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Nutrition and Inflammation: are centenarians calorie-restricted like individuals?

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Running title: Are 100+ calorie-restricted like people?

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Abstract

Individuals capable of reaching the extreme limit of human life such as centenarians are characterized by an exceptionally healthy phenotype, *i.e.* low number of diseases, low blood pressure, optimal metabolic and endocrine parameters, gut microbiota with increased diversity, and are epigenetically younger than their chronological age. We present data suggesting that such a remarkable phenotype is largely similar to that found in calorie restricted adults. Centenarian's interviews and historical data on nutritional and lifestyle habits of Italians in the last century suggest that centenarians lived in an environment which was "non-obesogenic" when they were children and adults but at the same time did not favour malnutrition. Centenarians appears to be creatures of habit and we argue that the regularity in meals timing favoured their maintenance of circadian rhythms, including sleep. Finally we argue that the centenarian's chronic inflammatory status we dubbed inflammaging is peculiar, likely adaptive and less detrimental.

1. Longevity, Geroscience and Inflammaging

While aging has been studied since long time it is only recently that longevity attracted the interest of scientists. Ilya Metchnikoff pioneered this topic, as many others, and, in 1908, published a famous book entitled "The Prolongation of Life: Optimistic Studies"(81). In this book, Metchnikoff explicitly mentioned the "centenarians" and suggested that a peculiar nutritional habit, *i.e.* that of eating "yahourth" and thus combating the putrefaction which occurs in the gut, was likely a main reason why they were able to reach such a remarkable age. A main interest of Metchnikoff which gained him the 1908 Nobel prize for Physiology and Medicine was the cell type he discovered and dubbed "phagocyte", we usually name macrophage. Metchnikoff hypothesized the presence of a physiological inflammatory status where macrophage play a crucial role, i.e. a condition to efficiently combat infectious diseases and microbial challenge (124).

As usual, in our scientific activity we mount on the shoulders of giants, and Metchnikoff is one of them as for the first time he investigated and connected tree topics, i.e. longevity, innate immunity and nutrition/gut microbiota (GM) that were separately addressed and remained separated until few years ago. When we started our studies on human longevity the data on centenarians were very few and anecdotal, and the role of nutrition and GM, anticipated by Metchnikoff, had remained neglected and unexplored. In this review we will illustrate how the most recent data fit and extend the original Metchnikoff's vision.

The study of aging and, more recently, the study of longevity and chronic age-related diseases followed parallel tracks, and the connections among them were few and sporadic. A main reason is that the study of aging in the last thirty years has been mainly focused on tractable animal models such as yeast, worms and flies where knowledge of the age-related pathology, was (and still is) quite poor. In this scenario, mice are much better because murine pathology is a well-developed field, but the common use of few inbred strains and the artificial laboratory conditions - so different from human anthropological and cultural context – raise doubts about the possibility to easily translate the results obtained in murine models to humans. These considerations are reinforced

taking into account the differences between humans and mice regarding genetics, immunology, nutrition/metabolism, among others. Accordingly, we surmise that the study of the relationship between nutrition and aging/longevity in humans represents a unique opportunity to disentangle such a complex connection and to let emerge topics and specific targets that can eventually be thoroughly and mechanistically studied in mice and other animal models. As we will see in the next sections, this is for example the case of the epigenetic clock (55) and of the change occurring with age in the gut microbiota (GM) which has been firstly described in humans (9) and later confirmed and extended in mice (43).

To fill the gap between longevity, innate immunity and nutrition/GM we will adopt the conceptual framework of the new Geroscience whose starting assumptions are the following: i) aging is the major single risk factor for the development of most if not all major age-related chronic diseases such as neurodegeneration, cancer, metabolic and cardiovascular diseases; ii) aging and age-related diseases share the same, basic molecular mechanisms, such as inflammation, accumulation of macromolecular damage, adaptation to molecular and psychological stressors, epigenetic changes, metabolic dysfunction, alteration of proteostasis and defective stem cell function (62). These mechanisms are highly conserved in evolution, and this is the reason why animal models are so informative, with the above-mentioned limitations. What is even more important is that these basic mechanisms do not work separately but are highly interconnected and forms complex networks where each one has a different – more or less important - role and weight in the rate of aging and in the different chronic age-associated diseases. Thus, the distinction between aging and age-related diseases becomes less clear-cut than previously thought, and the novel message is that we have to combat aging in order to combat the age-related diseases altogether and not one by one, as it occurs at present, owing to the exponential increase of medical specialties.

The variety of external and internal stressors, i.e. pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), respectively, that humans are exposed to lifelong elicit inflammatory stimuli,) sensed by a few, evolutionary highly conserved sensors (Toll-

like Receptors, TLRs; NOD-like receptors, NLRs; Rig-like receptors, RLRs, cGAS-STING), which in turn activate a limited number of molecular cascades, eventually resulting in the release of high levels of pro-inflammatory mediators whose role is to neutralize "damages" and to repair the involved tissues. Within this physiological scenario crucial for survival, increasing post-reproductive age, is characterized by an increase of PAMPs and DAMPs inflammatory stimuli, concomitantly with a decreased efficiency of the mechanisms devoted to their neutralization and disposal. The net result is a progressive increase of such physiological "inflammatory tone" that can reach a threshold over which a variety of pathological outcomes can ensue. Such a situation has been conceptualized as "inflammaging" (36). Inflammaging encompasses the peculiar low-grade, chronic, and "sterile" inflammatory state which characterizes old age (36) and is believed to substantially contribute to the progression of the aging process and the pathogenesis of many, if not all, age-associated diseases (37). In particular, senescent cells accumulating with age in many tissue, secrete pro-inflammatory mediators (Senescence Associated Secretory Phenotype, SASP) and fuel aging and age-related diseases by spreading the senescent phenotype to neighboring cells ("senescence by senescence") (37). Another mechanism fueling inflammaging involves mitochondria that, when damaged, release mitochondrial DAMPs, i.e. cardiolipin and circulating mitochondrial DNA (mtDNA). mtDNA shares evolutionarily conserved features with bacterial PAMPs and is able to activate innate immunity and induce the production of pro-inflammatory cytokines (103). We recently argued that the most common inflammatory stimuli fueling inflammaging and capable of activating macrophages and innate immunity are endogenously produced by cells, organs and systems, globally indicated as "garbage" (cell debris, misplaced molecules) (39). Finally, the GM and other microbial constituent of the human body are able to regulate host-pathogen balance and to produce systemic pro-inflammatory stimuli. The lifelong antigenic load represented by foods and bacteria/bacterial products leads to a profound remodeling of the GM and these changes are emerging as a driving force of the functional homeostasis of the immune system and as an important source of inflammatory stimuli during aging (8, 9).

Further, inflammaging appears to be more complex than originally thought, as the pro-inflammatory side of the phenomenon is accompanied by a concomitant modulation of anti-inflammatory responses (38). Inflammation is an ancestral, very efficient mechanism to maintain physiological homeostasis but owing to its potential destructive power, has to be accurately and precisely down-regulated. The most recent data suggest that impaired resolution of inflammation likely plays a major role in inflammaging and its deleterious effects (146). It is also emerging that inflammaging and immunosenescence, are basically adaptive mechanisms occurring lifelong, within a more general remodeling of the body that occurs with age at molecular, cellular and systemic levels as anticipated by the remodeling theory of aging (42). Accordingly, only taking into account the entire/integrated "immunobiography" of each individual it is possible to evaluate the biological role (positive or negative) played by inflammaging (41).

Thus, the understanding of the inflammaging process requires a lifetime perspective capable of integrating the lifelong series of the above-described sources of inflammatory stimuli, including those related to nutritional habits and derived from the GM specifically addressed in the present review.

2. Centenarians as a model of Longevity and Healthy Aging

2.1 Longevity as a recent, historical and dynamic phenomenon

H. sapiens appeared on the stage about 300,000 years ago and until about a century ago life expectancy was about 50-55 years in advanced countries, and did not change much from hunter gatherers until the XX century. Then a demographic revolution started, first in the developed countries but spreading soon worldwide. Life expectancy started to grow at about three months per year, and average life expectancy at birth in leading countries is at present more than 87 years for women and about 84 years for men (129). Thus, for hundreds of millennia longevity was a very rare event, and extreme longevity likely even more rare if it existed at all, apart from few exceptions that must be carefully investigated and validated. When we started the studies on centenarians in Italy at about 1990 the centenarians were born at the end of XIX century and were about 3000, while at

present (2017) after two decades they are born at the beginning of XX century and they are 18765 (of which 3000 are men), and are born at the beginning of XX century (57). Thus, from a demographic point of view, extreme longevity is a highly dynamic phenomenon, and the high number of centenarians present worldwide (about 434,000) (130) has to be considered a recent, largely unpredicted demographic phenomenon. Centenarians undergo rapid changes not only regarding their number but also their phenotype, as they belong to different cohorts and spent their long life in a rapidly changing world. Accordingly, we can predict that the centenarians of the future will be different from the one we are studying now.

2.2 Phenotype and health status of Centenarians

Centenarians are living 20–30 years longer than members of the same birth cohort and are considered the best example of successful aging given that they reached the extreme limit of human life span largely escaping, postponing or surviving to the major age-related diseases. In the last 20 years, the exceptional phenotype of centenarians has attracted the attention of a number of scientists all over the world, shedding light on basic mechanisms of aging and longevity in humans. Table 1 shows the results of the most representative studies on centenarians conducted on populations different for geography, lifestyle, nutrition and genetics, and their main phenotypic characteristics may be summarized as follow:

i) a quite good mental status, with a relatively non-existent anxiety and depression and a good self-reported quality of life (109); ii) an incidence of chronic illness (CVD, BPCO, hypertension, renal disease, stroke, malignancy, hypercholesterolemia and diabetes) lower than in old people (47, 63, 108, 113, 150); iii) a better cardiovascular risk profile with lower levels of triglycerides, total cholesterol and LDL cholesterol and a preserved glucose tolerance and insulin sensitivity with respect to elderly controls (1, 4, 10, 99, 100, 120); iv) presence of typical signs of inflammaging without most of its deleterious consequences and presence of a complex/peculiar balancing between pro- and anti-inflammatory factors (38, 94). Centenarians presented an augmented plasma levels of inflammatory molecules such as interleukin (IL)-6, interleukin (IL)-18, interleukin (IL)-15, C-

Reactive protein (CRP), fibrinogen, Von Willebrand factor, and leukotrienes(1, 12, 18, 19, 46, 50, 73, 120), but this was counterbalanced by a concomitant large amount of anti-inflammatory molecules (*i.e.* adiponectin, Transforming Growth Factor (TGF)-β1, interleukin (IL)-1 receptor antagonist (IL-1RA), cortisol, anti-inflammatory arachidonic acid compounds) (4, 10, 18, 48, 49); v) lower levels of serum IGF-I, confirming data on animals model where the down-regulation of IGF-I/insulin signaling significantly extends survival (31, 137, 138); vi) significantly higher serum TSH concentrations compared to younger controls (2) - supporting previous observations showing that it increases progressively with age – and lower serum free T3 levels than elderly controls (75), without any increase of serum thyroid autoantibodies (74). The age-associated increase of TSH levels and decrease of total and free T3 (FT3) concentrations were also observed in a recent study on Chinese centenarian's families confirming that a decreased thyroid function, and thus a lower basal metabolic rate and a reduction of oxidative stress, are peculiar traits of longevity (53); vii) signs of hypercoagulability (heightened coagulation enzyme activity, plasma levels of the activation peptides of prothrombin, factor IX, factor X, and thrombin-antithrombin complexes, enhanced formation of fibrin) which paradoxically seem to be compatible with health and longevity, being not accompanied by an augmented risk of arterial or venous thrombosis (73); viii) peculiar immune profile characterized by a well preserved complement (classical and alternative pathway) system (7); an increase in peripheral blood of $CD28^-$ T cells with a phenotype compatible with memory cells (up-regulated CD2 and CD11a; CD62L absent) predominantly among cytotoxic $CD8^+$ T cells, suggesting that they may constitute armed effector cells toward pathogens of intracellular origin (27, 28); a strong decrease of naive $CD95^-$ T cells capable of mounting responses versus novel pathogens; a residual thymic function and/or the presence of life-long naïve T cells (93).

3. Nutrition, gut microbiota and life style

3.1 Nutritional habits of centenarians

Longevity is a complex and multifactorial trait resulting from an intriguing combination of 'Nature' and 'Nurture', i.e. the unique reciprocal interaction between environmental, genetic, epigenetic and

stochastic factors, each contributing to the overall phenotype. Centenarians are the final result of a number of biological processes that exert their effects lifelong, from birth (and even *in utero*) until the extreme limits of human life. Accordingly, the concept of "immunobiography" has been suggested in order to comprehensively cover all the stimuli that have impinged lifelong on the immune system and contributed to inflammaging (41). Thus, the study of centenarians represents a sort of 'historical probing' that allows to trace the above-mentioned complexity (96). As far as we know, no longitudinal dataset is at present available regarding nutrition and other environmental lifestyle/stimuli that centenarians have followed or have been exposed to lifelong. However, we can fill this gap by using historical data on nutritional and lifestyle habits of people of the same cohorts, social-economic status and education of centenarians. In this review we will refer to data available in Italy which represent a reliable proxy to re-construct the nutritional and social environment of centenarians which underwent profound changes during the last century and their life. This methodology can be applied to other populations and geographical/historical environments (148).

The cohorts of centenarians analyzed in Italian studies were born from the end of 1800 to the beginning of the 1900. In particular, those belonging to the cohorts 1899-1909, had on average a low grade of education (6.5 ± 4.9 years) and most of them (65%) did not go beyond the low secondary school (8 years of education). Accordingly, most of them were housewives, sellers, farmers, fishermen, artisans, workers, among other elementary occupations, while managers, self-employees and clerks overall were less than 25%. An historical focus on the social, economic and nutritional situation of Italy during the last century is reported in Box 1. Briefly, Italian centenarians lived in a transition period, when deep social and economic changes occurred, as they survived two World Wars and years of considerable deprivation. The most important characteristic of Italian centenarian's nutrition is the high heterogeneity among them and in the different periods of their life (Table 2). However, some common features can be identified and summarized as follow: i) for most of their life they followed a pro-vegetarian diet rich of vegetables and legumes, eggs and cheese, and relatively poor of meat, highly overlapped with a Mediterranean diet and lifestyle

(MedDiet) (76), complemented by an everyday active life until late (walking, bicycle, domestic and/or agricultural and/or factory work). Centenarians and their family members report that centenarians were used to eat small portions of carefully and slowly prepared meals, distributed regularly at rather fixed hours of the day, usually consumed together with their family. For most of their life and especially during the first forty-fifty years, the ingredients of the food consumed by centenarians were local and fresh, and respected the seasonality because of the difficulty of storing (lack of refrigerators) and transporting food. Thus, food was quite different (e.g. no massive chemical treatment for vegetables and no antibiotics for animals, among others) and more rich in fibers than that of today. Even more important, the great majority of centenarians are remarkably creatures of habit and have been extremely methodical throughout their lives, especially regarding meals timing and recipes. Such regular attitudes and meal's timing, which are strong cultural and anthropological habits shared by their community, continued even after the great nutritional changes occurred in Italy in the last decades, thus largely preserving the centenarians from the disorderly consumption of nutrient rich, high calorie foods which became available in the second part of their life.

3.2 Mediterranean Diet as healthy aging and longevity strategy

Diet and nutrition represent pervasive mechanism able to finely modulate the phenotype lifelong and the pro- and anti-inflammaging balance (13). The heterogeneity in dietary and nutritional history and status of centenarians worldwide suggests that the dietary patterns promoting exceptional longevity may be quite different across time and space. As mentioned before, Italian centenarians for most of their life likely followed a nutritional/lifestyle pattern close to MedDiet (76, 95). A plethora of observational studies and clinical trials concordantly show that MedDiet prevents morbidity and mortality (64). MedDiet is characterized by consistent intake of vegetables, fruits, nuts and legumes, whole grains, fish (especially marine species) and extra-virgin olive oil, and moderate consumption of eggs, dairy products, lean meats, and red wine. MedDiet recommends a reduction of saturated fats (butter and other animal fats), red meat, refined carbohydrates, and

sweets. On the whole, MedDiet is a well-balanced diet providing an equilibrated mix of nutrients with antioxidant, anti-inflammatory, and prebiotic effects (76). The consumption of low glycemic index carbohydrates (vegetables, fruit, whole grains and legumes) reduces the increase of postprandial glycaemia and maintains insulin secretion under control. The abundance of dietary fibers (β-glucans, arabinoxylans, galactomannans, pectins) contributes to satiety and control of body weight, decreases systemic inflammation and have a central role in the maintenance of gut health, by providing selective substrate for "health-promoting bacteria" such as *Bifidobacterium* and *Lactobacillum*. Frequent consumption of marine fish provides high levels of ω3 polyunsaturated fatty acids (PUFA) competing with ω6 PUFAs for the same series of enzymes, reducing the production of arachidonic acid–derived pro-inflammatory eicosanoids (prostaglandin E2, leukotriene B4, thromboxane 2-series), with a chemotactic and pro-coagulant action, and increases the synthesis of anti-inflammatory eicosanoids (prostaglandin E3, leukotriene B5, and thromboxane 3-series) with immunomodulatory effects (18, 88, 141). On the whole, MedDiet is capable of fine tuning the balance between pro- and anti-inflammaging, delaying the detrimental effects of inflammaging and the onset of chronic age-related diseases (76, 95).

In a recent review, our group conceptualized the MedDiet as a form of chronic hormetic stress, similar to what has been proposed regarding calorie restriction (CR), the most thoroughly studied nutritional intervention able to increase lifespan of different organisms, as well as physical activity (76). Hormesis is the adaptive, nonmonotonic, biphasic dose-response relation following an initial disruption in homeostasis. The MedDiet contains compounds (resveratrol, quercetin, olive oil secorroids, phenolic antioxidants, terpenoids, carotenoids, and allium derived sulfur compounds to mention a few) collectively dubbed "hormetins" (106), able to stimulate/upregulate a variety of cellular and molecular defense and maintenance pathways (nuclear factor erythroid 2, Nrf2; nuclear factor kappa-light-chain-enhancer of activated B cells, NF-κB; mammalian target of rapamycin, mTOR; and sirtuins) involved in and mimicking CR (76, 97).

3.3 The peculiar gut microbiota (GM) of centenarians

The study of the GM of exceptionally long-lived individuals provides insights into how this symbiotic microbial community successfully adapts all along the lifespan to the progressive age-related environmental (lifestyle, diet, etc.) and endogenous changes, and promotes healthy survival by contributing to the maintenance of metabolic and immunological homeostasis and promoting survival (8). The comparison of GM among young adults, elderly, and centenarians has shown that the trajectory of the modifications in the composition and diversity of the gut ecosystem do not follow a linear trajectory with age, remaining highly stable between young adults and seventy year-olds and markedly changing in the last decades of life (9). In centenarians, after 100 years of symbiotic association with the human host, the GM displays a profound and adaptive remodeling (114). Bacteroidetes and Firmicutes still dominate the GM of centenarians as in adult and elderly individuals, but Firmicutes subgroups go through specific changes with a decrease in the contribution of *Clostridium cluster XIVa*, an increase of Bacillus species, and a rearrangement of the *Clostridium cluster IV* composition (9). The GM of centenarians is enriched in facultative anaerobe bacteria mostly belonging to Proteobacteria which have been redefined as ‘‘pathobionts’’ because, in some circumstances, *e.g.* inflammation, they may escape surveillance, prevail over mutualistic symbionts and induce pathological conditions (112). GM shapes intestinal immune responses during health and disease, and the age-related remodeling of GM may contribute to systemic inflammaging, which in turn can, directly or indirectly affect the composition of GM in a sort of self-sustaining loop. Indeed, the changes in GM profile observed in centenarians (enrichment in Proteobacteria and a decrease in butyrate-producing bacteria) correlate with a systemic increase in pro-inflammatory cytokines (IL-6 and IL-8) (9).

A phylogenetic GM analysis of the human microbiota trajectory of a consistent number of Italian young, elderly and extremely long-lived subjects (centenarians and semi-supercentenarians, *i.e.* persons who reach the age of 105 years) ranging from 22 to 109 years of age, showed that the core GM comprised of dominant symbiotic bacterial taxa (Ruminococcaceae, Lachnospiraceae, Bacterodaceae) loses diversity and relative abundance with age. However, in extreme longevity this

shrinkage is counterbalanced by an increase in longevity-adapted and possibly health-promoting subdominant species (*Akkermansia, Bifidobacterium, Christensenellaceae*) as well as in their co-occurrence network. As a consequence, unexpectedly, an increased diversity in the composition of GM species composition is observed in centenarians and semi-supercentenarians GM, at variance with most if not all diseases characterized by a decreased GM diversity (8). This major characteristics is not peculiar to Italian centenarians but has been reported also in Chinese and Japanese centenarians, despite the genetic, lifestyle and dietary differences with Italian centenarians, suggesting that it is a remarkable signature of longevity per se and a key health indicator (114).

3.4 Do nutritional habits impinge upon the maintenance of circadian rhythms in centenarians?

Circadian rhythms, metabolism, and nutrition are intimately linked (59). Mammalian circadian rhythms are driven by a master clock, within the suprachiasmatic nuclei (SCN) of the hypothalamus and is mainly entrained by light signals and transduced by specialized photoreceptors present in the retina (25). In addition, peripheral clocks are located throughout the body, in peripheral organs such as liver, intestine and heart (115), and their function contributes to regulate homeostasis and physiological responses (86).

The clock molecular machinery is characterized by a complex transcriptional–translational feedback loop that ensures 24-h oscillation in gene expression. The positive arm of the mammalian clock machinery is comprised of CLOCK and BMAL1, two transcriptional activators that heterodimerize and induce the expression of clock-controlled genes (*CCGs*). Cryptochromes (*Cry1*, *Cry2*) and Period genes (*Per1*, *Per2*, *Per3*) are CCGs encoding proteins that form the negative arm of the circadian machinery. PER and CRY proteins are classically thought to translocate into the nucleus to inhibit CLOCK:BMAL1-mediated transcription, thereby closing the negative feedback loop. Moreover, the nuclear related orphan receptors (RORs) and REV-ERB-α/β represent additional layers of circadian regulation through the control of Bmal1 rhythmicity (52). For the

circadian system to function optimally, individual clocks must be synchronized to one another and to the external environment. Abnormal circadian rhythms or defects in the synchronization of the pathways result in circadian misalignment or desynchrony, in turn associated with poor health and metabolic disorders (104). The SCN synchronize peripheral clocks through neuronal pathways, hormone rhythms, core body temperature, and behaviors such as the cycle of feeding and fasting (115). Photic cues are of primary importance for resetting human rhythms (69). However, in non-human species regularly timed non-photic cues, can regulate rhythms, *e.g*. temporal restriction of food availability resets the phase of rodent peripheral clocks (21, 79). Human studies have revealed that post-prandial responses are dependent on meal timing (60, 89), but little is known on the ability of meals per se to alter the timing of human circadian rhythms. It has been recently demonstrated that meal timing exerts a variable influence over human physiological rhythms, with notable changes occurring in glucose homeostasis (143). A 5-h delay in meal times induced a comparable delay in the phase of circadian plasma glucose rhythms, accompanied by a 1-h delay in the phase of PER2 rhythms in the white adipose tissue, but no change in markers of the SCN clock (melatonin, cortisol), rhythms of plasma insulin and triglyceride, or clock gene rhythms in whole blood (143). Overall, available data suggest that timed meal can have a strong impact on health outcomes. As mentioned above, centenarians had very regular meal timing due to personal lifestyles and cultural and social habits (Table 2), and we surmise that such regularity likely contributed to their overall good health status.

Recent evidence has shown that also GM displays circadian fluctuation, which is mainly driven by diurnal food intake, leading to rhythmic abundance of microbial metabolites (67, 127). The systemic oscillation of GM-derived metabolome reprograms the circadian transcriptome both locally and distally, thereby regulating host physiology such as metabolic function and drug detoxification (67, 126). Bacterial adherence to the epithelium shows temporal fluctuations, which also correlate to host transcriptional oscillations. Thus, the disruption of GM oscillatory activity as a result of antibiotic treatment or time disordered dietary intake leads to disorganization of host

rhythmicity (126), indicating that GM serves as a circadian organizer of peripheral clocks. This transcriptional reprogramming appears to function through nuclear receptors that occupy a pivotal position in the process of integrating GM-derived signals into the circadian network (92). Although the core clock machinery robustly oscillates independently of microbial effect, the expression pattern of canonical clock genes is influenced by GM presence and composition (51, 67). Altogether, the host–microbe interaction appears to be essential in keeping the host clock timed in an appropriate manner, in order to be integrated with fluctuating environmental signals. In turn, a functional clock impacts the time-of-day oscillations of the microbial structure. Because the commensal bacteria compete with the invading pathogens, the compositional oscillation of GM contributes to the circadian variation of host defense against invading pathogens. The circadian disruptions induced by modern lifestyles may lead to dysbiosis, which may predispose the host to metabolic disorders and inflammation (128).

Several bacterial components and metabolites have been shown to stimulate intestinal satiety pathways. Regular nutrient infusion into the colon stimulates immediate bacterial growth that lasts 20 min. Bacterial molecules and metabolites, whose production depends on bacterial growth phases, regulate intestinal release of satiety hormones, consequently systemic bacterial molecules directly activate central appetite pathways that might integrate the energy status of both the host and its GM. This short-term bacterial growth-linked modulation of intestinal satiety can be coupled with long-term regulation of appetite, controlled by the neuropeptidergic circuitry in the hypothalamus (29). The peculiar GM composition of centenarians may also contribute to the regulation of their appetite. The 24-hour sleep-wake cycle is one of the most prominent outputs of the circadian clock system. Circadian or sleep disturbance, i.e. misalignments between their phasing, can lead to different types of diseases, metabolic derangements, including accelerated aging (101) and increase of inflammation/inflammaging (56). Rhythmic behaviors fragment with age, suggesting that aging has an adverse effect on the circadian clock, and vice versa their alteration likely contributes to aging. The best example of such rhythmic behavior impairment is sleep, which often becomes less

consolidated as age progresses, possibly contributing to inflammaging (56). Insomnia symptoms have been related to advanced epigenetic age in women (16), at variance with semi-supercentenarians who have a younger epigenetic age (55). Although the SCN is relatively resistant to age at the level of the molecular clock, it undergoes significant age-related degradation at the network level.

In animal models (Drosophila) clock mutants period and timeless (homologs of mammalian Per and Cry) are less sensitive to the lifespan-extending effects of CR while CR strengthens circadian oscillations in peripheral tissues (61) suggesting that deterioration of circadian molecular clock shorten lifespan. In centenarians the quality and the quantity of a major circadian clock such as sleep, appear to be well preserved. It has been reported that 48 Italian (Calabrian) centenarians go to sleep early in the evening, have no problems in falling asleep, wake up early in the morning, take a nap in the afternoon and do not take pills before going to bed (119). Another study on a relatively large group of 180 centenarians from Rome demonstrated the existence of a positive correlation between sleep quality, survival and successful aging (123). Also the amplitude of the nocturnal peak and/or the persistence of a prevalent nocturnal secretion of melatonin is preserved in centenarians (72). Moreover, studies conducted in different populations such as Brazilians (78) and Chinese (17) confirmed that centenarians maintained strictly regular sleep-wake schedules and that cognitive impairment is associated with poor quality, longer sleep latency, and lower sleep efficiency percentage. These finding are in agreement with the interview we had with 6 centenarians (Table 2).

3.5 Are centenarians calorie restricted-like individuals?

CR is the most thoroughly studied nutritional intervention to modulate aging and increase healthspan and lifespan in a variety of animal models from the unicellular yeast to primates (77). Overall, the results suggest that the effect of CR is highly evolutionary conserved (22) and involves common pathways across taxa. Although the molecular mechanisms underpinning the effect of CR have not been fully understood, there is consensus that CR involves the down-regulation of insulin

and insulin-like signaling (IIS), as well as of the mTOR-S6 kinase pathway, the glucose signaling Ras-protein kinase A (PKA) and SIRT 1 activation (66), largely via activation of autophagy, stress defense mechanisms, and survival pathways while attenuating pro-inflammatory responses (6). It has been proposed that CR activates these longevity-promoting pathways by acting as a mild stressor that promotes hermetic responses (110). Major targets of CR are mitochondria, which undergo a mild functional impairment, and in turn counter-intuitively promote longevity by cross-talking with the nucleus and secreting a variety of mitokines (111). Indeed, mitochondria in primary fibroblasts from centenarians show a mild functional impairment (116), and FGF21, a master mitokine in metabolism, is increased in centenarian's plasma (125). Therefore, the data on centenarians fit experimental evidence that mitochondria with mild impairment promote longevity, being paradoxically more beneficial than perfectly working mitochondria (111).

Within this scenario, in order to answer the question whether centenarians are CR-like individuals, it is important to stress that CR is a very complex topic and that a number of variables, such strain/genetic background, feeding regimens, diet composition (protein versus carbohydrate versus fat; natural or purified ingredients) and its age of onset, as well laboratory differences, among others, can impinge upon the final outcome (133). Among the different types of macronutrient restriction, reduced intake of proteins and amino acids is the most effective pro-longevity regimen (83). In particular, the restriction of a single essential amino acid in a normal diet is able to increase the lifespan. A tryptophan-restricted diet capable of promoting longevity and reducing the age-dependent deterioration has mainly been explored for neurological benefits, due to the role of this amino acid in serotonin synthesis (66). Interestingly, tryptophan levels in serum of centenarians are lower than in younger subjects (18), and this finding is in agreement with an age-related amplified abundance of genes involved in the tryptophan metabolism pathway in GM (105). Modifications to the composition of GM across the lifespan may deeply affect the availability of tryptophan and the gut-brain axis during aging (114).

Moreover, recent data suggest that the timing distribution of meals (different types of fasting) can result in outcome mimicking classical CR (66). The point within the life cycle in which CR is initiated can deeply affect the outcomes, with differences across species, and is therefore critical to evaluate the results of CR in the literature. Overall the data in mice suggest that CR has to start early in life to prolong lifespan but healthspan can benefit (improvement of cognitive performances and motor skills) also when CR is started later on (117, 149). Genetics and sex are also critical variables, and they play a pivotal role in the modulation of the longevity- and health-promoting effects of CR (84). All the previously mentioned studies regard animal models, including mammals (mice), but what about primates? Two independent CR longitudinal studies on non-human primates (monkeys) were conducted at the National Institute of Aging in 1987 and at University of Wisconsin in 1989. Despite differences regarding feeding regimen, age of onset, diet composition, and genetic background of the monkeys, the two studies are concordant in suggesting that CR has beneficial effects on health span, but they differ regarding the capability to extend the lifespan, this difference likely being a consequence of the different age of onset of the two diet regimens (77).

The data on CR in humans are relatively scanty, and derive mostly by natural experiments in adult volunteers who followed CR regimens for several years, and from observations in populations that fits the CR paradigm, i.e. CR without malnutrition, such as Okinawan centenarians (147, 148). The older generation living in Okinawa, a small island in Japan, reported consumption of a reduced calorie but nutrient dense diet since their younger ages compared to age-matched elderly in other regions of the world including mainland Japan (147, 148). Compared to relevant reference elderly populations from around the world, the Okinawan elderly exhibited a better health status in terms of metabolic and cardiovascular risk markers as well as a lower prevalence of age-related diseases (148). It is also worthwhile to mention the Biosphere experiment of 1991 involving eight adults sealed inside a self-contained ecological space (Biosphere 2) who accidentally received a CR diet for almost 2 years. Improvement of blood pressure, hematological, biochemical and metabolic parameters were described (140). There is also evidence of positive effects of CR from a study of

voluntary practitioners, the CR Society members. These volunteers were consuming approximately 800 kcal fewer per day compared to age- and gender-matched subjects eating typical Western diets and had a significantly lower mean body fat mass, core body temperature, blood pressure, and triiodothyronine (T3) level (30). A notable NIH-sponsored controlled randomized study is CALERIE (Comprehensive Assessment of the Long- term Effects of Reducing Intake of Energy). Two studies were performed, the first was a 12 months of 30% CR involving a total of 130, 24–42 years old non obese and overweight subjects, and the second was a two years 25% CR involving a total of 220 healthy men and women aged 21-50 years old, with a BMI between 22-28 kg/m^2 (22).The main findings are summarized in Table 3, compared with data characteristic of the phenotype of centenarians.

On the whole, the data shown in Table 3 suggest that the phenotype of centenarians is remarkably similar to that observed in human adults volunteers who followed different CR regimens, even if the centenarians never purposely followed a specific CR regimen. Such a convergence between CR and longevity suggests the following consideration and hypothesis: i) CR appears to induce a healthy multi-parameter phenotype even in humans, even if CR studies until now have been conducted in relatively young people, i.e. healthy or over-weighted adults, ranging between 21 and 60 years of age, and lasted a maximum of two years. Thus, we do not know the possible long term effects of CR in humans, and particularly in the last decades of human life; ii) to this regard the centenarians can be assumed, *bona fide,* as an example of a nutritional habit followed lifelong, and also during the last decades of life, which attains results similar to those observed after a CR regimen; iii) the CR-like phenotype of centenarians is mirrored by their consistently younger DNA methylation age (on average 8.7 years younger than expected based on their chronological age) and a GM composition characterized by increased diversity, two characteristics that, as far as we know, have not been studied yet in CR humans. Recent studies suggest that CR mice have a younger epigenetic age, and that CR affects the gut microbiome (122, 142). It is also interesting to note that MedDiet favorably affects GM composition (71).

Within this scenario a major difference emerge between centenarians and CR adults, regarding several inflammatory parameters, which decrease after CR interventions and are high in centenarians (significantly higher than those found in younger elderly). However, in centenarians a variety of anti-inflammatory compounds are also high in plasma, suggesting that a peculiar balance/equilibrium between inflammaging and anti-inflammaging is reached. The measure of anti-inflammatory compounds (not only cytokines but also arachidonic acid cascade products, among others) has not been sufficiently investigated in CR humans. Moreover, as shown in Figure 1, the plasma levels of IL-6, named the "cytokine for geriatricians" (26), as the single most powerful predictor of morbidity and mortality in the elderly, loses its predictive power in centenarians. Recently it has been argued that inflammaging, being part together with immunosenescence, of the complex remodeling which occurs in the body of old people, might also be interpreted as a sign of positive/successful adaptation to the variety of stressors that humans are exposed to lifelong (44). Such a consideration could be particularly relevant for people like centenarians who reached extreme ages avoiding or postponing major age-related diseases, where we can hypothesize that a unique, integrated/comprehensive inflammatory equilibrium is reached.

4. Conclusions

The study of centenarians reveals important cues to longevity even regarding nutrition, a pillar to attain healthy aging and longevity (13). The lesson of centenarians can be summarized as follows:

i) Centenarians were physically active lifelong and even at advanced age, and most of them, and particularly men, are and were lean, while women have never been obese.

ii) Centenarians spent most of their early life in a non-obesogenic environment where the usual meal was relatively poor of meat and animal fat, and the portions were not large. Later, after the World War II, a nutritional transition occurred but those individuals who later became centenarians, as creature of habit, largely continued to follow their previous nutritional habits. Overall, centenarians avoided overnutrition and nutrient excess characteristic of the present

Western diet, which favor obesity and visceral fat deposition, metaflammation and inflammaging (35).

iii) Centenarian's are characterized by their regularity in meal timing. We surmise that such nutritional habit exerted profound effects on many other circadian rhythms of centenarian's body, including GM composition and sleep. Maintenance of circadian rhythms is considered a main characteristics/ prerequisite for attaining healthy aging and longevity.

iv) Centenarians have a peculiar composition/signature of GM, characterized by an increased diversity. This unique trait is likely the result of a lifelong adaptive process which might have played a major role in attaining longevity, owing to the cross-talk of GM with other organs of the body and its pervasive physiological effects.

v) The combination of the above-mentioned nutritional habits of centenarians can explain most of their peculiar phenotype, remarkably overlapping that of CR adults, despite centenarians never purposely followed a calorie restricted diet. At variance with CR adults, centenarians have a peculiar inflammaging, characterized by concomitant high plasma levels of pro- and anti-inflammatory cytokines, suggesting that an integrated, structured inflammatory process occurs (90), where high circulating levels of cytokines such IL-6 lose their predictive power regarding morbidity and mortality.

Altogether the nutritional habits of centenarians likely contributed substantially to their longevity and healthy aging of centenarians. However, many members of the same demographic cohort of the centenarians followed similar nutritional patterns but most of them did not attain 100 years of age. Thus, a major question is: do centenarians have other characteristics not shared with most of the members of the same cohort? The answer is probably YES, as suggested by the following circumstantial evidence: i) centenarians do have a specific genetics, even if this topic is quite difficult owing to the complex interaction between genetics, environment and individual lifestyle/habits (14, 23). All these "ingredients" of longevity can be different in different populations, and vary in time and space, being longevity a dynamic, context-dependent

phenomenon. The genetics of longevity cannot be addressed here and the reader is referred to (14, 23); ii) centenarians and their offspring have a peculiar metabolomics (18, 88) and epigenetic profile (55), two parameters which are considered a robust mirror of the gene-environment/lifestyle interaction. In particular, DNA methylation clock shows that centenarians and their offspring are consistently younger than their chronological age, suggesting that this trait runs in families (55). These epigenetic data fit and complement those showing that centenarians have a CR-like phenotype.

On the whole centenarians appear to have a younger biological age and a younger phenotype. The unique combination of peculiar genetics and epigenetics together with the peculiar nutritional habits and lifestyle, in a specific favorable environment (Figure 2) is likely the "secret" of centenarians. However, the complexity of human longevity deserves more studies on new cohorts of centenarians worldwide (40).

Box 1. Historical perspective on social, economic and nutritional situation in Italy over the XX° century

At the beginning of 1900, Italy was still an agricultural, peripheral and backward country. The vast majority of the families followed a subsistence and self-produced food consumption characterized by a nearly vegetarian diet lower in calories, fat and protein compared to the European context. Then, before the beginning of the First World War, the Italian economy started to take off with an increase of food consumption and an expansion of the range of foods in the diet of population including the subordinate classes. Products as sugar, meat, olive oil, wine and milk begun to appear on the table of laboring and farmer families. The First World War paradoxically accelerated this process. In cities, the consumption of equine and sheep meat increased, while in trenches, millions of peasants had the opportunity to taste, albeit in a dramatic context, meat, pasta, wheat bread, wine and coffee. These foods entered the collective heritage because they were included in the daily meal. The soldiers came in contact with several specialty foods of the domestic and regional tradition that families sent them to the front, which were exchanged and put together, so that thousands of young men were forced to leave their specific nutritional habits and to live side by side, comparing different cultural and food realities. Thus, an "Italian" diet pattern expanded to the lower social strata (87). Even if the intake of animal proteins and fats remained scarce, the First World War did not provoke significant recession in the nutrition of Italians due to the relatively low destructive intensity of the conflict, the growth of large industry and the acceleration of manufacturing and agricultural development (118). During the Fascist period (1922-1943) a significant reduction of the annual availability of the main foods of the Italian diet occurred (>5% for grains, 8% for fats, 14% for meat, >30% for sugar and fruit), and the average daily calorie intake decreased considerably (about 7.5%) due to the contraction of all essential dietary components (proteins, fats, carbohydrates). The propaganda of the Fascist regime promoted food sobriety (reuse of leftovers) and proposed bread as the symbol of the 'new man' of fascism, opposed to the American myths of consumerism. The regime provided national and economic alternatives to expensive imported products (e.g. karkadè instead of tea, barley or chicory infusion instead of coffee, fish instead of meat, rice instead of pasta, and vegetal margarine instead of butter). Thus, even if Italian nutrition at the time of Fascism was quite similar to a typical MedDiet, Italian situation was worse than in the early decades of 1900. Fascism, particularly for the lowest social classes, meant a significant deterioration of nutrition standards with a return to a diet composed mainly of carbohydrates with a further reduction of animal lipids and proteins (ISTAT, 1958). In addition, the disparities between North and South strengthened regarding the consumption of beef, milk, eggs, cheese, poultry and sugar (118). With World War II, the situation further worsened due to the growing inability of the Fascist regime in addressing the agricultural sector to sustain the troops and to ensure the urban population with minimal food availability. For their survival, farmers bypassed the public storage system and rationing and stimulated the illegal food market by contributing to the collapse of the organization devoted to food collection and distribution. Thus, all over the national territory, the reduction of calorie intake was combined with a qualitative impoverishment of the diet due to the depletion of animal proteins, fats and sugars. Even the consumption of bread, pasta, potatoes, rice, pulses and rye which were the main foods of the working classes and the main source of calories, collapsed during and immediately after the war. At the end of the World War II, Italian population was exhausted from the economic and social point of view, with heavily damaged productive and infrastructure systems and a dramatically impoverished society (5). In 1945 Italians welcomed the Allies with enthusiasm not only for the reasons of anti-fascism and democracy, but also in the hope that the United States would guarantee the end of this nutrition collapse which had been much more serious than the destruction of factories, transport and housing. The European Recovery Program (Marshall Plan) made available millions of dollars for food aid to Italy, like to other European countries, providing over 20% of daily average calories per inhabitant in post-war Europe, and was essential to increase the energy value of the daily diet (24). However, the Inquiry on Misery in Italy (1951) describes a country fallen again in its darkest past with 23.4% of the families (about 12 million Italians) living in miserable or disadvantaged conditions of absolute or relative poverty, i.e. four people per room, or in basement/caves/cabins, never or almost never eating meat, wine and sugar and having a minimum degree of education. The diet provided an absolute predominance of carbohydrates, low protein intake and very low fat. A stable and adequate access to pasta and bread was unwarrantable, and most families were in danger of malnutrition. Poverty was concentrated in Southern Italy, where more than 50% of the miserable and disadvantaged families lived. The difference regarding the average daily calorie intake between Northern Italy, characterized by industry, commerce and services, and Southern regions and the islands, still predominantly dependent on agriculture and fisheries, was over 450 kcal. Moreover, public assistance was concentrated in the North of the country and was rather absent in the Southern regions (11). The decade 1951-60 was the starting of the so called Italian economic “miracle”, characterized by a strong economic growth, and Italian nutrition reached the levels and standards of the advanced Western countries. This positive trend was

accompanied by a reduction in discrepancies in the quantitative and qualitative composition of diet among the Italian regions (24). Between 1951 and 1980s, the amount of food consumed by the families increased considerably and also the quality of the diet improved significantly. The progressive affirmation of dried pasta as national food present daily on the Italian's table stabilized the intake of cereals and, concomitantly, the consumption of beef, pork, poultry, vegetables, milk, sugar, olive oil, fresh and preserved fish exponentially raised. The maize, which had been the main component of the diet for Northern and Central Italy farmers, disappeared and the presence of lard, the poorest raw fat, pulses, potatoes and rice decreased significantly. What turned out was a combination between a European diet with high intake of animal proteins and a Mediterranean one with pasta, fruit and vegetables. In the 1980s a gradual reduction of the consumption of wheat, wine, sugar, a stabilization of meat intake and an increase of fruit and vegetables consumption occurred.

Starting from about 1960 a progressive ”Americanization" of lifestyles occurred and became popular particularly in adolescent and young adults (24). However, the Italian families have mostly maintained the habit to prepare daily meals in home kitchen thus preserving the traditional and cultural aspects of the Italian cuisine.

The stable accessibility to food and the improving of the quality of diet due to the daily consumption of animal protein combined with a significant extension of the range of food products entering the diet have led to the improvement of the living conditions of the Italian population. As a result, the mass malnutrition has disappeared, mean height has increased, infant mortality has drastically reduced to almost disappear, and average life has increased of almost 30 years since the early 1900s (58).

FIGURES

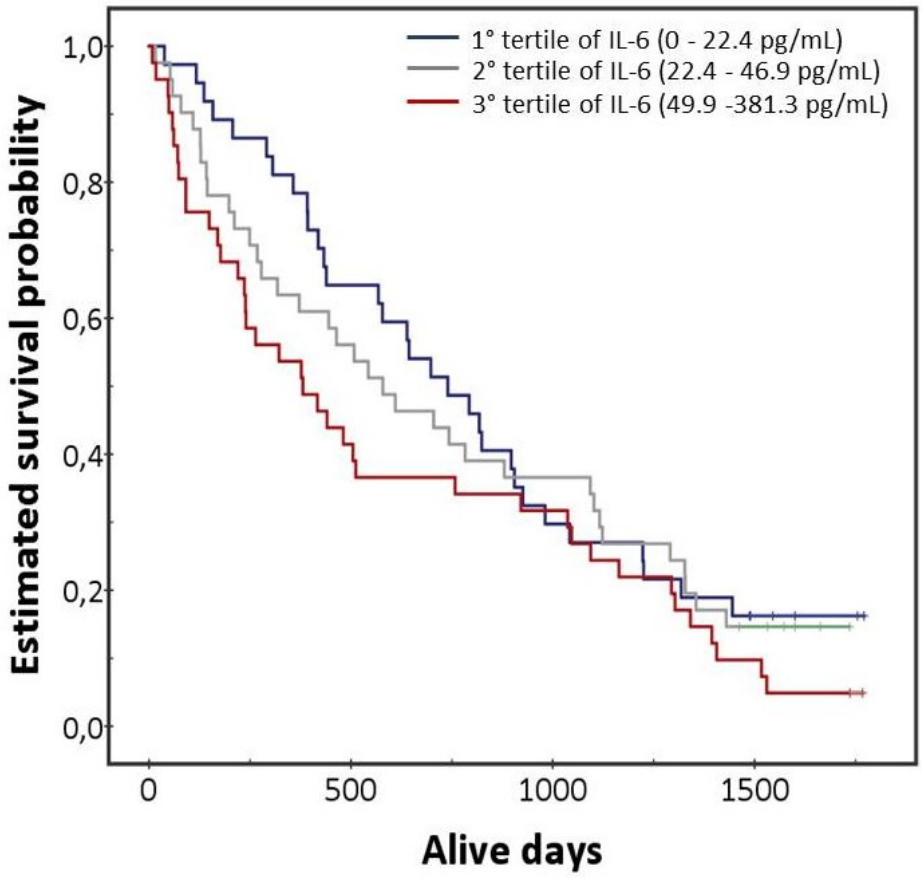

Figure 1. Kaplan–Meier survival curves displaying five-year all cause mortality in 119 Italian centenarians (27 men and 92 women) according to tertiles of IL-6 plasma levels.

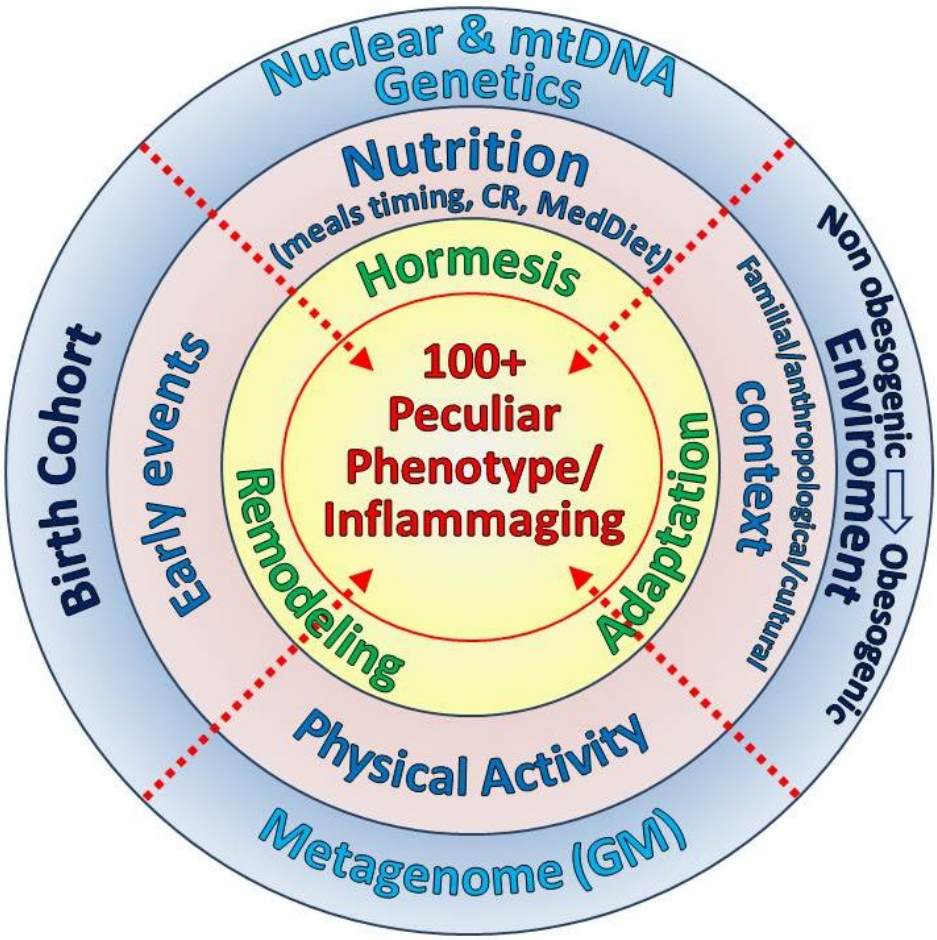

Figure 2. Centenarians are exceptional individuals because they live several decades longer than the members of the same demographic cohorts and escaped, postponed or survived most of the age-related diseases. Centenarians are epigenetically younger than their chronological age and are similar to people who followed CR regimens. Such an exceptional phenotype is the result of a unique lifestyle - characterized by nutritional habits such as lifelong moderate food consumption, diet largely similar to MedDiet and regularity in meals timing - combined with active life until extreme age, a peculiar GM and a particular genetic background (still largely unexplored). Longevity is historically- and context-dependent and thus the specific geographical, anthropological, familial, cultural and socio-economic environment(s) where the people who later became centenarians lived has a crucial importance.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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TABLES

Table 1. The exceptional phenotype of centenarians.

Phenotypic characteristics	Method	Population	Reference
Mental Status			
Relatively non-existent anxiety and depression. Good quality of life.	14-item Hospital Anxiety and Depression Scale (HADS). Quality of Life Scale.	Australian	(109)
Health Status & Diseases			
Lower incidence of chronic illness (CVD, BPCO, hypertension, end-stage renal disease, malignancy, diabetes) than octogenarians and nonagenarians	Medical examination	Male U.S. veterans	(63)
Low incidence of severe diseases (CVD, hip fracture, stroke, malignancy, diabetes, hypertension)	Medical examination	Swedish	(113)
Lower prevalence of hypertension than in the entire population of old people	Multiple measurements with a mercury sphygmomanometer	Polish	(150)
Low prevalence of hypertension	Multiple measurements with a mercury sphygmomanometer	Southern Italy (Calabria)	(47)
Lower prevalence of hypertension and hypercholesterolemia than general elderly population (≥75 years old)	Blood pressure: multiple measurements with Omron automated sphygmomanometer. Cholesterol: finger-prick blood sample	Australian	(108)
Better cardiovascular risk profile than middle-aged individuals	Medical examination	Polish	(4)
Metabolism, Hormones & Inflammation			
Lower levels of triglycerides, HDL-cholesterol, albumin and transferrin, lower BMI and higher serum C-Reactive protein and plasma IL-6 than elderly controls	Standard methods BMI=weight/(height)^2 Chemiluminescent enyme immunoassay	Japanese	(1)
Similar Lipoprotein (a) serum level respect to elderly controls	ELISA	Italian	(3)
Lower insulin resistance and preserved β-cell function	Insulin: radioimmunoassay; Glucose oxidase method; HOMA-IR	Italian	(99).
Preserved glucose tolerance and insulin action	Oral glucose tolerance test and euglycemic hyperinsulinemic glucose clamp	Italian	(100)
Lower glucose, alanine transaminase (ALT), total cholesterol and platelet, higher urea nitrogen than healthy old subjects	Standard methods	Italian	(68)
Higher serum concentration of all isoforms of adiponectin, lower fasting glucose, insulin, HOMA-IR, total cholesterol, LDL cholesterol and triglycerides than elderly subjects	ELISA and standards methods	Polish	(10)
Lower plasma concentrations of leptin and NPY and higher levels of adiponectin than elderly and obese subjects.	Radioimmunoassay (RIA)	Polish	(4)
Lower fasting plasma glucose, total cholesterol and hemoglobin and higher fibrinogen and CRP than adults.	Standard methods	Italian	(120)
Higher plasma levels of IL-6, TNF-α and sTNFR-II than octogenarians, adult and young subjects.	ELISA	Danish	(12)
Higher serum levels of IL-6 and lower	High sensitivity ELISA	Italian	(50)

serum levels of sIL-6R and sgp130 than 65-79 years old women.		women	
Higher serum levels of IL-18 and IL-18BP than adult and elderly controls and chronic ischemic syndrome patients.	ELISA	Italian	(45)
Elevated serum levels of sTNF-RI and sTNF-RII compared to old and young subjects. Mean sCD30 serum levels are four times those of younger groups.	ELISA	Italian	(49)
Lower serum level of IGF-I and IGF-II and higher insulin sensitivity than their offspring.	Chemiluminescence immunoassay (CLIA) and ELISA respectively	Italian	(138)
Higher IGF-I/IGFBP-3 molar ratio than aged subjects.	RIA	Italian	(98)
Higher serum TSH than younger controls	Chemiluminescent immunometric assay	Ashkenazi Jewish	(2)
No age-dependent increase in prevalence of serum thyroid autoantibodies	Passive hemagglutination	Italian	(74)
Lower levels of serum free T3 than elderly	Column adsorption chromatography and immunoassay respectively	Italian	(75)
Higher plasma levels of cortisol, ACTH and CRH than young subjects	ECLIA, IRMA and RIA respectively	Italian	(48)
Vitamin D deficiency, low serum calcium, hyperparathyroidism and osteopenia	Standard methods	Northern Italian	(102)
Decreased serum level of tryptophan and increased serum concentration of specific glycerophospholipids. Increased urine excretion of phenylacetylglutamine (PAG) and p-cresol sulfate (PCS)	Targeted liquid chromatography–mass spectrometry (LC-MS/MS) metabonomics Untargeted ^{1}H-NMR metabonomics	Northern Italian	(18)
Distinctive serum metabolic phenotype with unique changes in lipids biosynthesis (41 differently abundant phospho/sphingolipidsspecies with respect to elderly subjects).	MS/MS shot gun lipidomics ^{1}H NMR Spectroscopy	Northern Italian	(88)
Alteration of N-glycans in plasma and Igs fraction	DSA-FACE technology	Italian	(131, 132)
Typical N-glycan profile from plasma proteins: increased multi-branched and highly sialylated N-glycans as well as agalacto- and/or bisecting N-glycans and descreased biantennary N-glycans respect to aged and young controls	Liquid Chromatography/Multiple Stage Mass Spectrometry (LC/MSn)	Japanese (105+)	(85)
Immunology			
Maintaining of a reservoire of $CD45RA^{+}$ in $CD4^{+}$ (about 20%) and in $CD8^{+}$ (about 50%) T lymphocytes	Cytofluorimetric analysis	Italian	(20)
Higher percentages and absolute numbers of $CD28^{-}$ in $CD4^{+}$ and in $CD8^{+}$ T lymphocytes respect to elderly and young controls	Cytofluorimetric analysis	Italian	(27)
Lower naive $CD95^{-}$ T cells count respect to younger subjects	Cytofluorimetric analysis	Italian	(28)
High reactivity against human beta 2 glycoprotein I but no vascular events associated with anti-phospholipids syndrome	ELISA	Italian	(80)

Coagulation			
Well preserved complement (classical and alternative pathway) system	Functional assay	Italian	(7)
Increased Von Willebrand Factor in comparison to adult controls	ELISA	Italian	(19)
Higher plasma concentrations of fibrinogen and factor VIII than in controls but not elevation of other coagulation factors	Functional assay and ELISA	Italian	(73)
Epigenetics			
According the "epigenetic clock", centenarians are younger (8.6 years) than expected based on their chronological age.	Prediction method for biological age based on the DNA methylation levels of 353 CpGs	Italian	(55)

Table 2. Summary of 6 detailed interviews conducted with centenarians (born between 1914 to 1916) in an extraordinary intact cognitive and health status describing their familial situation, life experiences and body shape, physical activity, nutritional habits in young and adult age.

Gender, birth year, age at the interview	Family	Education and Occupation	Body shape	Physical activity	Nutrition	Meals timing and portions
			in young/adult age			
Male, 1915, 101 years old	Farmers, 7 siblings, living in the countryside. After the World War II, he moved to the city (Bologna).	5 years of education, primary school license. Farmer until he was 25, then soldier for 5 years during the World War II. After the end of the war, railway worker.	Thin	Daily walking	Pasta, white bread, vegetables, fruit, cheese, pulses, potatoes, eggs, sweets once a week, meat (pork, poultry and rabbit) 2-3 times a week, little red wine at meals. During the War, he suffered from hunger.	Very regular, 2 meals per day, early dinner
Female, 1914, 102 years old	Butcher (the father) and housewife (the mother), no siblings, living in the city (Bologna).	8 years of education, low secondary school license. Clerk in the family butchery.	Curvy	Daily walking or cycling	Pasta, meat (red meat until 20 years then only white meat), eggs, white bread, vegetables, beans, sweets once a week, fish once a week, milk, cheese, fruit.	Regular, 3/4 meals per day
Female, 1916, 100 years old	Grocery shop, 8 siblings, living in a village on the Apennines (900m). When she was 17 years old, she moved to the city (Rome and then Bologna).	5 years of education, primary school license. She took care of her younger siblings, she was maid in a family and then she left her job to take care of her disabled son.	Curvy	Daily walking for long distance, housework	In her infancy and adolescence: pasta, meat (pork, poultry), eggs, white bread potatoes, vegetables, pulses, nuts, fruit, cheese (cow and sheep). She suffered from hunger during her youth and the first years after she married.	Very regular, 3 meals per day, small portions
Male, 1913, 103 years old	Farmers, 3 siblings, living in the countryside. When he was 17 years old, he moved to the city (Bologna).	4 years of education. Artisan, mechanical, warehouse worker.	Thin	Daily walking or cycling for long distance, physical work	Pasta, white bread, milk, tomatoes, beans, eggs. Rarely: sweets, butter, cheese, poultry and pork meat.	Very regular, early dinner, 3 meals per day, small portions
Female, 1915, 101 years old	Orphan of father (dead during the First World War), living with mother (seamstress), grandparents and 2 sisters in a small village near the city (Bologna).	6 years of education, primary school license. Laborer for 20 years and then office worker for 15 years.	Curvy and strong	Daily cycling, housework	Pasta, milk, white bread, meat (pork and poultry), parmesan cheese, beans, butter, olive oil, vegetables, fruit and little white wine. Sweets once a week.	Very regular, 3 meals per day, small portions
Female, 1916, 101 years old	Farmer (mother) and carpenter (father), 5 siblings, living in the countryside, after wedding: 2 years in Germany and 4 years in Belgium with her husband (miner), then she moved to city.	5 years of education, primary school license. Farmer until wedding, then cook, greengrocer and maid in a family	Medium	Walking, physical work	Pasta, milk, white bread, little meat (pork and poultry), little cheese and few eggs, pulses, vegetables, little wine. Sweets once a week.	Very regular, 3 meals per day, small portions

Table 3. Comparison between inflammatory, metabolic, hormonal and phenotypical adaptations observed in calorie restricted humans and phenotypical characteristics of centenarians.

		Calorie restricted humans (21-60 years old)	**Centenarians (100+)**	References
Concordant				
Glucose metabolism	Glucose	↓	↓	(4, 10, 31, 33, 54, 68, 70, 99, 100, 107, 120, 135, 136, 138, 140)
	Insulin	↓	↓	
	Insulin sensitivity	↑	↑	
Blood pressure	Systolic blood pressure	↓	↓	(47, 63, 107, 108, 113, 134, 138, 140, 150)
	Diastolic blood pressure	↓	↓	
Thyroid	T3	↓	↓	(54, 65, 75, 136, 140)
Lipid profile	Total cholesterol	↓	↓	(1, 10, 33, 107, 120, 138–140)
	LDL cholesterol	↓	↓	
	Triglycerides	↓	↓	
Body composition	BMI	↓	↓	(1, 33, 100, 102, 107, 135, 136, 138–140, 144, 145)
	Fat free mass	↓	↓	
	Bone mineral density	↓	↓	
Metabolism	Cortisol	=↑	↑	(4, 10, 18, 65, 121, 135, 140, 144)
	Adiponectin	↑	↑	
	Leptin	↓	↓	
	Tryptophan	↓	↓	
Discordant				
Metabolism	Vitamin D	↑	↓	(1, 32, 34, 98, 102, 135, 138)
	HDL cholesterol	=↑	↓	
	IGF-I	=	↓	
	IGFBP-3	=	↓	
	IGF-I/IGFBP-3 ratio	=	=↑	
Inflammation	CRP	↓	↑	(1, 12, 15, 31, 33, 45, 49, 50, 82, 91, 102, 107, 120, 144)
	IL-6	↓	↑	
	TNF-α	↓	↑	
	TGF-β1	=↓	↑	