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Nutrition and Inflammation: Are Centenarians Similar to Individuals on Calorie-Restricted Diets?

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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—that is, a low number of diseases, low blood pressure, optimal metabolic and endocrine parameters, and increased diversity in the gut microbiota—and they are epigenetically younger than their chronological age. We present data suggesting that such a remarkable phenotype is largely similar to that found in adults following a calorie-restricted diet. Interviews with centenarians and historical data on the nutritional and lifestyle habits of Italians during the twentieth century suggest that as children and into adulthood, centenarians lived in an environment that was nonobesogenic, but at the same time the environment did not produce malnutrition. Centenarians appear to be creatures of habit, and we argue that their habit of eating meals at the same time each day favored the maintenance of circadian rhythms, including their sleep cycle. Finally, we argue that centenarians' chronic inflammatory status, which we dubbed inflammaging, is peculiar, likely adaptive, and less detrimental than in younger people.

Contents

1. LONGEVITY, GEROSCIENCE, AND INFLAMMAGING

While aging has been studied for many years, it is only recently that longevity has attracted the interest of scientists. Ilya Metchnikoff (82) pioneered studies in this area, and in 1908 published *The Prolongation of Life: Optimistic Studies*. In this book, Metchnikoff explicitly mentioned centenarians and suggested that a peculiar nutritional habit—that is, eating "yahourth" and thus combating the putrefaction that occurs in the gut—was likely one of the main reasons why they were able to reach such a remarkable age. Another of Metchnikoff's interests was the cell type he discovered and dubbed the phagocyte, which we usually call the macrophage, for which he was awarded the 1908 Nobel prize for Physiology and Medicine. Metchnikoff hypothesized the presence of a physiological inflammatory status in which macrophages have a crucial role—that is, they efficiently combat infectious diseases and microbial challenges (125).

As usual, in our scientific activity we stand on the shoulders of giants and Metchnikoff is one of them because he was the first to investigate and connect three topics that had been separately addressed and remained separated until about 10 years ago: longevity, innate immunity, and nutrition and gut microbiota (GM). When we started our studies on human longevity, the data on centenarians were sparse and anecdotal, and the role of nutrition and the GM, anticipated by Metchnikoff, had remained neglected and unexplored. In this review, we illustrate how the most recent data fit into and extend Metchnikoff's original vision.

The study of aging and, more recently, the study of longevity and chronic age-related diseases had been following parallel tracks, and the connections among them were few and sporadic. One reason for this is that during the past 30 years the study of aging has been mainly focused on tractable animal models, such as yeast, worms, and flies, for which knowledge of age-related pathology is quite poor. However, mice are much better because murine pathology is a well-developed field, but the common use of a few inbred strains and the artificial laboratory conditions—so different from the human anthropological and cultural contexts—raise doubts about the possibility of translating the results obtained in murine models to humans. These considerations are reinforced when taking into account the differences between humans and mice in terms of genetics,

immunology, and nutrition and metabolism, among others. Accordingly, we surmise that studying the relationship between nutrition and aging and longevity in humans represents a unique opportunity to disentangle complex connections and to foster the emergence of topics and specific targets that may eventually be thoroughly and mechanistically studied in mice and other animal models. As we show in the following sections, this is true in the case of the epigenetic clock (55) and of the changes that occur with age in the GM, which were first described in humans (9) and later confirmed in mice, in which the knowledge was extended (43).

To fill the gaps between longevity, innate immunity, and nutrition and the GM, we have adopted the conceptual framework of the new field of geroscience, for which the starting assumptions are the following: (*a*) Aging is the most important single risk factor for the development of most, if not all, major age-related chronic diseases, such as neurodegeneration, cancer, and metabolic and cardiovascular diseases; and (*b*) aging and age-related diseases share the same basic molecular mechanisms, such as inflammation, the accumulation of macromolecular damage, adaptation to molecular and psychological stressors, epigenetic changes, metabolic dysfunction, alteration of proteostasis, and defective stem cell function (63). These mechanisms are highly evolutionarily conserved, and this is 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 form complex networks in which 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 all age-related diseases together and not combat them one by one, as they are addressed at present, owing to the exponential increase in medical specialties.

The variety of external and internal stressors—that is, pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), respectively—that humans are exposed to throughout their life elicit inflammatory stimuli that are sensed by a few evolutionarily highly conserved sensors (e.g., Toll-like receptors, NOD-like receptors, Rig-like receptors, and the cGAS–STING pathway), which, in turn, activate a limited number of molecular cascades, eventually resulting in the release of high levels of proinflammatory mediators whose role is to neutralize damage and to repair the involved tissues. Within this physiological scenario that is crucial for survival, increasing postreproductive age is characterized by increased PAMP and DAMP inflammatory stimuli concomitant with a decreased efficiency in the mechanisms devoted to their neutralization and disposal. The net result is a progressive increase in a physiological inflammatory tone that can reach a threshold that, once crossed, ensures a variety of pathological outcomes ensue. Such a situation has been conceptualized as inflammaging (36). Inflammaging encompasses the peculiar, low-grade, chronic, and sterile inflammatory state that 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 that accumulate with age in many tissues secrete proinflammatory mediators (which is known as the senescence-associated secretory phenotype) and fuel aging and age-related diseases by spreading the senescent phenotype to neighboring cells (known as senescence by senescence) (37). Another mechanism fueling inflammaging involves mitochondria that, when damaged, release mitochondrial DAMPs—that is, cardiolipin and circulating mitochondrial DNA. Mitochondrial DNA shares evolutionarily conserved features with bacterial PAMPs and is able to activate innate immunity and induce the production of proinflammatory cytokines (104). One year ago, we 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 termed garbage (e.g., cell debris, misplaced molecules) (39). Finally, the GM and other microbial constituents of the human body

are able to regulate host–pathogen balance and to produce systemic proinflammatory stimuli. The lifelong antigenic load represented by foods, bacteria, and 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 proinflammatory side of the phenomenon is accompanied by a concomitant modulation of anti-inflammatory responses (38). Inflammation is an efficient ancestral mechanism for maintaining physiological homeostasis, but owing to its potentially destructive power, it has to be accurately and precisely downregulated. The most recent data suggest that impaired resolution of inflammation likely plays a major part in inflammaging and its deleterious effects (147). It also seems that inflammaging and immunosenescence are basically adaptive mechanisms that occur throughout life within the context of a more general remodeling of the body that occurs with age at the molecular, cellular, and systemic levels, as anticipated by the remodeling theory of aging (42). Accordingly, only by taking into account the entire, integrated immunobiography of each individual is it possible to evaluate the biological part (positive or negative) played by inflammaging (41).

Thus, understanding 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, which are specifically addressed in this review.

2. CENTENARIANS AS MODELS OF LONGEVITY AND HEALTHY AGING

2.1. Longevity as a Recent, Historical, and Dynamic Phenomenon

Homo sapiens appeared on the stage about 300,000 years ago, and until about a century ago, life expectancy was about 50–55 years in developed countries, and it did not change much from that of hunter-gatherers until the twentieth century. Then a demographic revolution started, first in developed countries, but soon spreading worldwide. Life expectancy started to increase at about 3 months per year, and the average life expectancy at birth in developed countries is now more than 87 years for women and about 84 years for men (130). Thus, for hundreds of millennia longevity was a rare event, and extreme longevity was likely even more rare, apart from few exceptions that must be carefully investigated and validated. When we started studying centenarians in Italy in about 1990, the centenarians had been born at the end of the nineteenth century, and there were about 3,000 of them; two decades later, in 2017, they were born at the beginning of the twentieth century and there are 18,765 of them (of which 3,000 are men) (58). Thus, from a demographic standpoint, extreme longevity is a highly dynamic phenomenon, and the high number of centenarians worldwide currently (about 434,000) (131) must be considered a recent, largely unpredicted phenomenon. Centenarians undergo rapid changes not only regarding their number but also their phenotype, as they belong to different cohorts and spend their long life in a rapidly changing world. Accordingly, we can predict that the centenarians of the future will be different from the ones 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 have reached the extreme limit of the human life span while largely escaping, postponing, or surviving the major age-related diseases.

During the past 20 years, the exceptional phenotype of centenarians has attracted the attention of scientists all over the world, thus shedding light on basic mechanisms of aging and longevity in humans. **Table 1** shows the results of the most representative studies of centenarians conducted on populations in different areas and with different lifestyles, nutritional habits, and genetics. Their main phenotypic characteristics are summarized as follows.

- They have quite good mental status, with relatively nonexistent anxiety and depression, and a good self-reported quality of life (110).
- Their incidence of chronic illnesses (such as cardiovascular disease, chronic obstructive pulmonary disease, hypertension, renal disease, stroke, malignancy, hypercholesterolemia, and diabetes) is lower than in other elderly people (47, 64, 109, 114, 151).

Table 1 The exceptional phenotype of centenarians

Phenotypic characteristics	Assessment method	Population	Reference
Mental status			
Relatively nonexistent anxiety and depression; good quality of life	14-item Hospital Anxiety and Depression Scale (HADS); Quality of Life Scale	Australian	110
Health status and diseases			
Lower incidence of chronic illness (CVD, chronic obstructive pulmonary disease, hypertension, end-stage renal disease, cancer, diabetes) than octogenarians and nonagenarians	Medical examination	Male US veterans	64
Low incidence of severe diseases (CVD, hip fracture, stroke, cancer, diabetes, hypertension)	Medical examination	Swedish	114
Lower prevalence of hypertension than in the entire population of elderly people in Poland	Multiple measurements with a mercury sphygmomanometer	Polish	151
Low prevalence of hypertension	Multiple measurements with a mercury sphygmomanometer	Southern Italy (Calabria)	47
Lower prevalence of hypertension and hypercholesterolemia than the general elderly population (≥75 years)	Blood pressure: multiple measurements with Omron automated sphygmomanometer Cholesterol: finger-prick blood sample	Australian	109
Better cardiovascular risk profile than middle-aged individuals	Medical examination	Polish	4
Metabolism, hormones, and 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 enzyme immunoassay	Japanese	1
Similar lipoprotein(a) serum level with respect to elderly controls	ELISA	Italian	3
Lower insulin resistance and preserved β-cell function	Insulin: radioimmunoassay, glucose oxidase method, HOMA-IR	Italian	100
Preserved glucose tolerance and insulin action	Oral glucose tolerance test and euglycemic–hyperinsulinemic glucose clamp	Italian	101
Lower glucose, alanine transaminase, total cholesterol, and platelets, and higher urea nitrogen than healthy elderly participants	Standard methods	Italian	69

(*Continued*)

Table 1 (*Continued*)

Phenotypic characteristics	Assessment method	Population	Reference
Higher serum concentrations of all isoforms of adiponectin; lower fasting glucose, insulin, HOMA-IR, total cholesterol, LDL cholesterol, and triglycerides than elderly participants	ELISA and standards methods	Polish	10
Lower plasma concentrations of leptin and neuropeptide Y, and higher levels of adiponectin than elderly and obese participants	Radioimmunoassay	Polish	4
Lower fasting plasma glucose, total cholesterol, and hemoglobin, and higher fibrinogen and C-reactive protein than adults	Standard methods	Italian	121
Higher plasma levels of IL-6, TNFα and sTNFR II than octogenarians, adults, and young participants	ELISA	Danish	12
Higher serum levels of IL-6 and lower serum levels of sIL-6R and sgp130 than 65–79-year-old women	High-sensitivity ELISA	Italian women	50
Higher serum levels of IL-18 and IL-18BP than adult and elderly controls and patients with chronic ischemic syndrome	ELISA	Italian	45
Elevated serum levels of sTNFR-I and sTNFR-II compared with old and young participants; mean sCD30 serum levels four times higher than those of younger groups	ELISA	Italian	49
Lower serum level of IGF-1 and IGF-2, and higher insulin sensitivity than their offspring	Chemiluminescent enzyme immunoassay and ELISA, respectively	Italian	139
Higher molar ratio of IGF-1/IGFBP-3 than elderly participants (75–99 years)	Radioimmunoassay	Italian	99
Higher serum TSH than younger controls	Chemiluminescent enzyme immunoassay	Ashkenazi Jewish	2
No age-dependent increase in prevalence of serum thyroid autoantibodies	Passive hemagglutination	Italian	76
Lower levels of serum free T3 than elderly controls	Column adsorption chromatography and immunoassay	Italian	75
Higher plasma levels of cortisol, ACTH, and CRH than young participants	Electro-chemiluminescent assay, immunoradiometric assay, and radioimmunoassay, respectively	Italian	48
Vitamin D deficiency, low serum calcium, hyperparathyroidism, and osteopenia	Standard methods	Northern Italian	103
Decreased serum level of tryptophan and increased serum concentrations of specific glycerophospholipids; increased urine excretion of phenylacetylglutamine and *p*-cresol sulfate	Targeted liquid chromatography–mass spectrometry metabolomics and untargeted ^{1}H-NMR metabolomics	Northern Italian	18
Distinctive serum metabolic phenotype with unique changes in lipid biosynthesis (41 differently abundant phospho- and sphingolipid species compared with elderly participants)	MS/MS shotgun lipidomics and ^{1}H NMR spectroscopy	Northern Italian	89

(*Continued*)

Table 1 (*Continued*)

Phenotypic characteristics	Assessment method	Population	Reference
Alteration of *N*-glycans in plasma and immunoglobulin fraction	DSA-FACE	Italian	132, 133
Typical *N*-glycan profile from plasma proteins: increased multibranched and highly sialylated *N*-glycans, as well as agalacto- and/or bisecting *N*-glycans, and decreased biantennary *N*-glycans compared with elderly and young controls	Liquid chromatography–multiple stage mass spectrometry	Japanese (aged >105 years)	86
Immunology			
Maintenance of a reservoir 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 compared with elderly and young controls	Cytofluorimetric analysis	Italian	27
Lower naive $CD95^-$ T cell count compared with younger participants	Cytofluorimetric analysis	Italian	28
High reactivity against human β2 glycoprotein I, but no vascular events associated with antiphospholipid syndrome	ELISA	Italian	81
Coagulation			
Well-preserved complement system (both classical and alternative pathways)	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 no elevation of other coagulation factors	Functional assay and ELISA	Italian	74
Epigenetics			
According the epigenetic clock, centenarians are 8.6 years younger than expected from their chronological age	Prediction method for biological age based on the DNA methylation levels of 353 CpGs	Italian	55

Abbreviations: ACTH, adrenocorticotropic hormone; BMI, body mass index; BP, binding protein; CRH, corticotropin-releasing hormone; CVD, cardiovascular disease; DSA-FACE, DNA sequencer-assisted fluorophore-assisted carbohydrate electrophoresis; ELISA, enzyme-linked immunosorbent assay; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; IGF, insulin-like growth factor; IL, interleukin; LDL, low-density lipoprotein; MS/MS, tandem mass spectrometry; NMR, nuclear magnetic resonance; sCD30, soluble CD30; sIL-6R, soluble interleukin 6 receptor; sgp130, soluble gp130; sTNFR, soluble tumor necrosis factor receptor; T3, triiodothyronine; TNF, tumor necrosis factor; TSH, thyroid-stimulating hormone.

- They have a better cardiovascular risk profile, with lower levels of triglycerides, total cholesterol, and low-density lipoprotein (LDL) cholesterol, and their glucose tolerance and insulin sensitivity are preserved when compared with other elderly controls (1, 4, 10, 100, 101, 121).
- They have typical signs of inflammaging without most of its deleterious consequences, and there is a complex and peculiar balance between pro- and anti-inflammatory factors (38, 95). Centenarians had elevated plasma levels of inflammatory molecules [e.g., interleukin (IL)-6, IL-18, IL-15, C-reactive protein, fibrinogen, von Willebrand factor, and leukotrienes] (1,

12, 18, 19, 46, 50, 74, 121), but this was counterbalanced by a concomitantly large amount of anti-inflammatory molecules [i.e., adiponectin, transforming growth factor (TGF)-β1, IL-1 receptor antagonist, cortisol, anti-inflammatory arachidonic acid compounds] (4, 10, 18, 48, 49).

- Their lower levels of serum insulin-like growth factor (IGF)-1 confirm data from animal models in which the downregulation of IGF-1 and insulin signaling significantly extends survival (31, 138, 139).
- Centenarians have significantly higher serum concentrations of thyroid-stimulating hormone (TSH) compared with younger controls (2), supporting previous observations that TSH increases progressively with age. They also had lower serum free triiodothyronine (T3) levels than elderly controls (75), without any increase in serum thyroid autoantibodies (76). The age-associated increase in TSH levels and decrease in total and free T3 concentrations were also observed in a study of Chinese centenarians' families published three years ago, confirming that decreased thyroid function—and thus a lower basal metabolic rate and a reduction in oxidative stress—is a peculiar trait of longevity (53).
- They have signs of hypercoagulability (i.e., heightened coagulation enzyme activity; plasma levels of the activation peptides prothrombin, factor IX, factor X, and thrombin–antithrombin complexes; and enhanced formation of fibrin), which paradoxically seem to be compatible with health and longevity, not being accompanied by an augmented risk of arterial or venous thrombosis (74).
- Centenarians also have a peculiar immune profile that is characterized by a well-preserved complement system (classical and alternative pathways) (7); an increase in peripheral blood $CD28^-$ T cells, with a phenotype compatible with memory cells (upregulated CD2 and CD11a, with CD62L absent) predominantly among cytotoxic $CD8^+$ T cells, suggesting that they may constitute armed effector cells aimed toward pathogens of intracellular origin (27, 28); a strong decrease in naive $CD95^-$ T cells capable of mounting responses against novel pathogens; and residual thymic function or the presence of lifelong naive T cells (94), or both.

3. NUTRITION, GUT MICROBIOTA, AND LIFESTYLE

3.1. Dietary Habits of Centenarians

Longevity is a complex and multifactorial trait resulting from an intriguing combination of Nature and nurture—that is, 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 throughout life, 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 had an effect throughout life on the immune system and contributed to inflammaging (41). Thus, the study of centenarians represents a sort of historical probing that allows this complexity to be traced (97). As far as we know, no longitudinal data set is available that addresses nutrition and other environmental and lifestyle factors and stimuli that centenarians have been exposed to throughout their life. However, we can fill this gap by using historical data about the nutritional and lifestyle habits of people from the same cohorts, socioeconomic status, and with the same educational background. In this review, we refer to data from Italy that represent a reliable proxy for reconstructing the nutritional and social environments of centenarians who underwent profound changes during the twentieth century and their life (see the sidebar titled Historical Perspectives

HISTORICAL PERSPECTIVES MAY BE HELPFUL WHEN COLLECTING DATA FROM CENTENARIANS

In **Table 3** we report on interviews with six centenarians who had good cognitive status as representatives of the more than 1,000 centenarians we have studied during the past 30 years. Despite being aware of the crucial importance of lifelong nutritional habits in attaining healthy longevity, detailed and robust data are not available from a large number of the Italian centenarians whom we have thoroughly analyzed. On different occasions and in different settings, we organized investigations aimed at collecting data about the lifelong diets and nutritional habits of centenarians, but the results were always unsatisfactory. We encountered a similar failure when we tried to obtain information about the sex life of centenarians. The main reason for these disappointing results was that the centenarians had difficulties (including cultural ones) in remembering details that were so remote in time, and the memories of family members were not particularly helpful or reliable, likely owing to the long period of time involved and the profound changes that had occurred throughout Italy during a century that saw two world wars. Taking into account other data collected on centenarians (i.e., place of birth and living, educational level completed, occupation, socioeconomic status), a historical perspective on the nutritional situation in Italy over the twentieth century can be helpful.

May Be Helpful When Collecting Data from Centenarians). This methodology can be applied to other populations and geographical and historical environments (149).

The cohorts of centenarians analyzed in Italian studies were born from the end of the 1800s to the beginning of the 1900s. In particular, those belonging to the cohort born during 1899–1909 on average had a low level of education (6.5 $\pm$ 4.9 years), and most of them (65%) had about 8 years of education. Accordingly, most of them had occupations that required only elementary school education, such as housewives, peddlers, farmers, fishermen, artisans, and laborers; overall, less than 25% were employed as managers or clerks, or were self-employed. The social, economic, and nutritional situations in Italy during this time are discussed in Section 3.2. Briefly, these Italian centenarians lived during a transitional period when deep social and economic changes occurred, and they survived two world wars and many years of considerable deprivation. The most important characteristics of the Italian centenarians' diet is that there is high heterogeneity among them and during different periods of their life (**Table 2**). However, some common features can be identified: For most of their life, they followed a provegetarian diet, rich in vegetables and legumes, and eggs and cheese, and relatively poor in meat; this diet overlaps with the Mediterranean diet and lifestyle (77). Their diet was complemented by an active everyday life (walking, bicycling, and domestic, agricultural, or factory work, or some combination of these) that lasted until old age. Centenarians and their families report that centenarians eat small portions of carefully and slowly prepared meals at fixed times of day and that these meals are usually consumed with their family. For most of their life and especially during the first 40–50 years, the ingredients of the food consumed by centenarians were local and fresh, and they respected seasonality because of the difficulty of storing (i.e., lack of refrigerators) and transporting food. Thus, food was quite different (e.g., no chemical treatment of vegetables and no antibiotics for animals, among others) and richer in fiber than the food of today. Even more remarkable, the great majority of centenarians are creatures of habit and have been extremely methodical throughout their lives, especially regarding the timing of meals and the recipes they use. Such regular habits and meal timing, which are strong cultural and anthropological habits shared by their community, continued even after the great nutritional changes that occurred in Italy during the past decades, thus largely shielding the centenarians from the consumption of energy-dense foods that became available during the second part of their life.

Table 2 Summary of six detailed interviews conducted with centenarians (born between 1914 and 1916) with extraordinarily intact cognitive and health status, describing their familial situation, life experiences, body shape, physical activity, and nutritional habits when younger and as adults

Sex, birth year, age at time of interview	Family background	Education and occupation	Body shape	Physical activity	Diet	Meal timing and portion size
Male, 1915, 101 years	Parents were farmers, 7 siblings, living in the countryside After the Second World War, he moved to the city (Bologna)	5 years of education, primary school certificate Farmer until he was 25, then soldier for 5 years during 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/day, early dinner
Female, 1914, 102 years	Parents were a butcher (the father) and housewife (the mother), no siblings, living in the city (Bologna)	8 years of education, low secondary school certificate Clerk for the family's business	Curvy	Daily walking or cycling	Pasta, meat (red meat until 20 years previously, then only white meat), eggs, white bread, vegetables, beans, sweets once a week, fish once a week, milk, cheese, fruit	Regular, 3–4 meals/day
Female, 1916, 100 years	Parents owned grocery shop, 8 siblings, living in a village in the Apennines (altitude, 900m) When she was 17 years old, she moved to the city (Rome and then Bologna)	5 years of education, primary school certificate She took care of her younger siblings, was a maid for a family, and then left her job to take care of her disabled son	Curvy	Daily long-distance walking, 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/day, small portions
Male, 1913, 103 years	Parents were farmers, 3 siblings, living in the countryside When he was 17 years old, he moved to the city (Bologna)	4 years of education Artisan, mechanic, warehouse worker	Thin	Daily long-distance walking or cycling, physical work	Pasta, white bread, milk, tomatoes, beans, eggs Rarely: sweets, butter, cheese, poultry, and pork	Very regular, early dinner, 3 meals/day, small portions
Female, 1915, 101 years	Father died during the First World War Lived with mother (seamstress), grandparents, and 2 sisters in a small village near the city (Bologna)	6 years of education, primary school certificate 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 a little white wine; sweets once a week	Very regular, 3 meals/day, small portions
Female, 1916, 101 years	Farmer (mother) and carpenter (father), 5 siblings, living in the countryside After wedding spent 2 years in Germany and 4 years in Belgium with her husband (miner), then she moved to city (Bologna)	5 years of education, primary school certificate Farmer until wedding, then cook, greengrocer, and maid for a family	Medium	Walking, physical work	Pasta, milk, white bread, little meat (pork and poultry), little cheese, few eggs, pulses, vegetables, little wine; sweets once a week	Very regular, 3 meals/day, small portions

3.2. Historical Perspective on the Social, Economic, and Nutritional Situations in Italy During the Twentieth Century

At the beginning of 1900, Italy was still an agricultural, peripheral, and undeveloped country. The majority of families ate a subsistence diet of food that they grew themselves, which was nearly vegetarian and lower in calories, fat, and protein compared with the rest of Europe. Then, before the beginning of the First World War, the Italian economy started to improve, and there was an increase in food consumption and an expansion in the range of foods available to all, including the lowest social classes. Products such as sugar, meat, olive oil, wine, and milk began to appear on the tables of laborers and farmers. Paradoxically, the First World War accelerated this process. In cities, the consumption of horsemeat and lamb increased, while in the trenches, millions of peasants had the opportunity to taste, albeit in a dramatic context, meat, pasta, whole wheat bread, wine, and coffee. These foods entered the collective heritage because they were included in daily meals. Soldiers came into contact with specialty foods, both domestic and regional, that families sent to their colleagues at the front, and these were exchanged and combined, so that thousands of young men were forced to abandon their usual eating habits and come to terms with different cultural realities. Thus, an Italian dietary pattern expanded to the lower social strata (88).

Even if animal protein and fats remained scarce, the First World War did not provoke significant declines in nutrition in Italy due to the relatively low destructive intensity of the conflict, the growth of large industry, and the acceleration of the development of manufacturing and agriculture (119). During the Fascist period (1922–1943), significant reductions in the seasonal availability of the staple foods in the Italian diet occurred (decreases of more than 5% for grains, 8% for fats, 14% for meat, and more than 30% for sugar and fruit), and the average daily calorie intake decreased by about 7.5% due to the contraction in the availability of all essential dietary components (proteins, fats, carbohydrates). The propaganda of the Fascist regime promoted responsible food consumption, including the use of leftovers, and also promoted bread as the symbol of the "new man" of Fascism as opposed to the American ethos of consumerism. The regime provided national and economical alternatives to expensive imported products [e.g., karkadè (hibiscus tea) instead of black tea, barley or chicory infusion instead of coffee, fish instead of meat, rice instead of pasta, and vegetable margarine instead of butter]. Thus, even if Italian nutrition at the time of Fascism was quite similar to the typical Mediterranean diet, the Italian situation was worse than in the early decades of the 1900s.

Fascism, particularly for the lowest social classes, meant a significant deterioration in nutritional standards, with a return to a diet composed mainly of carbohydrates and with less animal fats and proteins (57). In addition, the disparities between the North and the South increased in terms of the consumption of beef, milk, eggs, cheese, poultry, and sugar (119). With the Second World War, the situation further worsened due to the growing inability of the Fascist regime to manage the agricultural sector not only to sustain the troops but also to ensure at least the minimal availability of food for the urban population. To survive, farmers bypassed the public storage system and rationing, which stimulated the illegal food market and contributed to the collapse of organizations devoted to food collection and distribution. Thus, all over the country, the reduction in 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 staple foods of the working classes and their main sources of calories—collapsed during and immediately after the war. At the end of the Second World War, the Italian population was exhausted both economically and culturally, and the country was left with heavily damaged production and infrastructure systems and a dramatically impoverished society (5).

In 1945, Italians enthusiastically welcomed the Allies not only because Fascism had been defeated but also in the hope that the United States would help reverse the collapse of the food

production industry, which had been much more serious than the destruction of factories, transport, and housing. The European Recovery Program (known as the Marshall Plan) made available to Italy and other European countries millions of dollars in food aid, providing more than 20% of the average daily caloric intake per inhabitant in postwar Europe, and it was essential in increasing the energy value of the daily diet (24).

However, the Inquiry on Misery in Italy (1951) (11) describes a country fallen again into its darkest past, with 23.4% of families (about 12 million Italians) living in miserable or disadvantaged conditions of absolute or relative poverty, for example, living four people per room; or living in basements, caves, or cabins; never or almost never eating meat, wine, and sugar; and having a low level of education. These impoverished Italians ate mostly carbohydrates and little protein and fat. Families did not have stable and adequate access to pasta and bread, and most families were in danger of malnutrition. Poverty was concentrated in Southern Italy, where more than 50% of the disadvantaged families lived. The difference in 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 fishing—was more than 450 kcal. Moreover, public assistance was concentrated in the north of the country and was absent in the southern regions (11). The decade 1951–1960 was the beginning of the so-called Italian economic miracle, which was characterized by strong economic growth, and Italian nutrition reached the levels and standards of developed countries in the west. This positive trend was accompanied by reductions in discrepancies in the quantity and quality of food among the Italian regions (24). Between 1951 and the 1980s, the amount of food consumed by families increased considerably, and the quality of the food improved significantly. The progressive acceptance of dried pasta as a national food present daily on the Italians' tables stabilized the intake of cereals, and concomitantly, exponential increases were seen in the consumption of beef, pork, poultry, vegetables, milk, sugar, olive oil, and fresh and preserved fish. Maize—which had been the main component of the diet of farmers in Northern and Central Italy—disappeared from the Italian diet, and significant decreases were also seen in the consumption of lard (the poorest raw fat), pulses, potatoes, and rice. What developed was a combination of the European diet, with its high intake of animal proteins, and the Mediterranean diet, with pasta, fruit, and vegetables. In the 1980s, there was a gradual reduction in the consumption of wheat, wine, and sugar; a stabilization of meat intake; and an increase in fruit and vegetable consumption.

Starting from about 1960, a progressive Americanization of lifestyles occurred and became popular, particularly among adolescents and young adults (24). However, Italian families have mostly maintained the habit of preparing meals at home, thus preserving the traditional and cultural aspects of Italian cuisine.

Stability in access to food and improvements in the quality of the food eaten, due to the daily consumption of animal protein, combined with a significant expansion in the range of food products entering the diet have led to improvements in the living conditions of the Italian population. As a result, mass malnutrition has disappeared, mean height has increased, infant mortality has drastically declined and almost disappeared, and average life expectancy has increased by almost 30 years since the early 1900s (59).

3.3. The Mediterranean Diet as a Strategy for Ensuring Healthy Aging and Longevity

Diet and nutrition represent pervasive mechanisms that are able to finely modulate the phenotype throughout life and the pro- and anti-inflammaging balance (13). The heterogeneity in the dietary and nutritional history and status of centenarians worldwide suggests that the dietary patterns

promoting exceptional longevity may be quite different across time and place. As mentioned before, Italian centenarians for most of their life likely followed a nutritional and lifestyle pattern that was close to the Mediterranean diet (77, 96). A plethora of observational studies and clinical trials concur that the Mediterranean diet prevents morbidity and enhances longevity (65).

The Mediterranean diet is characterized by the consistent intake of vegetables, fruits, nuts, legumes, whole grains, fish (especially marine species), and extra virgin olive oil, and the moderate consumption of eggs, dairy products, lean meats, and red wine. People following a Mediterranean diet eat less saturated fats (butter and other animal fats), red meat, refined carbohydrates, and sweets. On the whole, the Mediterranean diet is a well-balanced diet providing a balanced mix of nutrients with antioxidant, anti-inflammatory, and prebiotic effects (77). The consumption of low-glycemic-index carbohydrates (vegetables, fruit, whole grains, and legumes) reduces the increase in postprandial glycemia and controls insulin secretion. The abundance of dietary fiber (β-glucans, arabinoxylans, galactomannans, pectins) contributes to satiety and to controlling body weight, decreases systemic inflammation, and has a central role in maintaining gut health by providing selective substrates for health-promoting bacteria, such as *Bifidobacterium* and *Lactobacillus*. Frequent consumption of marine fish provides high levels of omega 3 polyunsaturated fatty acids (PUFAs) that compete with omega 6 PUFAs for the same enzymes, thus reducing the production of arachidonic acid–derived proinflammatory eicosanoids (prostaglandin E_2, leukotriene B_4, and the thromboxane 2 series) that have chemotactic and procoagulant actions, and increasing the synthesis of anti-inflammatory eicosanoids (prostaglandin E_3, leukotriene B_5, and the thromboxane 3 series) that have immunomodulatory effects (18, 89, 142). On the whole, the Mediterranean diet is capable of fine-tuning the balance between pro- and anti-inflammaging, and delaying the detrimental effects of inflammaging and the onset of chronic age-related diseases (77, 96).

In a review published last year, our group conceptualized the Mediterranean diet 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 the lifespan of different organisms, along with physical activity (77). Hormesis is the adaptive, nonmonotonic, biphasic dose–response relationship that occurs following an initial disruption in homeostasis. The Mediterranean diet contains compounds—resveratrol, quercetin, olive oil secorroids, phenolic antioxidants, terpenoids, carotenoids, and allium-derived sulfur compounds, to mention a few, collectively dubbed hormetins (107)—that are able to stimulate and upregulate a variety of cellular and molecular defense and maintenance pathways [e.g., nuclear factor erythroid 2 (Nrf2), nuclear factor kappa–light-chain-enhancer of activated B cells (NF-κB), mammalian target of rapamycin (mTOR), and sirtuins (SIRT)] that are involved in and mimic CR (77, 98).

3.4. The Peculiar Gut Microbiota of Centenarians

The study of the GM of exceptionally long-lived individuals provides insights into how this symbiotic microbial community successfully adapts throughout the life span to progressive, age-related environmental (e.g., lifestyle and diet) and endogenous changes and how it promotes healthy survival by contributing to the maintenance of metabolic and immunological homeostasis (8). Comparisons of the GM of young adults (25–40 years), elderly people (59–78 years), and centenarians (99–104 years) have shown that the trajectory of modifications with age in the composition and diversity of the gut ecosystem is not linear, but remains highly stable between young adulthood and the seventies, markedly changing during the last decades of life (9). After 100 years of symbiotic association with the human host, the GM displays a profound and adaptive remodeling (115). Bacteroidetes and Firmicutes continue to dominate the GM of centenarians as they do in adults and elderly individuals, but the Firmicutes subgroups undergo specific changes, with a decrease in the contribution of *Clostridium* cluster XIVa, an increase in *Bacillus* species, and a rearrangement

of the composition of *Clostridium* cluster IV (9). The GM of centenarians is enriched in facultative anaerobic 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 (113). The GM shapes intestinal immune responses during health and disease, and the age-related remodeling of the GM may contribute to systemic inflammaging, which in turn may directly or indirectly affect the composition of the GM in a sort of self-sustaining loop. Indeed, the changes in the GM profile observed in centenarians (enrichment in Proteobacteria and a decrease in butyrate-producing bacteria) correlate with a systemic increase in proinflammatory cytokines (IL-6 and IL-8) (9).

A phylogenetic analysis of the trajectory of the human GM in a number of young, elderly, and extremely long-lived Italians (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, which is composed of dominant symbiotic bacterial taxa (Ruminococcaceae, Lachnospiraceae, and Bacteroidaceae), 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 their co-occurrence networks. An unexpected consequence is increased diversity in the composition of the GM species observed in centenarians and semi-supercentenarians, which is contrary to most, if not all, diseases characterized by decreased GM diversity (8). This major characteristic is not peculiar to Italian centenarians, but has been reported also in Chinese and Japanese centenarians, despite the genetic, lifestyle, and dietary differences seen in Italian centenarians, suggesting that it is a remarkable signature of longevity per se and a key health indicator (115).

3.5. Do Nutritional Habits Affect the Maintenance of Circadian Rhythms in Centenarians?

Circadian rhythms, metabolism, and nutrition are intimately linked (61). Mammalian circadian rhythms are driven by a master clock within the suprachiasmatic nuclei (SCN) of the hypothalamus, and they are mainly entrained by light and transduced by specialized photoreceptors in the retina (25). In addition, peripheral clocks are located throughout the body in organs such as the liver, intestines, and heart (116), and their function contributes to the regulation of homeostasis and physiological responses (87).

The molecular machinery of these clocks 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 composed of CLOCK and BMAL1, two transcriptional activators that heterodimerize and induce the expression of clock-controlled genes. Cryptochrome genes (*Cry1*, *Cry2*) and period genes (*Per1*, *Per2*, *Per3*) are clock-controlled genes, 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 and REV-ERBα and -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), which is, in turn, associated with poor health and metabolic disorders (105). The SCN synchronize peripheral clocks through neuronal pathways, hormone rhythms, core body temperature, and behaviors such as the cycle of feeding and fasting (116). Photic cues are of primary importance for resetting human rhythms (70). However, in nonhuman species, regularly timed, nonphotic cues

can regulate rhythms; for example, the temporal restriction of food availability resets the phase of rodent peripheral clocks (21, 80). Human studies have revealed that postprandial responses depend on meal timing (60, 90), but little is known about the ability of meals per se to alter the timing of human circadian rhythms. A paper published last year demonstrated that meal timing exerts a variable influence over human physiological rhythms, with notable changes occurring in glucose homeostasis (144). A 5-h delay in meal times induced a comparable delay in the phase of circadian plasma glucose rhythms, which was accompanied by a 1-h delay in the phase of PER2 rhythms in white adipose tissue, but there was no change in markers of the SCN clock (melatonin, cortisol), rhythms of plasma insulin and triglyceride, or clock gene rhythms in whole blood (144). Overall, data suggest that the timing of meals can have a strong impact on health outcomes. As mentioned above, the centenarians we interviewed had very regular meal timing due to their lifestyles and cultural and social habits (**Table 2**), and we surmise that such regularity likely contributed to their good health status overall.

Evidence has shown that the GM also displays circadian fluctuation, which is mainly driven by diurnal food intake and leads to rhythmic abundance of microbial metabolites (68, 128). The systemic oscillation of the GM-derived metabolome reprograms the circadian transcriptome both locally and distally, thereby regulating host physiology, such as metabolic function and drug detoxification (68, 127). Bacterial adherence to the epithelium shows temporal fluctuations, which also correlate with host transcriptional oscillations. Thus, disruption of the GM oscillatory activity as a result of antibiotic treatment or time-disordered dietary intake leads to disorganization of host rhythmicity (127), indicating that the 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 (93). Although the core clock machinery robustly oscillates independently of microbial effect, the expression pattern of canonical clock genes is influenced by the presence and composition of the GM (51, 68). Altogether, the host–microbe interaction appears to be essential to keeping the host clock timed in an appropriate manner to integrate with fluctuating environmental signals. In turn, a functional clock impacts the time-of-day oscillations of microbial composition. Because commensal bacteria compete with invading pathogens, the compositional oscillation of the 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 (129).

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 for 20 min. Bacterial molecules and metabolites, whose production depends on bacterial growth phases, regulate the intestinal release of satiety hormones; consequently, systemic bacterial molecules directly activate central appetite pathways that may integrate the energy status of both the host and its GM. This short-term bacterial growth–linked modulation of intestinal satiety may be coupled with long-term regulation of appetite, which is controlled by the neuropeptidergic circuitry in the hypothalamus (29).

The peculiar GM composition of centenarians may also contribute to regulating their appetite. The 24-h sleep–wake cycle is one of the most prominent outputs of the circadian clock system. Circadian or sleep disturbance—that is, misalignments between the sleep and wake phases—can lead to different types of diseases; metabolic derangements, including accelerated aging (102); and an increase in inflammation, or 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. Sleep provides the best example of impairment in behavioral rhythms because it often becomes less consolidated as age progresses, possibly contributing to inflammaging

(56). Insomnia symptoms have been related to advanced epigenetic age in women (16), contrary to findings in 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*), the clock mutants period and timeless (homologs of the mammalian *Per* and *Cry*) are less sensitive to the life span–extending effects of CR, while CR strengthens circadian oscillations in peripheral tissues (62), suggesting that the deterioration of the circadian molecular clock shortens life span. In centenarians, the quality and the quantity of sleep, a major circadian clock, appear to be well preserved. It has been reported that 48 Italian (Calabrian) centenarians go to sleep early in the evening, have no problem falling asleep, wake up early in the morning, take a nap in the afternoon, and do not take sleeping pills before going to bed (120). Another study evaluated a relatively large group of 180 centenarians from Rome and demonstrated the existence of a positive correlation between sleep quality, survival, and successful aging (124). Additionally, the amplitude of the nocturnal peak or the persistence of a prevalent nocturnal secretion of melatonin, or both, is preserved in centenarians (73). Moreover, studies conducted in different populations of centenarians, such as Brazilians (79) and Chinese (17), confirmed that they maintained strictly regular sleep–wake schedules and that cognitive impairment is associated with poor-quality sleep, longer sleep latency, and lower percentage of sleep efficiency. These finding are in agreement with the interviews we conducted with six centenarians (**Table 2**).

3.6. Are Centenarians Similar to Individuals on Calorie-Restricted Diets?

CR is the most thoroughly studied nutritional intervention considered to modulate aging and to increase health span and life span in a variety of animal models, from the unicellular yeast to primates (78). Overall, results suggest that the effect of CR is highly evolutionarily conserved (22) and involves common pathways across taxa. Although the molecular mechanisms underpinning the effects of CR are not fully understood, there is consensus that CR involves the downregulation of insulin and insulin-like signaling, as well as of the mTOR/S6 kinase pathway and the glucose-signaling Ras protein kinase A, and activation of SIRT1 (67), largely via autophagy, stress defense mechanisms, and survival pathways, which attenuate proinflammatory responses (6). It has been proposed that CR activates these longevity-promoting pathways by acting as a mild stressor that promotes hormetic responses (111). Major targets of CR are mitochondria, which undergo mild functional impairment and, in turn, counterintuitively promote longevity through cross talk with the nucleus and by secreting a variety of mitokines (112). Indeed, mitochondria in primary fibroblasts from centenarians show mild functional impairment (117), and fibroblast growth factor 21 (FGF21), a master mitokine in metabolism, is increased in the plasma of centenarians (126). Therefore, the data on centenarians fit the experimental evidence that mitochondria with mild impairment promote longevity, being paradoxically more beneficial than perfectly working mitochondria (112).

To answer the question of whether centenarians are similar to individuals with restricted calorie intake within this scenario, we emphasize that CR is a complex topic and that a number of variables—such as strain and genetic background, feeding regimens, diet composition (protein versus carbohydrate versus fat; natural or purified ingredients), and the age of onset, as well laboratory differences, among others—can affect the final outcome (134). Among the different types of macronutrient restriction, the reduced intake of proteins and amino acids is the most effective pro-longevity regimen (84). In particular, restricting a single essential amino acid in a normal diet extends the life span. A tryptophan-restricted diet capable of promoting longevity and reducing age-dependent deterioration has mainly been explored for its neurological benefits due

to the role of this amino acid in serotonin synthesis (67). Interestingly, tryptophan levels in the serum of centenarians are lower than in younger participants (18), and this finding is in agreement with an age-related amplified abundance of genes involved in the tryptophan metabolism pathway in the GM (106). Modifications to the composition of the GM across the life span may strongly affect the availability of tryptophan and the gut–brain axis during aging (115).

Moreover, data suggest that the timing of meals (i.e., different types of fasting) can result in outcomes mimicking classical CR (67). The point in the life cycle in which CR is initiated can strongly affect the outcomes, with differences across species, and, therefore, it is critical to consider this when evaluating the results of trials of CR. Overall, data in mice suggest that CR has to start early in life to prolong the life span, but the health span may also benefit (e.g., with improvements in cognitive performance and motor skills) when CR is started later (118, 150). Genetics and sex are also critical variables, and they have a pivotal role in modulating the longevity- and health-promoting effects of CR (85). All of the previously mentioned studies used animal models, including mammals (mice), but what about primates? Two independent longitudinal studies of CR on nonhuman primates (monkeys) were conducted at the US National Institute on Aging in 1987 and at the University of Wisconsin in 1989 (78). Despite differences in the feeding regimens, 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 its capability of extending the life span, with this difference likely being a consequence of the different age of onset of the two diet regimens (78).

The data on CR in humans are relatively scant, and they are derived mostly from natural experiments in adult volunteers who followed CR regimens for several years and from observations in populations that fit the CR paradigm—that is, CR without malnutrition, such as is seen in Okinawan centenarians (148, 149). Compared with age-matched elderly controls in mainland Japan and other regions of the world, the older generation living in Okinawa, a small island in Japan, reported consuming a reduced-calorie but nutrient-dense diet from the time they were young (148, 149). Compared with relevant reference elderly populations from around the world, the Okinawan elderly exhibited better health status in terms of metabolic and cardiovascular markers of risk, as well as a lower prevalence of age-related diseases (149). It is also worth mentioning the 1991 Biosphere experiment, during which eight adults were sealed inside a self-contained ecological space (Biosphere 2) and accidentally consumed a calorie-restricted diet for almost 2 years. Improvements were described in their blood pressure, and hematological, biochemical, and metabolic parameters (141). There is also evidence of the positive effects of CR from a study of voluntary practitioners, members of the CR Society. These volunteers were consuming approximately 800 kcal fewer per day than age- and sex-matched participants eating typical Western diets, and they had significantly lower mean body fat, core body temperature, blood pressure, and T3 levels (30). A randomized controlled trial sponsored by the US National Institutes of Health is known as CALERIE (Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy). Two CALERIE studies were performed. The first assessed 12 months of 30% CR and involved 130 participants aged 24–42 years who were nonobese and not overweight; the second assessed 2 years of 25% CR and involved 220 healthy men and women aged 21–50 years with BMI between 22 and 28 (22). The main findings are summarized in **Table 3** and compared with data on 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 adult 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 considerations and hypothesis.

- CR appears to induce a healthy multiparameter phenotype even in humans and even if CR studies until now have been conducted in relatively young people—that is, healthy or overweight adults, ranging between 21 and 60 years of age—and lasted a maximum of 2 years. Thus, we do not know the possible long-term effects of CR in humans, particularly in the last decades of human life.
- Thus, the centenarians can be assumed to be bona fide examples of a nutritional habit followed throughout life and also during the last decades of life, which attains results similar to those observed from a CR regimen.

Table 3 Comparison between inflammatory, metabolic, hormonal, and phenotypical adaptations observed in people on calorie-restricted diets and phenotypical characteristics of centenarians

Adaptation	Parameter	Calorie-restricted diet (people aged 21–60 years)	Centenarians (>100 years)	References
Concordant				
Glucose metabolism	Glucose	↓	↓	4, 10, 31, 33, 54, 69, 71, 100, 101, 108, 121, 136, 137, 139, 141
	Insulin	↓	↓	
	Insulin sensitivity	↑	↑	
Blood pressure	Systolic	↓	↓	47, 64, 108, 109, 114, 135, 139, 141, 151
	Diastolic	↓	↓	
Thyroid	T3	↓	↓	54, 66, 75, 137, 141
Lipid profile	Total cholesterol	↓	↓	1, 10, 33, 108, 121, 139–141
	LDL cholesterol	↓	↓	
	Triglycerides	↓	↓	
Body composition	BMI	↓	↓	1, 33, 101, 103, 108, 136, 137, 139–141, 145, 146
	Fat-free mass	↓	↓	
	Bone mineral density	↓	↓	
Metabolism	Cortisol	= or ↑	↑	4, 10, 18, 66, 122, 136, 141, 145
	Adiponectin	↑	↑	
	Leptin	↓	↓	
	Tryptophan	↓	↓	
Discordant				
Metabolism	Vitamin D	↑	↓	1, 32, 34, 99, 103, 136, 139
	HDL cholesterol	= or ↑	↓	
	IGF-1	=	↓	
	IGFBP-3	=	↓	
	IGF-1/IGFBP-3 ratio	=	= or ↑	
Inflammation	C-reactive protein	↓	↑	1, 12, 15, 31, 33, 45, 49, 50, 83, 92, 103, 108, 121, 145
	IL-6	↓	↑	
	TNFα	↓	↑	
	TGFβ1	= or ↓	↑	

↓ indicates decrease; ↑ indicates increase; = indicates no change with calorie-restricted diet or no difference between centenarians and younger controls.
Abbreviations: BMI, body mass index; BP, binding protein; HDL, high-density lipoprotein; IGF, insulin-like growth factor; IL, interleukin; LDL, low-density lipoprotein; T3, triiodothyronine; TGF, transforming growth factor; TNF, tumor necrosis factor.

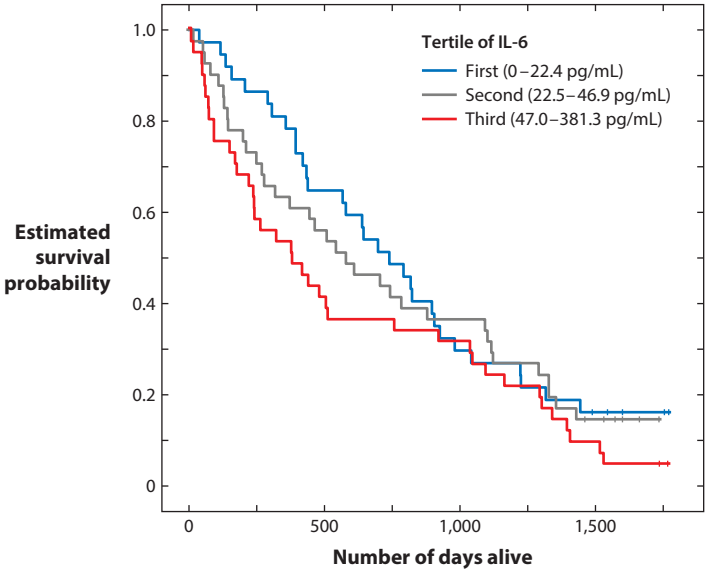

Figure 1

Kaplan–Meier survival curves of 5-year all-cause mortality in 119 Italian centenarians (27 men and 92 women) by tertile of interleukin (IL)-6 plasma level.

- 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 have not been studied in humans following CR regimens. Studies published last year suggest that mice on CR regimens have a younger epigenetic age and that CR affects the GM (123, 143). It is also interesting that the Mediterranean diet favorably affects the GM composition (72).

Within this scenario major differences emerge between centenarians and adults on a CR regimen in terms of several inflammatory parameters, which decrease after CR interventions but are high in centenarians (significantly higher than those found in younger elderly). However, centenarians have high levels of a variety of anti-inflammatory compounds in their plasma, suggesting that a peculiar balance is reached between inflammaging and anti-inflammaging. The levels of anti-inflammatory compounds (not only cytokines but also arachidonic acid cascade products, among others) have not been sufficiently investigated in humans on CR regimens. Moreover, as shown in **Figure 1**, the plasma levels of IL-6—called the cytokine for geriatricians (26)—which is the single most powerful predictor of morbidity and mortality in the elderly, lose their predictive power in centenarians. A review published this year argued that inflammaging—which with immunosenescence is part of the complex remodeling that occurs in the bodies of older people—might also be interpreted as a sign of successful adaptation to the variety of stressors that humans are exposed to throughout life (44). This consideration could be particularly relevant for people such as centenarians who have reached extreme ages while avoiding or postponing major age-related diseases, for whom we can hypothesize that a unique, integrated, and comprehensive inflammatory equilibrium is reached.

4. CONCLUSIONS

The study of centenarians reveals important clues to longevity, especially regarding nutrition, a pillar to attaining healthy aging and longevity (13). The lessons of centenarians can be summarized as follows.

1. Italian centenarians were physically active throughout their life and even at advanced ages, and most of them, particularly the men, are and were lean, while the women have never been obese.
2. Italian centenarians spent most of their early life in nonobesogenic environments where the usual meal contained relatively little meat and animal fat, and the portions were not large. After the Second World War, a nutritional transition occurred in Italy, but those individuals who later became centenarians, being creatures of habit, largely continued their previous nutritional habits. Overall, centenarians avoided overnutrition and the nutrient excess that are characteristics of the present Western diet, which favors obesity and visceral fat deposition, and inflammaging (35).
3. One habit of centenarians is regularity in meal timing. We surmise that this nutritional habit exerted profound effects on many other circadian rhythms, including the composition of the gut microbiota (GM) and sleep. The maintenance of circadian rhythms is considered one of the main characteristics of and a prerequisite for attaining healthy aging and longevity.
4. The GM of centenarians has a peculiar composition that is characterized by increased diversity. This unique trait is likely the result of a lifelong adaptive process that may have played a major part in the centenarians' longevity, owing to its pervasive physiological effects and the cross talk of the GM with organs of the body.
5. The nutritional habits of centenarians may explain most of their peculiar phenotype and the remarkable overlap with that of adults on CR diets, despite centenarians never purposely following a calorie-restricted diet. In contrast to adults on CR diets, centenarians have a peculiar inflammaging that is characterized by concomitantly high plasma levels of pro- and anti-inflammatory cytokines, suggesting that an integrated, structured inflammatory process occurs (91) in which high circulating levels of cytokines such as IL-6 lose their predictive power regarding morbidity and mortality.
6. Altogether, the nutritional habits of centenarians likely contributed substantially to their longevity and healthy aging. However, many members of the same demographic cohort of centenarians followed similar nutritional patterns, but most of them did not live to be 100 years of age. Thus, a major question is, Do centenarians have other characteristics not shared by most members of the same cohort? The answer is probably yes, as suggested by the following circumstantial evidence. First, centenarians do have specific genetics, even if this topic is quite difficult to unravel owing to the complex interaction between genetics, environment, and individual lifestyles and habits (14, 23). All of these ingredients of longevity may be different in different populations, and they may vary in time and place, making longevity a dynamic, context-dependent phenomenon. The genetics of longevity cannot be addressed here and the reader is referred to References 14 and 23. Second, centenarians and their offspring have peculiar metabolomics (18, 89) and epigenetic profiles (55), two parameters that are considered to be robust mirrors of the gene–environment–lifestyle interaction. In particular, DNA methylation clocks show 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.
7. 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 their distinctive

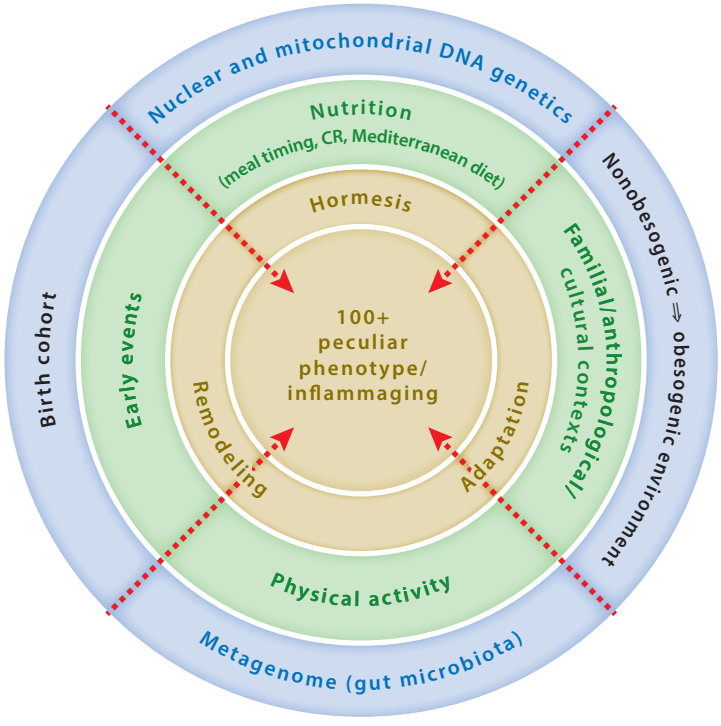

Figure 2

Centenarians are exceptional individuals because they live several decades longer than members of the same demographic cohort, and they escape, postpone, or survive most age-related diseases. Centenarians are epigenetically younger than their chronological age and are similar to people who have followed calorie-restriction (CR) regimens. Such an exceptional phenotype is the result of a unique lifestyle, one that is characterized by specific nutritional habits—such as lifelong moderate food consumption, a diet largely similar to the Mediterranean diet, and regularity in meal timing—combined with living an active life until extreme old age, a peculiar gut microbiota, and a particular genetic background (still largely unexplored). Longevity depends on history and context, and thus the specific geographical, anthropological, familial, cultural, and socioeconomic environments where people who later became centenarians live are crucially important.

nutritional habits and lifestyle in a specifically favorable environment (**Figure 2**) is likely the secret of the centenarians. However, the complexity of human longevity deserves more studies on new cohorts of centenarians worldwide (40).

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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