in) on a evolutionary-reduced set of building blocks (core) capable of converting them into a wide variety of outputs (fan out). In particular, in Fig.2 the fan in is represented by the large variety of self, quasi-self and non-self damage stimuli which have the capability to bind to a limited number of evolutionary-conserved innate immunity sensors (core), whose activation produce a large number of inflammatory compounds (fan out).

There are several examples of this degeneracy or promiscuity in immune-response receptors that can also be activated by compounds in nutrients. One such example is the ability of saturated fatty acids to activate both TLR2 and TLR4, which are involved in pathogen recognition and activation of the innate immune response, and trigger the release of proinflammatory mediators [39,40]. Another example is that the organic compounds curcumin (from turmeric), helenalin (extracted from Arnica), cinnamaldehyde (from cinnamon) and sulforaphane (extracted from cruciferous vegetables, such as broccoli), containing α,β-unsaturated carbonyl or isothiocyanate group, can inhibit TLR4 activation by interfering with TLR4 receptor dimerization [41]. In addition, gliadin (a component of gluten) can activate the TLR signalling pathway in vitro [42]. Furthermore, morphine [43], glucuronic acid (found in natural gum), the ethanol metabolite ethyl-glucuronide [44], polyphenol epigallocatechin-3-gallate (found in green tea) [45], phenethyl isothiocyanate (found in cruciferous vegetables) and parthenolide (extracted from the plant, feverfew) can all activate TLRs [46]. Finally, during ageing a diet rich in saturated fatty acids activates pattern recognition receptors, and together with debris from dead cells (necroptosis) [47] which behave as DAMPs activate an inflammatory response that is similar to that seen during an infection.

A study published in 2016 showed that self nucleic acids and nucleic acid sensing receptors have a fundamental role in promoting inflammation associated with diet-induced obesity and in the regulation of glucose homeostasis and insulin signalling in obesity [48]. Diet-induced obesity promotes excess release and diminished clearance of nucleic acids, and mishandling of nucleaic acids activates visceral adipose tissue macrophages, via TLR7 and TLR9, to promote inflammation. Moreover, inhibiting TLR7 and TLR9 improves obesity-related inflammation and glucose homeostasis. Another study showed that obesity-related adipocyte degeneration causes the release of single-stranded and double-stranded cell-free DNA into the plasma, which promotes macrophage accumulation in adipose tissue (via TLR9 activation) and stimulates chronic adipose tissue inflammation and insulin resistance [49]. The degeneracy of TLR9 sensing of mitochondrial DNA (mtDNA) is described in **BOX 2**.

[H2] Stimuli that fuel inflammaging

A major focus in ageing research is the identification of the stimuli that fuel inflammaging. A variety of data suggests that besides persistent viral (such as CMV) and bacterial infections (such as periodontitis infections [50]), cell debris, misplaced self-molecules and misfolded and oxidized proteins are major contributors to inflammaging [36,51]. Within this framework, the gut microbiota is of particular and crucial importance as it is at the boundary between diet, metabolism and the innate immune response [52] and also because it undergoes profound remodelling with age [53–56]. Host–bacteria symbiosis (in particular the co-evolution of the gut microbiota and its human host), preserves the mutually advantageous co-existence where the host gains nutrients from the digestion of certain foods by the microbiota and the commensal microbiota feed on the host's meals. As beneficial and potentially pathogenic microbes share similar epitopes, evolutionary forces provided by the microbiota itself might have shaped many immunological features in the host in order to produce mechanisms of immunological tolerance [57–59].

The human gut microbiota is a highly diverse ecosystem made up of trillions of bacteria that establish a complex, multi-species 'new organ'. Every component of this ecosystem has a specific role and is capable of responding to signals from its host or the environment (including the circadian rhythm; **BOX 3**) by altering its own activity [60]. There are several factors that can permanently change the composition and function of the gut microbiota, which results in large variabilities and heterogeneity of this ecosystem in the human host [61]. These factors that constitute a sort of biography of each person are divided into individual-based (age, gender, genetics, lifestyle, type of delivery, breastfeeding or formula feeding), population-based (ethnicity, cultural habits, nutrition, population genetic structure and ancestry), and environment-based (climate, use of antibiotics, immunological stimuli lifelong). The adaptive and plastic nature of the gut microbiota allows it to adjust the host's immune and metabolic pathways in response to dietary habits and energy requirements and it has a profound effect on health and disease [60]. The gut microbiota has a central role in immunity and metabolism due to its pervasive effect and continuous crosstalk with the other organs and tissues of the body. The intestinal immune responses during health and disease are shaped by the gut microbiota and the age-related remodelling of it might contribute to systemic inflammaging, which can directly or indirectly affect its composition in a self-sustaining loop [62] (**FIG. 3A**). In particular, the changes in the gut microbiota profile in centenarians, where there is an enrichment of *Proteobacteria* and a decrease in butyrate-producing bacteria, correlate with a systemic increase in levels of the pro-inflammatory cytokines IL-6 and IL-8[53]. *Proteobacteria* is a group containing many of those bacteria recently redefined as ''pathobionts''. These are considered

to be minor and opportunistic components of the human gut ecosystem that, under some circumstances, e.g. inflammation, may escape surveillance, overtake mutualistic symbionts and induce pathology. Butyrate is a short chain fatty acids representing a major energy source for the enterocytes and has been implicated in the protection against inflammatory bowel diseases [44]. Studies in mouse models showed that when gut microbiota from aged mice was inoculated into young germ free mice it induced inflammaging, increased the percentage of several T helper (T_H) cell subsets and the levels of inflammatory markers, such as TNFα [63,64]. This pro-inflammatory effect was associated with lower levels of *Akkermansia (a* well-known health-associated genera protecting against inflammation, and promoting a healthy metabolic homeostasis [65]*)*, and higher levels of TM7 bacteria and *Proteobacteria* (both associated with intestinal inflammation and the pathogenesis of Intestinal Bowel Disease [66,67]), which is probably linked with the increased inflammatory potential of the gut microbiota of aged mice [63].

A comprehensive phylogenetic analysis of the human gut microbiota of individuals from Italy (aged 22–109 years) showed that the core population of gut microbiota (which is comprised of the dominant symbiotic bacterial taxa *Ruminococcaceae*, *Lachnospiraceae* and *Bacterodaceae*) exhibits a decrease in diversity and relative abundance with age [56]. In extreme longevity (over 105 years), this decline is offset by an increase in longevity-adapted, and possibly beneficial, subdominant species of *Akkermansia, Bifidobacterium* and *Christensenellaceae*. Accordingly, an unexpected increase in diversity in the composition of the gut microbiota in comparison with young subjects was observed in centenarians from Italy, and also in those from China and Japan despite the differences in genetics, lifestyle and diet between people from these countries [60]. Such a remarkable signature of longevity, per se, is probably a key health indicator of centenarians, at variance with the charactersistic decrease in gut microbiota diversity that is associated with most, if not all, age-related diseases [56,60].

[H2] Stimuli that attenuate inflammaging

[H3] Calorie restriction

Evolution favours the emergence of mechanisms and phenotypes that ensure survival when faced with undernutrition or starvation, while countermeasures against energy or nutrient excess apparently do not develop [68]. This feature is probably why calorie restriction (by reducing calorie intake or alternate fasting) elicits conserved cell protective responses in nearly all tissues and organs

that extend the lifespan of simple model organisms, mammals and non-human primates [69]. As with all experiments, there are several variations and exceptions in the longevity models, which could be due to differences in strain or genetic background of the animals used, feeding regimens, diet composition (the ratio of protein: carbyohydrate: fat; natural or purified ingredients), age of onset (the age at which the calorie restriction was implemented), laboratory differences and other variables [69]. Overall, the results suggest that the effect of calorie restriction is highly conserved, even in mammals, and involves common pathways across phyla and species [70].

There is a consensus that calorie restriction involves the down-regulation of insulin, insulin-like signalling, the mammalian target of rapamycin (mTOR)–ribosomal protein S6 kinase (S6K) pathway and glucose signalling through Ras–protein kinase A (PKA) pathway as well as the activation of NAD-dependent protein deacetylase sirtuin 1 (SIRT1) [71]. These pathways activate autophagy, stress defence mechanisms and survival pathways while attenuating pro-inflammatory responses [72]. Calorie restriction has been proposed to activate these longevity-promoting pathways by acting as a mild stressor that promotes hormetic responses [73]. Major targets of calorie restriction are mitochondria, which undergo a mild functional impairment, which, counter-intuitively, results in the promotion of longevity by crosstalk with the nucleus and secretion of a variety of mitokines (such as FGF21, GDF15 and humanin) [74]. Different types of macronutrient restriction exist, but the reduction of dietary proteins and amino acids is most effective for promoting longevity [75]. In particular, dietary restriction of a single essential amino acid in a normal diet is able to increase lifespan. For instance, a tryptophan-restricted diet, which can promote longevity and reduce the effects of age-dependent deterioration, has been explored for its neurological benefits as tryptophan has a role in serotonin synthesis [71]. A general reduction of inflammation in the body is apparently a major advantage of calorie restriction, resulting in pervasive beneficial effects on ageing mechanisms (including insulin resistance, adult neurogenesis and neuronal plasticity, autophagy and mitochondrial biogenesis) [76]. In summary, cells perceive the reduced availability of nutrients and translate this information into integrated, adaptive or protective metabolic and immune responses. The relationship between nutrient intake and inflammatory processes is summarized in FIG. 2B.

[H3] Hibernation

Life-extending manipulations, such as calorie restriction, also decrease core body temperature. Temperature is a basic and essential property of any physical system, including living systems. From poikilotherms (organisms that cannot regulate their own body temperature) to homeotherms (organisms that can maintain thermal homeostatsis) there is a clear trend that animals with naturally

lower body temperatures have longer lifespans than those with higher body temperatures , both in wild animal populations and under laboratory conditions [77]. In 1997, a study suggested that the body's response to calorie restriction in mammals shows features similar to hibernation and concluded that the observations from calorie restriction studies conducted by gerontologists is due to a range of evolution-conserved responses to food deprivation and not a result of laboratory artifact [78]. The study suggests that the responses have adaptive value in the wild and activation of these mechanisms might involve the neuroendocrine system.

The previously discussed evolutionary-driven hierarchical redistribution of energy [20], which is a response to situations of undernutrition, starvation and infections, is a good example showing the similarities shared between hibernation, which involves complex neuroendocrine remodelling, and calorie restriction [78] for extending lifespan. On the physiological level, the similarities between fasting during hibernation and calorie restriction are manifested in several ways, for instance, decrease in blood glucose and blood insulin levels [78]. Fasting mice are prone to reduced body temperatures and enter a torpor-like state. Calorie restriction in mice and hibernation in the Arctic ground squirrel share many similar molecular signatures that are involved in shifting metabolic fuel use[79]. Examples of these signatures include the high expression of genes involved in gluconeogenesis and the low expression of genes involved in fatty acid biosynthesis, together with regulatory changes that suppress cell growth; all these changes seem to be largely driven by peroxisome proliferator-activated receptor α (PPARα)[79]. Changes to the expression of genes involved in the sleep–wake cycle-related and temperature response such as heat shock proteins and cold-inducible RNA-binding protein (CIRBP) led to the hypothesis that the torpor–arousal cycle is the result of the expression of peripheral clock genes situated in peripheral tissues but not the central circadian clock genes within the suprachiasmatic nuclei (SCN) of the hypothalamus, that persists in a non-temperature compensated manner during hibernation (core clock genes are arrested and stop oscillating during hibernation) [80] . Circadian oscillations are indeed generated by a set of genes forming a transcriptional autoregulatory feedback loop. In mammals, these include: Clock, Bmal1, Per1, Per2, Cry1, and Cry2 [81].

[H1] Inflammaging and metaflammation

[H2] Nutrition and Inflammation

The most advanced and convincing mechanism linking nutrient excess and inflammation is the so-called 'metaflammation' (metabolic inflammation) process, which is a low-grade chronic and sterile inflammatory status, sustained by high nutrient intake that alters the inflammatory milieu of

metabolic cells, tissues and organs [6]. The concept of metaflammation was developed from studies on the effect of overfeeding in animal models[5]. The increase of the low-grade physiological inflammation under conditions of high nutrient intake is a critical contributor to the onset of insulin resistance. The result is an increased activation of inflammatory responses that affects a variety of organs (such as the adipose tissue, liver, pancreas, muscle and brain). The basis of metaflammation is the physiological inflammatory activation elicited by the ingestion of any meal (where lipids have a central role) by an organism (**BOX4**). Postprandial lipoproteins are involved in the inflammatory process that preceeds the development of cardiometabolic diseases (such as atherosclerotic cardiovascular disease, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis and T2DM). During the postprandial state, remnants of chylomicrons and VLDL (also called triglyceride-rich lipoproteins) bind to endothelial cells and circulating leukocytes [82–86]. The binding of the triglyceride-rich lipoproteins results in acute activation of the cells that elevates levels of adhesion molecules, cytokines, oxidative stress and ultimately fuels inflammation. Moreover, triglyceride-rich lipoproteins and free fatty acids (produced by the hydrolysis of triglyceride-rich lipoproteins) stimulate the expression of vascular cell adhesion protein 1 (VCAM1) in human aortic endothelial cells, which stimulates monocytes adhesion [87–89].

[H2] Bacterial endotoxins

During a state of nutrient overload and excessive exposure to meals rich in pro-inflammatory fatty acids the innate immune system is exceedingly stimulated, which contributes to homeostatic failure (which is loss of capacity of the cells to properly respond to changes of the external environment) that in turn leads to metabolic disorders. High-fat meals in healthy subjects results in an increased neutrophil count and raised levels of IL-6 and hydroperoxides [90]. In healthy individuals, a high-fat diet also increases serum levels of bacterial endotoxin (lipopolysaccharide), which might cause leukocyte activation and inflammation [91]. Bacterial endotoxins are potent inflammatory compounds that circulate at low concentrations in the blood, which mimics a sort of chronic low-grade bacterial infection. The abundance of bacterial endotoxins in the gut depends on which bacteria are present. Postprandial elevation of lipopolysaccharide in the circulation contributes to ‘metabolic endotoxemia’ (which is defined in this way because initiated by the food intake and not by a primary infection by bacteria) and low-grade inflammation [92], which seem to have a substantial role in the development and progression of cardiometabolic diseases [93] and in promoting ageing phenotypes (such as muscle decline and sarcopenia)[94]. A high-fat diet can cause gut microbiota dysbiosis that exaggerates the physiological production of lipopolysaccharide and dysregulation in meal timing contributes to such metabolic and inflammatory dysregulation (**BOX 3**).

[H2] Adipocytes

From a mechanistic point of view adipose tissue is at the core of metaflammation. Adipocytes exposed to nutrient-dense diets increase in size until they reach a structurally critical condition [95] **.** Consequently, vascularization of adipose tissue is reduced and adipocytes are exposed to hypoxic conditions. Moreover, obese mice adipocytes experience endoplasmic reticulum (ER) stress caused by the accumulation of unfolded proteins with the concomitant activation of the mTOR pathway and downregulation of AMP-activated protein kinase (AMPK) and SIRT pathways [96,97]. Thus, ER stress triggers inflammation through the activation of the unfolded protein response [98].

[H2] Similarities

The similarities and differences between inflammaging and metaflammation were comprehensively reviewed in 2017 [99]. The concepts of metaflammation and inflammaging emerged from two distinct research fields — the obesity and T2DM and the ageing and longevity field, respectively. However, few, if any, studies exist that cover both metaflammation and inflammaging or in which inflammaging originally observed and conceptualised in humans [100] has been mechanistically modelled and validated in animal studies. On the other hand metaflammation stems from study on animal models that identify mechanistic relationship between nutrient excess and increase in the inflammatory paths disregarding the role of inflammaging**.** It is remarkable that these two broad research fields have identified — independently and almost simultaneously — that a chronic and, most importantly, sterile inflammatory process is the critical aetiological momentum for metabolic diseases and age-related physiological decline, thus likely playing a critical role in all the major age-related diseases. It is also noteworthy that, although the stressors that are the basis of these inflammatory processes are different, the mechanisms that sustain chronic inflammation are largely shared by inflammaging and metaflammation. Indeed, the following processes all have a central role in inflammaging and metaflammaging: the increase of senescent cells and their accumulation; hyperactivation of the innate immune response through TLR signalling, inflammasome and cGAS–STING (Cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS) detects intracellular DNA and signals through the adapter protein STING (stimulator of interferon genes)) pathway activation; and the accumulation and systemic spillover of cellular debris as a consequence of the exacerbation of cell death in target tissue and organs and mitochondrial dysfunction. Accordingly, metaflammtion can contribute to inflammaging and be considered a nutrient excess-driven accelerated ageing, supporting the conceptualizationof a continuum of ageing and age-related diseases [101].

Both metaflammation and inflammaging, separately and/or together, exert a pro-inflammatory systemic effect, which suggests that both phenomena can interact and synergize and probably interfere with inter-organ communication and crosstalk. Understanding the inter-organ interaction represents one of the most intriguing and complex scientific issues that needs to be urgently addressed. The 'garbaging theory', proposed in 2017, goes to the heart of this issue from an ageing and age-related diseases perspective [36]. 'Garbaging' proposes that, besides persistent viral and bacterial infections, inflammaging is largely caused and sustained by the age-related progressive impairment of the 'cleaning' of misplaced and/or damaged self-molecules (cell debris) produced by the physiological or pathological cell death processes [36,51]. The systematic study of circulating mediators such as microRNAs [102], micro-vesicles [103], nano-vesicles and lipids in the elderly and during nutrient excess conditions could help in understanding how the communication between organs and tissues is altered by inflammaging and metaflammation, and if and how such alterations subsequently contribute to sustain chronic age-related and metabolic diseases.

[H1] Integration of biomarkers

The unifying hypothesis that metaflammation might precede and contribute to inflammaging and *vice versa*, and that metabolic age-related dysfunctions and diseases could be considered manifestations of the acceleration of ageing (which in turn accelerate ageing itself) opens a new possibility regarding biomarkers that could and should be used to assess most metabolic diseases. In metabolic diseases, it is timely to apply a new set of informative and robust biomarkers that have emerged from studies of human ageing and can be used to measure the 'biological' age of individuals compared with their chronological age. The new markers should be integrated with the classic biochemical and hormonal parameters, including lipids as biochemical markers of immune-metabolic dysregulation. The following sections will describe in detail three biomarkers, while an overall summary of the biomarkers is reported in Table 1.

[H2]DNA methylation

A general alteration of the chromatin structure and the epigenetic signature of the genome is a major characteristic of the ageing process, which can be assessed by the newly available techniques that can scan the whole epigenome. DNA methylation is a biomarker that can be used to distinguish chronological age from biological age. To date, the biomarker that gives the best correlation with chronological age in humans is the DNA methylation levels of the CpG sites located in a CpG island in the promoter region of *ELOVL2* (which encodes omega 3 and omega 6 fatty acid elongase,

also known as ELOVL fatty acid elongase 2) [104]. However, studies from the past 7 years have developed a mathematical model (termed 'epigenetic clock') based on the DNA methylation levels of many CpG sites to estimate the biological age of individuals [105–108]. The epigenetic clock has been applied to different conditions. The most studied model is Horvath's epigenetic clock, which is based on the DNA methylation levels of 353 CpG sites across the human genome. Results from using the epigenetic clock provide information about the biological age of individuals, which could be associated with their health status and used to predict the potential occurrence of age-related health outcomes.

For instance, using the Horvath's clock on patients with Down syndrome or Werner syndrome (a rare adult premature ageing disease), the epigenetic clock showed an age acceleration for both conditions [109–111], while in centenarians and their offspring, the clock showed a consistently decelerated DNA methylation age [112]. To date different measures of epigenetic acceleration exists [113]and measuring the extrinsic epigenetic age acceleration allows the measurement of epigenetic ageing in immune-related components having a positive correlation with the amount of exhausted CD8 T cells and plasma blast cells) and predicts risk factors for cardiometabolic disease. Moreover, the analysis of extrinsic epigenetic age acceleration in post-menopause women between ages of 50 and 79 years enrolled in the WHY study (women's Health Initiative) showed a positive correlation with triglyceride levels, C-reactive protein and creatinine, and a negative association with education levels [114]. In a 2017 study, a mouse epigenetic clock, constructed from blood DNA methylation profiles, correctly estimated the biological age of a mouse cohort. This mouse clock was also able to measure the effect of calorie restriction and of knockout of specific gene on ageing and detect the rejuvenation of fibroblast-derived induced pluripotent stem cells [115]. To date, the relationship between DNA methylation-age (age determined by DNA methylation levels) and inflammaging have not been investigated. Studying the possible relationship of these two factors is of the utmost interest. The correlation between cytomegalovirus infection and higher methylation levels of *ELOLV2* provides evidence of a possible interaction between these factors [116]. Several studies that offer indirect observations on this subject showed a possible link between whole blood DNA methylation levels (in different CpGs sites) and plasma levels of different pro-inflammatory compounds (such as serum C-reactive protein or plasma IL-6 levels) [117–119] or chronic inflammatory conditions [120] .

[H2]Glycomics

Glycosylation is a frequent co- and post-translational modification of proteins which modulates a variety of biological functions including their conformation, solubility, antigenicity, activity, and recognition by glycan-binding proteins. The analysis of the sugar chains attached to protein at asparagine (Asn) residues by an N-glycosidic bond (N-glycans, collectively called N-glycome) identified new candidate biomarkers of aging such as N-glycans devoid of galactose residues on their branches[121]. Since the two seminal studies by Vanhooren and colleagues[122,123], enzymatic glycosylation has become one of the most promising biomarkers of biological age. The two studies demonstrate that the log-ratio of the relative abundance of two N-linked glycan species (namely agalacto, core-α-1,6-fucosylated diantennary glycan, NGA2F and digalacto core-α-1,6-fucosylated diantennary glycan, NA2F) increases progressively with age and is associated with features of healthy and unhealthy ageing. This combination of N-linked glycan signals was called 'GlycoAge test' and was tested in a number of human pathological conditions (Down syndrome, T2DM, hepatocellular carcinoma) to assess its ability to determine features of biological age. Of all the studies that tested GlycoAge, the result worth mentioning is the report of an accelerated glycomic age for individuals with Down syndrome, which is similar to the results from DNA methylation analyses [124].

Technological advancements has made it possible to measure the whole spectrum of N-linked glycans from peripheral tissues, such as serum or plasma [125] . In 2013, the use of N-**glycans** as a biomarker of inflammaging in the elderly was proposed[121]. Protein galactosylation is responsible for the anti-inflammatory function of IgG; thus when the galactosylated biantennary structures that decorate the Asn297 of Fc portion of IgG become with age devoid of galactose at both branches they are called IgG-G0 and become highly pro-inflammatory. In particular, the study proposed to use IgG-G0 as a biomarker of inflammatory conditions during ageing, where chronic low-grade inflammatory pathways negatively affect the glycosylation machinery of antibody-producing cells. The accumulation of IgG-G0 together with the loss of a2,6-sialilation of IgG glycans during ageing can contribute to inflammaging and to age-related diseases as they exert a pro-inflammatory effect through different mechanisms involved in inflammation and in process that sustain and amplify inflammatory signals including activation of the lectin pathway of complement through the mannose-binding lectin pathway, phagocyte activation by binding to Fcγ receptors, formation of autoantibody aggregates. Moreover, glycosylation promotes the recognition by DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) which increases expression of inhibitory FcγRIIB and is anti-inflammatory. Within this framework, age-related alterations of the N-glycome were suggested as a biomarker of biological age and inflammaging [121,124].

A study published in 2014 confirmed and extended the application of glycans as a biomarker of biological age [126] . The study includes data on the IgG glycosylation of 5,117 individuals from four European populations (Croatia island Vis, Croatian island Korcula, Orkney Island in Scotland and United Kingdom)**.** The study showed that several IgG glycans promoting inflammation (including FA2B, FA2G2 and FA2BG2) account for up to 58% of the variance in chronological age (considerably more than other biomarkers of age like telomere lengths), while the remaining variance strongly correlated with physiological parameters associated with biological age and metabolism (systolic and diastolic blood pressure*, forced vital capacity and peak expiratory flow,* albumin, waist circumference, *BMI,* glucose, insulin, cholesterol, triglycerides, LDL, HDL, creatinine and uric acid. . The association of IgG N-glycan variability with critical parameters, including the metabolic ones, to assess health status and burdens in the elderly is extremely promising and support the specific IgG N-glycan analysis to obtain innovative and informative markers to monitor health in elderly population. Following the hypothesis that protein glycosylation should predict higher cardiovascular risk by reflecting inflammatory pathways a study published in 2018 on 76 IgG glycosylation traits in 2970 women (age range, 40-79 years) from the TwinsUK cohort and replicated in 967 women from ORCADES cohort (Orkney Complex Disease Study) reported that glycosylation traits are independently associated with arterial lesion formation and subclinical atherosclerosis while one specific trait related to the sialylated N-glycan (GP18-the percentage of FA2BG2S1 glycan in total IgG glycans) is negatively correlated with cardiovascular disease risk, very-low-density lipoprotein and triglyceride serum levels, and presence of carotid plaque [127].

Available data also showed that the IgG N-glycosilation changes observed with ageing could be related not only to inflammation but also to the alteration of important metabolic parameters and pathways [121]. The first study on the relationship between metabolic diseases such as metabolic syndrome and T2DM and N-glycans was that of Testa and colleagues [128] on 562 T2DM patients (mean age 65.6±8.2 years) and 599 healthy control subjects (mean age, 58.5±12.4 years). Specific and significant changes in N-glycan composition in the sera of T2DM patients and in T2DM with complications were found. In particular, α (1,6)-linked arm monogalactosylated, core-fucosylated diantennary N-glycans (NG1(6)A2F) were significantly reduced in T2DM compared with control subjects. Macro vascular-complications were found to be related with decreased levels of NG1(6)A2F. Moreover, NG1(6)A2F and NG1(3)A2F levels were strongly negatively correlated with most of metabolic syndrome parameters (waist/hip ratio, triglycerides, glycemia, glycated

haemoglobin and the absolute number of neutrophils) while NG1(6)A2F levels are negatively correlated with HOMA and NG1(3)A2F levels are positively correlated with HDL. Furthermore NG1(6) A2F and NG1(3)A2F decrease more with the increased severity of patient phenotype from the extreme "healthy" (controls without MS) to the extreme "unhealthy" (T2DM+ with metabolic syndrome) [128] .

In a randomized, single-blind, placebo-controlled trial, 38 prediabetic subjects received metformin (1500 mg/day) or placebo for 2 months. Metformin significantly improved insulin sensitivity and metabolic parameters compared to baseline and favourably modified the plasma N-glycan profile compared to placebo [129] . On the same line, Keser and colleagues reported that increased complexity of plasma N-glycan structures is associated with higher risk of developing type 2 diabetes and poorer regulation of blood glucose levels [130]. Another study published in 2017 on a cohort of 1826 individuals demonstrated the potential of IgG1, IgG2 and IgG4 glycosylation as a biomarker for inflammation and metabolic health [131]. The results showed that a low level of galactosylation and sialylation and a high degree of core fucosylation associated with poor metabolic health and increased inflammation as assessed by increased C-reactive protein, low serum HDL and high triglycerides.

Finally, it is interesting to note that up to 50% of plasma glycome variability is estimated to be herirtable and a genetic control of IgG glycosylation is suggested by a genome-wide association study (GWAS) on liquid chromatography electrospray mass spectrometry (LC-ESI-MS)-measured IgG glycopeptides of 1,823 individuals in the Cooperative Health Research in the Augsburg Region (KORA F4) study cohort and replicated in 1,836 individuals from the Leiden Longevity Study (LLS). The results indicate that, in addition to genes encoding for glycosyltransferases (i.e., ST6GAL1, B4GALT1, FUT8, and MGAT3), other genetic loci have strong influences on the IgG glycosylation patterns, including the transcription factor RUNX3 [132].

As a whole, the age-related increase in aberrantly glycosylated IgG and other proteins can be taken as a robust marker of biological age and a factor contributing to inflammaging by its capability to activate the immune system toward a pro-inflammatory status.

[H2] Metabolomics and lipidomics

Metabolomics **[G]** and lipidomics **[G]**could provide biomarkers at the interface between metabolism, inflammation (including age-related changes in the composition of the gut microbiota) and disease risk. The use of high-throughput technologies, such as liquid chromatography tandem-mass spectrometry (LC-MS) and NMR allow the measurement of a wide range of endogenous small-molecule metabolites. A study published in 2014 showed for the first time that lipidomics

could be a possible source of biomarkers of biological age and longevity [133]. The researchers showed that centenarians had a peculiar lipid profile, with unique changes in 41 of 161 measured lipid species. The lipid profile emphasized that long-living individuals have marked features of anti-inflammatory molecules, such as increased levels of phenylalanine, which inhibits the nuclear factor-κB (NF-κB) pathway, and decreased levels of glycerophosphocholine (a circulating marker of cellular senescence). Moreover, the use of lipidomics in a longevity study was reported in 2013; 19 lipid species associated with female familial longevity were identified[134]. A profile that included high levels of phosphocholine and sphingomyelin and low levels of phosphoethanolamine and long-chain triglycerides species was found to be characteristic of healthy ageing. The study suggests that the longevity plasma lipidome reflects the antioxidant capacity, lower lipid peroxidation inflammatory state and β-oxidation function likely contributing to healthy aging of studied individuals. Additionally the same study reported several longevity-associated lipids that correlated with a reduced risk of age-related diseases, such as hypertension and diabetes mellitus.

Several studies have successfully used metabolomic profiling to identify biomarkers of metabolic diseases, such as T2DM [135–137] and obesity [138,139] . The metabolomic profile of extreme ageing was described in a study published in 2013 [52]. In this study, the comparison of NMR metabolomic profile from a cohort of centenarians and their offspring from Italy with the profiles of adults and young controls showed for the first time that from a young age to extreme longevity the relative concentration of analysed metabolites change with age with different trajectories. Centenarians plasma and urine showed changes in the levels of specific glycerophospholipids and sphingolipids and a decrease in tryptophan concentration [52]. The metabolomic profile of centenarians showed peculiar mechanisms of cellular detoxification, which was through the specific modulation of the arachidonic acid metabolic cascade and enhanced cytochrome P450 enzyme activity. In particular, the longevity phenotype of arachidonic acid synthesis displayed both pro-inflammatory and anti-inflammatory characteristics, such as a high concentration of leukotriene E4 (a molecule with vasodilatation properties) or a high concentration of 15-hydroxyeicosatetraenoic acid (a molecule with anti-inflammatory properties). This metabolomic profile is in line with the hypothesis that longevity results in complex remodelling of lipids, amino acid metabolism, and of gut microbiota Similarly, the metabolomic profile of centenarians was successfully correlated with the metagenomic profiles of semi-supercentenarians (105 years of age or above) in another study[56] . These results highlight that the different classes of biomarkers are tightly interconnected, as shown by a study published in 2016 that suggested that blood lipids influence in circulting cells [140]. The data suggest that by combining the different classes of molecules (*e.g.* DNA methylation status of specific genomic loci with tiglicerides levels) it is possible to obtain a new generation of biomarkers

that are effective in assessing health status in different domains of biological ageing and can be used to predict healthy and unhealthy ageing.

Such an approach, with the inclusion of validated metabolic health biomarkers, should help to improve the accuracy of identifying individuals who do not have overt metabolic disease, but who have pre-clinical alterations of metabolism and accelerated ageing — a preventative and personalized approach. These new biomarkers will also be useful in monitoring the efficacy of therapeutic interventions, including diet [141], physical exercise, prebiotics and probiotics. The relationship between local and systemic inflammaging and metaflammation in the onset of chronic age-related diseases is still unclear and represents a major challenge and research subject that will be critical for the identification of additional biomarkers.

[H1] Conclusions

Collectively, the nutrient composition and the quantity of meals, as well as when meals are eaten (timing, rhythmicity), have a strong effect on the gut microbiota and metabolism, thus contributing to sustain basal level of inflammation ("inflammatory tone" [4]) which can be increased by over-nutrition (metaflammation) and ageing (inflammaging). Accordingly, metaflammation (caused by nutrient excess) can be conceptualized as a specific case of a more general and physiological mechanism that encompasses the activation of the pro-inflammatory evolutionary-selected machinery (depicted in Figure 2), that is the activation of the innate immune system whenever danger signals are sensed. This physiological and unavoidable 'inflammatory tone' [4] that occurs in the young and adult increases over time and can become highly detrimental either at old age (which is largely unpredicted by evolution) or in a situation such as over nutrition and nutrient excess (which is also unpredicted by evolution). The conceptual integration of inflammaging and metaflammation within the geroscience perspective suggests that metaflammation can be considered a specific type of accelerated ageing and paves the way to the amalgamation of biomarkers, which until now were developed separately, thus contributing to improved preventive and personalized medicine for the elderly.

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Competing interests

The authors declare no competing interests.

Publisher's note

Key points

- According to geroscience, inflammation is one of the seven evolutionary-conserved mechanistic pillars of ageing that is shared by age-related diseases, including metabolic diseases.

- Inflammaging is the long-term result of the chronic physiological stimulation of the innate immune system, which can become damaging during ageing — a period of life largely unpredicted by evolution

- Inflammaging is the byproduct of the degeneracy of a few receptors that can sense a variety of non-self, self and quasi-self ‘damage’ signals (or ‘garbage’) and activate the innate immune system.

- Inflammaging and metaflammation largely share the same molecular mechanisms, where metaflammation can be conceptualized as a specific situation of chronic inflammation caused by nutrient excess.

- The gut microbiota has a central role in metaflammation and inflammaging as it can release inflammatory products and contribute to the circadian rhythms and crosstalk with other organs and systems.

- Biomarkers of ‘biological’ age, such as DNA methylation, glycomics, metabolomics and lipidomics, can be successfully applied to metabolic diseases.

[b1] **BOX 1- Inflammation, taste and chemosensory receptors**

Taste receptors, in particular G protein-coupled receptors, that detect bitter, sweet and savoury tastes, signal to the brain to orchestrate food behaviour (searching for, choosing of and consuming food) in response to a wide range of nutritional stimuli. The same receptors not only have a role in nutrient sensing, but also in immune detection and in inflammation. In the upper respiratory epithelium, bitter and sweet taste receptors influence antimicrobial innate immune defence responses. Lee and colleagues[142] hypothesized that D-amino acids, produced by various bacteria, activate TLR in taste receptor cells in the mouth, as well as in the airway. Previous studies showed that the bitter receptor T2R38 in the upper respiratory epithelium can recognize epitopes produced by *Pseudomonas aeruginosa* [143], which can be taken as an example of a receptor involved not only in the perception of bitterness but also in host defence by sensing infection [144]. TRPM5 (transient receptor potential cation channel subfamily M member 5), a cation channel essential for the transduction of bitter, sweet and umami flavours, is also expressed in intestinal cells called tuft cells. TRPM5-dependent signals activate tuft cells that are involved in the initiation of immune responses following parasitic infections by producing IL-25, which promotes the rapid expansion of type 2 innate lymphoid cells [145,146]. In *Drosophila melanogaster*, environmental sensing is involved in lifespan duration[147] and loss of pickpocket protein 28 (Ppk28), a water sensor defined as a gustatory gene, alters metabolic homeostasis by promoting the internal storage of lipids and water to extend lifespan [148]. These examples show that taste receptors and chemosensory receptors constitute not only sensory structure for food, but it can also be hypothesized that they behave as pattern recognition receptors. The study of the degeneracy of these receptors and their potential role in modulating inflammaging and metaflammation is therefore a subject of interest for future research on ageing, longevity and metabolic diseases.

[b2] BOX 2. **The degeneracy of cell-free mtDNA sensing and inflammation**

The capacity of the limited number of sensors depicted in Fig 2 to recognize a large variety of self, non-self and quasi-self molecular motifs makes them reasonable candidates for the evolutionary unpredicted development of inflammaging [32]. A clear example of such sensor degeneracy is the mitochondrial DNA (mtDNA), which is normally inside the mitochondria. A great amount of cell-free mtDNA (cf-mtDNA) can be found in the blood in a variety of pathological conditions (such as sepsis), as a result of cell damage and death [149]. We assumed that cf-mtDNA is also an example of inflammatory garbage, as large amounts of cf-mtDNA are present in the blood of the elderly and nonagenarians [150]. The amount of cf-mtDNA appears to be a familial or genetic trait and has a high inflammatory capability, probably due to the mitochondria's ancestral derivation from bacteria [150]. It is probably not by chance that mtDNA, which has characteristics of self, non-self and quasi-self molecules, is sensed by several of the sensors mentioned in Fig.2, such as TLR9 (Toll-like receptor 9), NLRP3 (NOD-like receptor family, pyrin domain-containing protein 3) and cGAS-STING [151]. This sensing redundancy is also indirect evidence that the recognition of mtDNA is biologically critical. In addition to mtDNA, TLR9 is also able to recognize bacterial and viral DNA, as well as synthetic oligonucleotides [152]. Moreover, the function and expression of the previously mentioned sensors, all capable of activating innate immunity and inflammation, are modulated by circadian rhythms (BOX3), which is the case for TLR9. A study investigated the in vivo daily variations in the responsiveness of TLR9 to its ligand and showed that the time of day determines disease severity in a TLR9-dependent sepsis mouse model. Moreover, it was demonstrated that timing of immunization determines TLR9 ligand-adjuvant vaccine responsiveness [153].

[b3] Circadian fluctuation of gut microbiota

Energy intake as food is used by the host for metabolic neads and concomitantly by gut bacteria for their growth [154]. The gut microbiota displays circadian fluctuation [155], mainly driven by diurnal food intake, that leads to rhythmic abundance of microbial metabolites [156,157]. The systemic oscillation of the gut microbiota-derived metabolome reprogrammes the circadian transcriptomes (both locally and distally) and thereby regulates host physiology, including metabolic function and drug detoxification [157,158]. Bacterial adherence to the epithelium shows temporal fluctuations, which also correlates to host transcriptional oscillations. Thus, the disruption of gut microbiota oscillatory activity, as a result of antibiotic treatment or disordered time of dietary intake, leads to disorganization of host rhythmicity [158], suggesting that the gut microbiota serves as a circadian regulator of peripheral clocks [159]. Altogether, the host–microbe interaction seems to be essential in keeping the host clocks timed in an appropriate manner, to integrate the fluctuating environmental nutritional signals. According to chronobiomics [160], this interaction is bidirectional and host clock influence microbial community configurations. Moreover as the commensal bacteria compete with the invading pathogens, the compositional oscillation of the gut microbiota contributes to the circadian variation of host defence against invading pathogens. The circadian disruptions induced by modern lifestyles might lead to dysbiosis, which could predispose the host to metabolic disorders and inflammation [81,161]. Regular nutrient infusion into the colon immediately stimulates bacterial growth for 20 minutes. Bacterial molecules and metabolites, whose production is regulated by bacterial growth phases, control the release of satiety hormones in the intestine. Therefore, systemic bacterial molecules could directly switch on central appetite pathways that might incoporate the energy status of both the host and its gut microbiota. This modulation of intestinal satiety by short-term bacterial growth can be coupled with long-term control of appetite, which is regulated by the neuropeptidergic circuitry in the hypothalamus [154].

[b4] The physiopathology of anti-inflammatory and pro-inflammatory lipids

Lipids, including high density lipoprotein (HDL) modulate metabolic inflammation and ageing by coupling nutrition to microbiota, triglyceride-rich lipoproteins and innate immunity. Phospholipids account for 40–60% of total lipids and the surface phospholipids, especially 1-palmitoyl-2-linoleoyl phosphatidylcholine (PC), modulate the anti-inflammatory properties of HDL by inhibiting the NF-kB pathway and inflammation in endothelial cells [162,163]. In addition, 1-palmitoyl-2-linoleyl phosphatidylcholine is responsible for the ability of HDL to inhibit the activation of T cells that is mediated by dendritic cells[164]. The fatty acid composition of PC influences the anti-inflammatory activity of HDL[165]. Specifically, 1-palmitoyl-2-linoleoyl phosphatidylcholine and 1-palmitoyl-2-arachidonoyl phosphatidylcholine inhibit VCAM1 expression in activated endothelial cells to a greater degree than 1-palmitoyl-2-oleoyl phosphatidylcholine, whereas dipalmitoyl phosphatidylcholine does not inhibit it at all. These differences seem to be related to the physical properties of phosphatidylcholines, as the increase of unsaturated fatty acid moieties increases their fluidity[166]. Hence, the HDL containing polyunsaturated fatty acid has enhanced anti-inflammatory properties, leading to the accelerated efflux of cell-derived pro-inflammatory lipids to more fluid particles as an underlying mechanism. HDL also promotes lipopolysaccharide clearance by stimulating the interaction of lipopolysaccharide with lipopolysaccharide-binding protein [167,167,168]. These mechanisms were proposed to underlie the HDL-mediated protection from sepsis. Apolipoprotein A1 (ApoA1) is the main apoprotein of HDL and is pivotal in the induction of cholesterol efflux from cells. The interaction of HDL or ApoA1 with cells causes cholesterol depletion and disturbance of intracellular signalling in specific cholesterol and sphingolipids-enriched membrane microdomains, named lipid rafts B[169,170]. Key immunological receptors are localized in lipid rafts, including TLR and T cell and B cell receptors[171,172]. Modification of lipid raft composition can modulate raft-dependent immunological signalling because of protein delocalization. ApoA1 and HDL substantially decrease lipid raft abundance in monocyte membranes because of rapid cholesterol efflux[167] and thus directly modulate inflammation.

FIGURE LEGENDS

Figure 1. *The seven pillars of ageing.* The seven pillars are inflammation, stem cell regeneration, macromolecular damage, stress, proteostasis, metabolism and epigenetics [1]. The relationship between the pillars is shown by the interconnected network. The pillars are shared by ageing and age-related diseases.

Figure 2. *The bow tie architecture of the inflammaging machinery*. (A) A heterogeneous and broad spectrum of exogenous and endogenous danger stimuli (Fan in) interacts with a limited repertoire of sensors expressed on the cell surface and in the cytoplasm (Core), and elicits a limited number of inflammatory responses (Fan out). Danger molecules can be non-self (pathogen-associated molecular patterns, PAMPs), self (damage-associated molecular patterns, DAMPs) and quasi-self (nutritional and metabolic products from the gut microbiota) and this multitude of stimuli converges on the same evolutionary selected 'promiscuous' sensors triggering inflammatory responses. This physiological inflammatory process is critical for survival until middle-age. (B) With age the pro-inflammatory readout usually increases and becomes detrimental in post-reproductive age. The summation of these responses produces a progressive increase of the inflammatory tone that can last several years or decades, eventually leading to inflammaging. This process is modulated by a variety of factors, including genetics, lifelong lifestyle habits, immunobiography and anatomical variables [61,173]. Nutrient excess and over-nutrition fits this general scenario, representing a particular type of stimuli fueling inflammaging. AHR, aryl hydrocarbon receptor; cGAS, cyclic GMP–AMP synthase; IFN, interferon; NF-κB, nuclear factor-κB; NLR, NOD-like receptor. TLR, Toll-like receptor. For simplicity, only some of the sensors and inflammatory compounds have been reported.

Figure 3. The *gut microbiota as a key modulator of nutrition and inflammation*. The gut microbiota transforms environmental signals and dietary molecules into signalling metabolites to communicate with different organs and tissues in the host, mediating meal-related inflammation. (A) The connection between the gut microbiota and three metabolically important organs (the liver, brain and adipose tissue) is shown. The intestine and liver form a bidirectional link, via the portal vein and bile duct, called the "gut–liver axis". The gut microbiota also establishes a strong bidirectional connection with the central nervous system named the 'gut–brain axis'. The gut microbiota also communicates with the host's adipose tissue. The crosstalk between the gut microbiota and the other organs is also regulated by circadian rhythms, which are driven by a master clock within the suprachiasmatic nuclei (SCN) of the hypothalamus and it is mainly entrained by light signals [178]. In addition, peripheral clocks are located throughout the body, in peripheral organs such as liver, intestine and adipose tissue

[179]. Feeding rhythm and the gut microbiota drive peripheral clock (circadian transcriptional regulation across organs) which in turn can contribute to the regulation of the central clock (transcript expression). Nutritional and metabolic 'garbage' contribute to metaflammation and inflammaging. (B) The levels of inflammation during periods of constant undernutrition (low calorie intake), normal nutrition (which has periods of feeding and fasting) and overnutrition (high calorie intake) are depicted.

Figure 4. *Inflammaging, age trajectories and age-related diseases.* (A) Inflammaging is the basis of ageing and many age-related chronic diseases, which in turn increase the rate of ageing. Accordingly, age-related diseases can be conceptualized as the manifestation of accelerated inflammaging or ageing. +++ indicates acceleration or increase. (B) The ageing process and inflammaging show a high degree of heterogeneity among individuals. Accordingly, the slopes of the different trajectories diverge as a consequence of early critical immunological experiences (including intrauterine stimuli, type of birth, neonatal feeding and the use of antibiotics in early childhood) and continue to be different depending on events in adulthood (such as geographical place of living, infections, diet and vaccinations) [61]. The change in the slope of each line suggests that a clinically manifest disease accelerates the rate of ageing. Some individuals, such as centenarians and semi-supercentenarians, escape or delay the onset of age-related diseases (indicated with the line on the right)

Table 1. Biomarkers emerged from the study of ageing and age-related diseases that can be translated to metabolic diseases

Biomarker	Molecules involved	Effect	Reference
Inflammatory cytokines	TNF IL-1 IL-6 IL-8	Circulating biomarkers of chronic inflammation	[51]
N-linked glycans profile	Serum IgG-G0 digalactosylated or agalactosylated N-linked glycan structures	Biological age (pathological versus non-pathological ageing)	[121]
DNA methylation	*ELOVL2* 353 CpG sites, used to construct the epigenetic clock to estimate the methylation age	Chronological age and biological age	[104,106]
Circulating miRNA	miR-155 miR-21 miR-146a	Systemic inflammation	[180]
Metabolomics and lipidomics	Glycerophosphoethanolamines Glycerophosphocholines Glycerolipids Gile acids Steroids Isoprenoids Fatty amides Sphingolipids Tryptophan levels L-carnitine esters	Healthy ageing (centenarians)	[52,181–183]
Circulating cell-free mtDNA	cf-mtDNA	Systemic inflammation	[150]

cf-mtRNA, cell-free mitochondrial DNA; miRNA, microRNA; mtDNA, mitochondrial DNA

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GLOSSARY

Geroscience: a research field that tries to understand the molecular relationship and link between ageing and age-related chronic diseases. The basic assumption is that the machanisms driving ageing and those driving age-related diseases largely overlap. A detailed description of the strategies are reported in the paper of Kennedy and colleagues [1]

Inflammasome. Inflammasome is a multiprotein intracellular complex that detects pathogenic microorganisms and sterile stressors

Immunosenescence: The term indicate all the most marked changes that occur with aging in the adaptive immune system [3]. Immunosenescence is responsible for the increased susceptibility of elderly to new infectious diseases and is also linked to inflammatory age-related diseases.

Poikilotherms A cold□blooded animals. These animals set biological strategies that allow them to elude or endure exposures to environmental temperatures that are below the freezing point of their body fluid. Longevity was demonstrated to be increased by temperature reduction in poikilotherms.

Homeotherms An organism having a body temperature that is constant and largely independent of the temperature of its surroundings. Longevity was demonstrated to be increased by temperature reduction in homeotherms

Metabolic endotoxemia: Low-grade, chronic elevation in plasma lipopolisaccaride () 10-50 times lower than during septic conditions. It has been observed in obesity studies. It has been suggested that metabolic endotoxemia relates to inflammation and may serve a key mediator of metabolic derangements observed in obesity

Metabolomics: metabolomics refers to the systematic identification and quantification of the small molecule metabolic products (the metabolome) of a biological system.

Lipidomics is the study of the structure and function of the complete set of lipids (the lipidome).

Mitokine: cytokine produced in response to a mitochondrial stress (oxidative stress, unfolded proteins, etc.). It can be either nuclear- or mitochondrial-DNA encoded. Other types of stresses can induce mitokine production as well, such as endoplasmic reticulum stress. Mitokines include FGF21 and GDF15 (nuclear encoded), Humanin and MOTS-c (mitochondrial encoded peptides). Mitokines were originally postulated by Dillin and co-workers as soluble elements responsible for spreading at systemic level the effects of a local (mitochondrial) stress [184–187].

References to highlight:

1) Kennedy, B. K. *et al.* Geroscience: Linking Aging to Chronic Disease. *Cell* 159, 709–713 (2014).
This paper identified 7 ageing research fields to expand geroscience research and to extend healthspan. The link between ageing and chronic disease is the central point of this paper.

2) Franceschi, C. et al. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann. N. Y. Acad. Sci. 908, 244–254 (2000).
This is the first paper that starting from evolutionary insight conceptualizes the inflammaging theory. The term inflammaging was used here for the first time.

3) Hotamisligil, G. S. & Erbay, E. Nutrient sensing and inflammation in metabolic diseases. Nat. Rev. Immunol. 8, 923–934 (2008). This paper describe for the first time the metabolically trigger inflammation (term "metaflammation") starting from a detailed evolutionary description of the evolutionary history of metabolic and immune responses.

4) Ottaviani, E., Malagoli, D., Capri, M. & Franceschi, C. Ecoimmunology: is there any room for the neuroendocrine system? Bioessays 30, 868–874 (2008).
The evolutionary considerations of this paper introduce the importance of the common origin of the immune and neuroendocrine system for the study of stress response and human health.

5) Edelman, G. M. & Gally, J. A. Degeneracy and complexity in biological systems. Proc. Natl. Acad. Sci. 98, 13763–13768 (2001).
Gerald Edelman (Nobel Price for Medicine in 1972) and Joseph Gally describe the concept of degeneracy crucial in many biological system (immune system among others). This paper makes an excursus of the degeneracy of many systems and that degenracy is a the importance also for natural selection.

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This review describes the point that still need to be address in calorie restriction and many crucial points (such as the relation between calorie restrictin and cognitive decline, the negative effect of calorie restriction, the role of genetics and gender in calorie restriction response) to consider in future studies.

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This paper investigates commonalities between physiologic events that occured during hibernation and under calorie restriction.

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This Review describes not only the role of pro-inflammatory mechanisms in ageing but also the importance of the balance with anti-inflammatory factors (called anti-inflammaging) in healhy aging.

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10) Vanhooren, V. et al. N-glycomic changes in serum proteins during human aging. Rejuvenation Res 10, 521-531a (2007).

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This Review described the data relevant to possible involvement of the gut bacteria in the regulation of host appetite, integrating the energy status of both the host and its gut microbiota

12) Thaiss, C. A. et al. Transkingdom Control of Microbiota Diurnal Oscillations Promotes Metabolic Homeostasis. Cell 159, 514–529 (2014).

This paper present the first example of how how a symbiotic community may synchronize its interdependent physiologic activities to the geophysical clock promoting homeostasis. Moreover it reveals that feeding rhythms direct microbiota diurnal oscillations and how external intervention (such as chronic jet lag) may lead to dysbiosis.

13) Zhang, Q. et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature 464, 104–107 (2010).

This paper described in details the consequences of the evolutionarily conserved similarities between DAMPs and bacterial PAMPs and their role in inflammation