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Parental longevity impacts on the health status of the offspring

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1. Introduction

1.1 Aging and Longevity Processes

1.1.1. Biodemography of Human Aging

Human senescence has been delayed by a decade. Most western countries have experienced large increases in mean life expectancy, from around 50 years to around 75–80 years. This has been due to a marked reduction in early life mortality during the first half of the twentieth century, followed by a almost twofold reduction in mortality at ages above 70 in the past 50 years (Vaupel et al., 1998). This finding is a fundamental discovery about the biology of human aging, and will have profound implications for individuals, society and the economy. Remarkably, the rate of deterioration with age seems to be constant across individuals and over time: it seems that death is being delayed because people are **reaching old age** in **better health**. Research by demographers, epidemiologists and other biomedical researchers suggests that further progress is likely to be made in advancing the frontier of survival — and healthy survival — to even greater ages. Mortality has been postponed considerably, as a result not of revolutionary advances in slowing the process of aging but of ongoing progress in improving health. The **debility** that often characterizes the last years of life also seems to have been delayed, although the evidence is mixed, in part because deterioration is difficult to define and measure.

Due to the improvement of health care and to a more correct life style it seems plausible that very long lives may be the probable destiny of younger people alive today. If progress in reducing mortality continues at the same pace as it has over the past two centuries then, in countries with high life expectancies most children born since the year 2000 will celebrate their 100th birthday — in the twenty-second century. These longer life spans will alter the way individuals want to allocate time during their lives and will

require drastic revision of employment, retirement, health, education and other policies.

Biological, epidemiologic, and demographic data have generated a number of theories that attempt to identify the cause and the process to explain aging and its inevitable consequence, death. These theories have been grouped by Weinert into several categories on the basis of levels of organization they affect (molecular, cellular, systemic and evolutionary) (Table I). In complex, multi-cellular organisms and particularly in humans, the study of interactions among **intrinsic** (*genetic*), **extrinsic** (*environmental*), and **stochastic** (*random damage to vital molecules*) factors provides a fruitful approach conducive to a comprehensive and realistic understanding of the aging process.

Table I. *Classification and brief description of main theories of aging*

Biological Level/Theory	Description
Evolutionary	
Mutation accumulation	Mutation that affect health at older ages are not selected against.
Disposable soma	Somatic cells are maintained only to ensure continued reproductive success; after reproduction soma becomes disposable soma.
Antagonistic pleiotropy	Genes beneficial at younger age become deleterious at older ages.
Molecular	
Gene regulation	Aging is caused by changes in the expression of genes regulating both development and aging.
Codon restriction	Fidelity/accuracy of m RNA translation is impaired due to inability to decode codons in m RNA
Error catastrophe	Decline in fidelity of gene expression with aging results in increased fraction of abnormal proteins.
Somatic mutation	Molecular damage accumulate, primarily to DNA /genetic material.
Dysdifferentiation	Gradual accumulation of random molecular damage impairs regulation of gene expression.
Cellular	
Cellular senescence-Telomere theory	Phenotypes of aging are caused by an increase in frequency of senescent cells. Senescence may result from telomere loss (replicative senescence) or cell stress (cellular senescence).
Free radical	Oxidative metabolism produces highly reactive free radicals that subsequently damage lipids, protein and DNA.
Wear-and-tear	Accumulation of normal injury.
Apoptosis	Programmed cell death from genetic events or genome crisis.
System	
Neuroendocrine	Alteration in neuroendocrine control of homeostasis results in aging-related physiological changes.
Immunologic	Decline of immune function with aging results in decrease incidence of infectious diseases but increased incidence of autoimmunity.
Rate-of-living	Assumes a fixed amount of metabolic potential for every living organism (live fast, die young).

Table I. *Classification and brief description of main theories of aging by Weinert et al.2003*

A major contribution of biodemography researcher has been to show greater variation in patterns of aging, including so-called **inverse senescence** — the decline of mortality and the improvement of health over all or most of adult life (Baudisch A., 2008)

A number of studies confirm that, for some species and some periods of adult life, mortality can decline with age and that changes in **diet** and other **environmental factors**, as well as **genetic changes**, can greatly alter age trajectories of survival. (Finch C. E., 1990; Rose M. R., 1994).

1.1.2. Progress in delaying debility

In contrast with death, health is difficult to measure and is often unreliably reported. The prevalence of diseases and morbid disorders among the elderly has tended to increase over time. In part this rise may be due to earlier diagnosis of, for example, type 2 diabetes, hypertension and some cancers. Moreover, aged individuals are more often reported to have multiple disorders. Disability is usually measured in terms of self-reported limitations concerning activities of daily living, such as dressing, bathing, shopping and so on. There is increasing evidence that the prevalence of disability may be decreasing (Christensen et al., 2009) reflecting better treatment and a postponement of senescence, but some studies find an increase in the prevalence of disability (Jagger et al., 2008; Parker et al., 2008). The prevalence of a disease or condition depends on the net balance between death and incidence, with death delayed by more effective treatment and with incidence increased not only by the greater frequency of the condition but also by improved screening. **Three states of health** can be distinguished: a person can be **healthy**, **unhealthy** or **dead**. To the extent that the unhealthy state is better than death, **greater prevalence of morbidity among the elderly may be a positive development**. The unhealthy state can be broken down into two states: having a chronic illness but being able to function, and being disabled. Although it does not reduce morbidity, effective rehabilitation reduces disability. Suffering from erratic heart rhythms but controlling them with a pacemaker, or suffering a stroke but

getting physical therapy, increases the prevalence of morbidity but is preferable to being incapacitated by illness, or death. Research on which countries are doing best in minimizing disability — and how — could greatly benefit the elderly.

With rising age, women make up an increasing share of the population (Passarino et al., 2002). In terms of various indices of health and disability, however, older men generally do better than coeval women. This is the **health–survival paradox**: men seem to be healthier than women, but they die younger (Oksuzyan et al., 2008). Social and biological factors interact to determine the prevalence of frail females and dead males, but the relative importance of specific mechanisms is not well understood (Austad S., 2006). **Males** tend to believe their health is better than it actually is and do not seek medical care as frequently as females: they have fewer appointments with general practitioners but require emergency treatment more often. **Females** seem to be better able to survive with poor health. Males tend to engage in reckless behavior. This tendency may be partly genetic in origin, having its basis in the different reproductive opportunities males face in comparison with females (Bonduriansky et al., 2008).

Lastly, to achieve life expectancies of 100 years or more, new knowledge will be needed. Research advances suggest that cardiovascular diseases, CVD, and malignant neoplasms will be prevented and treated much more effectively in the future. Two scourges of senescence are cognitive impairment, often due to Alzheimer's disease, and sensory deprivation. Progress in overcoming these scourges would greatly enhance old age. **Genetics research**, both in humans and in non-human species, will contribute to a deeper understanding of the mechanisms that cause senescence and may allow **individualized medical treatment** based on knowledge of **individuals' genomes**. Research on non-genetic interventions like dietary restriction (Mair et al., 2003) and other might also lead to strategies for delaying human aging. The emerging field of regenerative medicine shows great promise, and in the next decades it might be possible to rejuvenate organs and tissues. Nanotechnologies also might have a role in

postponing senescence. **Priority research** include study not only of very long lived subjects such as nonagenarians, centenarians and supercentenarians, but especially on **family longevity** , on male versus female health and survival, on how improvements in health and reductions in mortality in the young and the middle aged influence health and mortality in old age. (Vaupel, 2010).

1.2. Genetic Determinants of human longevity

1.2.1. Insights from model organisms

Lifespan is the outcome of complicated processes that might involve thousands of genes and non-genetic factors. Findings from animal studies have provided evidence that individual genes can have a significant effect on lifespan. Furthermore, human genetic studies have shown that common polymorphisms in one gene — apolipoprotein E (*APOE*) — influence lifespan, probably mainly through their association with disease. (Corder et al., 1996). Due to their short lifespan and the ability to control both environment and genotype invertebrate animal models have been largely used for studying genetic variants associated with longevity.

Several general facts about the biology of longevity have become clear from animal models: most of the genes involved are pleiotropic, specify increased stress resistance and result in increased robustness in older animals. Animal studies have also revealed a down side to increased lifespan: many **long-lived mutants** are **slow-growing**, with **reduced fecundity** and **fertility**, and **fail to compete in a changing environment** (Walker et al., 2000). Animal studies have also provided insights into the types of gene that can be involved in the regulation of lifespan. The first longevity mutant to be identified was the *C. elegans* gene *age-1* (Friedman et al., 1988) that encodes phosphatidylinositol 3-kinase (PI3K) (Ogg et al., 1997), which has a key role in a signaling pathway that is homologous to the mammalian insulin–IGF1 (insulin-like growth factor 1) pathway which regulates the expression of numerous downstream genes that mediate stress resistance, innate immunity, metabolic processes and toxin degradation (McElwee et

al., 2003; Murphy et al., 2003). Mutations that affect this pathway show notable effects on longevity in both invertebrates and mammals; several mouse longevity mutants alter key components of the insulin–IGF1 pathway, with one of the strongest lines of evidence being the increased lifespan of mice that are heterozygous for the IGF1 receptor knockout (Tatar et al., 2003). Longevity genes have also been identified in other animal models. Two key examples are *sir-2* and *Tor* (*Target of rapamycin*), which were identified in yeast and *Drosophila melanogaster*, respectively. *sir-2* encodes an NAD-dependent protein deacetylase, which might mediate the lifespan-extending effects of dietary restriction, whereas *Tor* encodes a protein that is involved in sensing amino-acid availability. The effects of these genes on lifespan indicate a link between nutrient intake and longevity, and both genes might be involved in the life-extension effects that are mediated by dietary restriction. Importantly, animal studies have shown that mortality is affected at some ages but not all (Johnson et al., 2001) so **human longevity genes** might also be **age-specific**.

Figure I. *Schematic representation of molecular pathways lengthening lifespan in C. elegans and in humans*

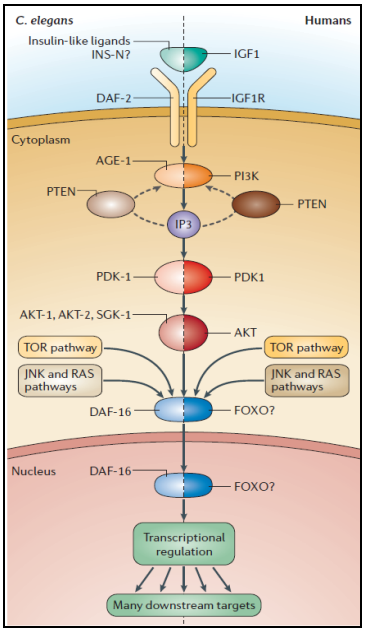

Figure I. Some of the molecular pathways that lengthen lifespan in *Caenorhabditis elegans* and the corresponding components in humans. The C. elegans insulin–IGF-1 (insulin-like growth factor 1) like signal transduction pathway is shown on the left, and human homologues of the proteins that are involved are shown on the right. This pathway involves a cascade of phosphorylation events that ultimately regulate the nuclear translocation of DAF-16. INS-N is an unknown insulin-like peptide, and DAF-2 is its cell-surface receptor, which has tyrosine kinase activity. AGE-1 encodes a phosphatidylinositol 3-kinase (PI3K). IP3 is phosphatidylinositol-3,4,5-trisphosphate (for simplicity, it is shown here as part of the pathway, although it is actually a membrane component), which is produced as a result of AGE-1 activity and activates PDK-1. PTEN is a phosphatase with IP3 substrate activity and suppresses AGE-1. PDK-1 is an IP3-dependent kinase that activates AKT-1, AKT-2 and SGK-1, which are serine/threonine kinases. DAF-16 is a forkhead class transcription factor that is homologous to the FOXO class of human transcription factors, and is probably orthologous to human FOXO3A. The target of rapamycin (TOR), JNK and RAS pathways also feed into the insulin-like signalling pathway at the level of DAF-16 regulation. TOR is a kinase that responds to intracellular amino acids, especially leucine, among other activities; RAS and JNK are involved in numerous signal-transductioncascades in mammals. Numerous other genes in which mutations lead to life extension in C. elegans (for example, sir-2 and mitochondrial genes) are not shown.

1.2.2. Human lifespan as a heritable trait

A striking and consistent series of epidemiological data from different populations (White Americans from New England, Mormons from Utah, Ashkenazi Jewish living in the United States, Icelanders, Japanese from Okinawa, Netherlanders from Leiden, Danish collected in the entire nation, Italians from Southern Italy) suggests the presence of a strong **familiar component of human longevity**.

1.2.2.1. Twin studies

Studying twins is of extraordinary interest because they share the same genetic background.

These studies have consistently found that for cohorts born around 100 years ago, approximately 25% of the variation in lifespan is caused by genetic differences (Herskind et al., 1996). Recent combined analyses of ~20,000 twins born in Nordic countries between 1870 and 1910 confirm this, but they also show that the genetic influences on lifespan are minimal before the age of 60 and only increase after that age. This finding provides support for the search or genes that affect longevity in humans, especially at advanced ages (Hjelmborg et al., 2006). Countries with larger socio-economic differences might be expected to have lower heritability estimates owing to larger environmental variance. In terms of frailty, it was estimated that the heritability of this trait is approximately 50% (Iachine *et al.,* 1998). The model indicates that the importance of survival attributes might increase with life expectancy.

1.2.2.2. Siblings of centenarians

These subjects have an advantage for survival and for attaining extreme longevity. Perls et al., found that the chances of survival until 80–94 years old for siblings of centenarians were about four times as high as those for siblings of individuals who died at 73 years of age (Perls et al., 2002). From the analysis of the pedigrees of 348 Okinawan centenarian families with 1142 siblings it resulted that both male and female centenarian siblings experienced approximately half mortality of their birth cohort-matched

counterparts of the general Okinawan population. (Willcox et al., 2006). Moreover, in families with at least two long-living siblings (men aged 89 years or more and women aged 91 years or more), the rest of their siblings, their parents, and their offspring, but not their spouses (husbands and wives), showed a higher survival and a mortality rate for all causes of death that was 35% less than in the general population Schoenmaker et al., 2006).

1.2.2.3. Centenarian's Offspring

Centenarian's offspring **represent** one of **the best models** to study the different components which contribute to human aging and longevity without the disadvantages inherent in the study of centenarians (rarity, lack of a control group of the same age, presence of frailty due to extreme age). In particular, centenarian's offspring appear to undergo an aging process "better" than that of subjects of the same age (cohort) born from non long-lived parents.

Centenarian's offspring are on average seventy years old, are more numerous than centenarians and it is possible to compare them with a demographically-matched control group (subjects matched for age, sex, ethnicity, parent year of birth, but born from non long-lived parents) thus avoiding cohort effects. As regards centenarians offspring literature data indicate that:

I. Centenarian's offspring had a 56% reduced relative prevalence of heart disease, a 66% reduced relative prevalence of hypertension, and 59% reduced relative prevalence of diabetes and that the median ages of onset for coronary heart disease, hypertension, diabetes, and stroke were significantly delayed compared with the age-matched controls. Moreover centenarians' offspring had a significantly lower risk of all-cause mortality, of cancer- specific mortality, and of coronary heart disease–specific mortality. (Dellara et al.,2003; Dellara et al., 2004a; Dellara et al., 2004b).

II. Men and women with fathers who died at age greater than 80 years showed a 20–30% lower risk of mortality from stroke, cardiovascular disease, and of all causes of mortality compared with those with fathers

whose age of death was lower than 60 years. A similar reduction was found when the age of death of mothers was greater than 85 years compared with those lower than 65 years. Furthermore, the risk reduction was more evident amongst persons with both parents long-lived compared with those with both parents short-lived, especially for death from cardiovascular disease (Ikeda et al., 2006).

III. Offspring of Ashkenazi Jewish centenarians had a favourable lipid profile compared with control groups. In particular, female offspring of centenarians had significantly higher plasma levels of high density lipoprotein-cholesterol, HDL-C, compared with controls, while male offspring of centenarians had higher plasma levels of HDL-C, and significantly lower low density lipoprotein-cholesterol, LDL-C compared with controls. These data suggested that a certain phenotypic lipid profile may be transmitted in families and that a favourable lipid profile may play a role in longevity (Barzilai et al, 2003).

IV. Offspring of long-lived parents had significantly lower prevalence of hypertension (by 23%), diabetes mellitus (by 50%), heart attacks (by 60%), and strokes than several age-matched control groups, (Atzmon et al., 2004)

V. Centenarians' offspring, after approximately 4 years follow-up, had a significantly—lower risk of myocardial infarction, of stroke, and of developing diabetes mellitus than the referent cohort. Additionally, centenarians offspring were significantly less likely to die than the referent cohort (Adams et al., 2008).

1.3. Aging, Immune System and Inflammation

A functional immune system (IS) is considered vital for the host's continued survival against the daily onslaught of foreign organisms and pathogens. In humans, as well as in many other species, it is becoming recognized that the immune system declines with age, a term known as **immunosenescence**, which leads to a higher incidence of infections, neoplasia and autoimmune diseases.

Figure II. *The effect of age on the different components of the innate and adaptive immune systems.*

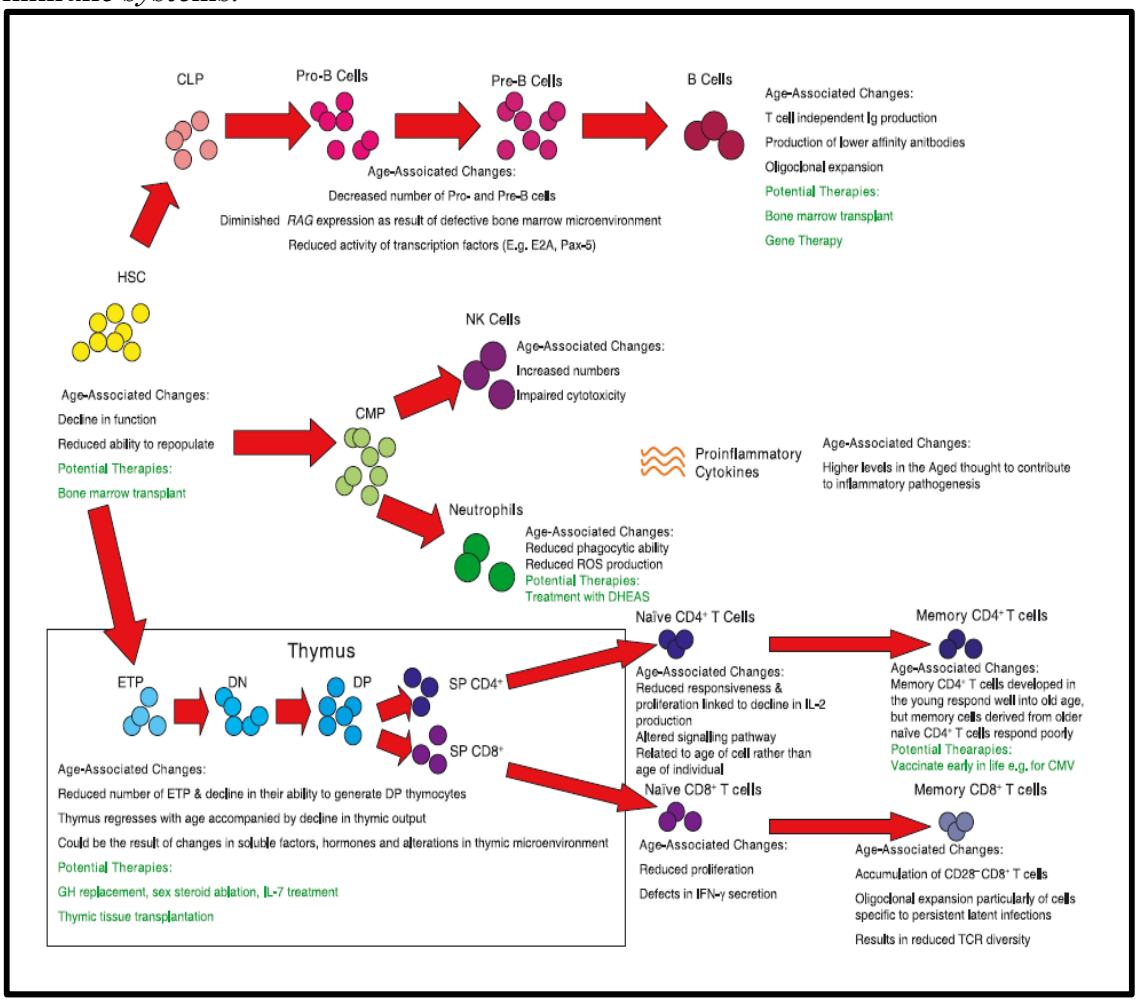

Figure II. The effect of age on the different components of the innate and adaptive immune systems. From Danielle Aw et al., 2006 Abbreviations: CLP, common lymphoid precursor; CMP, common lymphoid progenitor; DHEAS, dehydroepiandrosterone sulphate; DN, double negative; DP, double positive; ETP, early thymic progenitors; HSC, haematopoietic stem cell; Ig, immunoglobulin; IFN-c, interferon-c; NK, natural killer cell; RAG, recombination activating gene; ROS, reactive oxygen species; SP, single positive; TCR, T-cell receptor.

Investigations now suggest that **aging** is associated with the **increased** production of **pro-inflammatory cytokines** by macrophages and fibroblasts

for example. Elevated levels of these mediators are believed to be responsible for most of the age-associated diseases such as diabetes, osteoporosis and atherosclerosis because they all share an inflammatory pathogenesis. Termed **'inflamm-aging'**, it has been hypothesized that as a result of constant antigenic challenge, the continual production of inflammatory mediators could potentially trigger the onset of associated inflammatory diseases resulting detrimental for longevity. (De Martinis et al., 2005; Licastro et al., 2005). Indeed, emerging evidence suggests that the balance between pro- and anti-inflammatory cytokines can be used as a profile to indicate frailty and mortality in older individuals (van den Biggelaar et al., 2004).

1.3.1. Inflammatory markers in aging

Elevations in inflammatory markers have been observed with increasing age. The defined risk factors for these modest increases include visceral adiposity, lower sex steroid hormones, smoking, depression and periodontal disease. Among many of inflammatory markers studied in older adults, IL-6 is most robustly associated with incidence of diseases, disability and mortality. Elevated levels of inflammatory markers are also associated with a higher incidence of mobility limitation (Penninx et al., 2004) and subjects with higher levels of IL-6 were more likely to develop mobility disability and had a greater risk of activities of daily living, ADL, disability (Ferrucci et al., 2002). Elevated inflammatory markers may increase the risk of CVD (Tracy et al., 1997; Aviles et al., 2003; Cesari et al.,2003; Kalogeropoulos et al., 2010). In fact, most studies of cardio vascular disease, CVD and inflammation support a link between the two but it is difficult to infer a single causal path. In older adults, cardiovascular risk factors and prevalent CVD may help elevate pro-inflammatory markers (Ferrucci et al., 2005). This can lead to a cause-and-effect cycle with inflammation stimulating CVD and CVD stimulating inflammation. Chronic low-grade inflammation in older adults has been linked to cognitive decline and dementia, including vascular dementia and Alzheimer's disease (Yaffe et al., 2003).

Among the acute-phase proteins the C-reactive protein and serum amyloid A protein, A-SAA are implicated in several chronic inflammatory diseases. In fact, C-reactive protein, produced by the liver in response to elevations in IL-6, was reported to be positively correlated with resistin levels in several pathophysiological conditions (Axelsson et al., 2006) while A-SAA is implicated in several chronic inflammatory diseases, such as amyloidosis, atherosclerosis, and rheumatoid arthritis (Zhang et al., 2005).

Moreover with age, an increases of body fat with a shift from the periphery to more central abdominal or visceral location was observed. Adipose tissue acts as an active endocrine organ, capable of secreting several cytokines including IL-6 and TNF-alpha and adipokines such as adiponectin and leptin (Trayhurn and Wood, 2005). Levels of adiponectin are inversely correlated with body fat percentage in adults and reduced in metabolic conditions while they are increased in the presence of chronic inflammatory and autoimmune diseases (Fantuzzi, 2007; Ukkola et al., 2009). In human leptin influences energy homeostasis and regulates neuroendocrine function primarily in states of energy deficiency. As cytokine leptin affects thymic homeostasis and, similar to other proinflammatory cytokines, it promotes Th1 cell differentiation and cytokine production (Matarese et al., 2005).

Recently, several studies showed that resistin may also play a pivotal role in inflammation and process of inflammation-related diseases. Resistin strongly up-regulated IL-6 and TNF-α in human PBMC *via* NF-κB pathway (Bokarewa et al., 2005). More recently, the inflammatory markers were shown to be independently associated with circulating resistin levels in patients with chronic kidney disease.

1.3.2. The impact of advancing age on immunity

1.3.2.1. Innate immunity

Whereas age-related alterations of the components within the adaptive immune system are well documented, detailed analysis of the impact of advancing age on the innate immune system remains unresolved. An increasing number of studies have suggested that there is a decline in the

phagocytic capacity and reduced superoxide anion production in macrophages and neutrophils in the aged (Gomez et al., 2005). Other features of aged phagocytes include a reduced expression of Toll-like receptors on macrophages (Plowden et al., 2004). Regarding natural killer (NK) cells, it is reasonable to assume that the clinical manifestation of immunosenescence could be in part due to the age-related alteration of NK cell number and function because of NK cells play an essential role in immunity. The number of NK cells increase with age while regarding their function there are still conflict reports (Plackett et al., 2004; Mocchegiani et al., 2004; Sansoni et al., 1993). Moreover, different NK-cell subsets expanded with age; in particular in the elderly there is an increased proportion of the $CD56^-$ NK-cell subset; cells which exhibit lower cytolytic activity and have a reduced ability to secrete cytokines in comparison to the more abundant $CD56^+$ NK-cell subset (Borrego et al., 1999).

Also the humoral immunity in aged individuals is severely compromised. A marked decrease of peripheral B lymphocytes was observed in humans during age (Sansoni et al., 1993). In addition, the oligoclonal expansions of B cells associated with CD5 expression are known to occur in old individuals (Cancro et al., 2003) and to occupy niches, which then cannot be occupied by other B cells (Eaton-Bassiri et al., 2000). Many other intrinsic B-cell defects have also been reported in aged mice and humans, including reduction of co stimulatory molecules, (Zheng et al., 1997) defects in B-cell receptor signaling (Whisler et al., 1993) and low immunoglobulin titer and affinity. In addition, T-cell/B-cell interactions are known to be disrupted both in aged humans (Lazuardi et al., 2005). Such defects in T-cell helper function, which are known to occur during aging, (Rink et al., 1998) significantly affect humoral immunity because they are required for germinal centre formation and production of soluble factors.

1.3.2.2. Adaptive immunity

Of all age-associated changes in the immune system (IS), regression of the thymus is the most dramatic, ubiquitous and recognizable. Chronic thymic atrophy is now accepted as an ancient and conserved evolutionary process

and the impact on immunosenescence along with characterization of the stages and mechanisms concerned are under increasing scrutiny. The thymus is the primary site of T-cell development capable of generating self-tolerant, self major histocompatibility complex-restricted, immunocompetent T cells, (Miller., 1961). With age, there is a decrease in thymic epithelial space and thymic cellularity, collectively called **thymic involution**. In mice, loss of thymic epithelial space is caused by a gross reduction in thymus size, (Li et al., 2003) whereas in the human thymus there is an increase in perivascular space, which is progressively replaced with fat in the aging thymus (Flores et al., 1999; Ostan et al., 2008). Despite the reduction in functional thymic area, the aging thymus still demonstrates T-cell output, although at decreased rates (Douek et al., 1998). Continual persistence of T-cell receptor excision circle-positive ($TREC^+$) T cells, representing recent thymic emigrants (RTE), was found in the peripheral blood of elderly people being ever less evident with advancing age and in particular in centenarians (Jamieson et al., 1999; Nasi et al., 2006).

1.3.2.3. Age-dependent defects in peripheral T cells

Surprisingly there is little change in the number of peripheral T cells with age, especially given the reduction in thymic output in the aged (Hulstaert et al., 1994). The size of the peripheral T-cell pool is tightly regulated by several variables including homeostatic mechanisms. Both memory and naïve T cells undergo homeostatic control and in humans steady-state proliferation significantly contributes to the naïve T-cell receptor (TCR) repertoire. (Goronzy et al., 2005). It had been presumed that naïve and memory T-cell pools were maintained separately with different survival requirements that are considerably stricter for naïve T cells. (Tanchot et al., 1997). Yet an innovative study revealed that clonal expansion of $CD8^+$ T cells is the consequence of the diversity of the remaining T cells, particularly those that share the same TCR Vb element (Messaoudi et al., 2004). This could have a profound impact on TCR diversity. Analysis of the TCRVb chain presented a decreased antigen-recognition repertoire from approximately 108 in young adults to 106 in older individuals (Weng 2006;) with a drastic contraction in $CD4^+$ T-cell diversity in

the seventh and eighth decades of life (Naylor et al., 2005). Studies in mice have determined that a twofold to tenfold decrease in diversity is sufficient to jeopardize a T-cell-mediated immune response, thereby leaving the elderly more susceptible to new pathogens. Examination of lymphocyte lifespan has shown variations in subsets, but all are finite. Human $CD4^+$ T cells have around 33 population doublings in culture (Pawelec et al., 1996) whereas $CD8^+$ T cells have only around 23 (Perillo NL, et al., 1993). The restraint dictating lifespan is believed to be telomere-dependent and analysis of telomere length displays significantly shorter telomeres in old individuals among all T-cell subsets (Akbar et al., 2004). These cells, which have undergone replicative senescence, accumulate with age and many, particularly in the $CD8^+$ memory subset, are specific to only certain persistent infections (Akbar et al., 2005). Therefore immunosenescence is characterized by a progressive reduction of peripheral naïve T cells (Fagnoni et al., 2000), by an increase of memory T cells (Cossarizza et al., 1996) and of CD28- T lymphocytes (Fagnoni et al., 1996) accompanied by a clonal expansions of $CD8^+$ T lymphocytes specific for viral epitope (CMV, EBV) producing inflammatory cytokines (Vescovini et al., 2004) and filling the "immunological space" (Franceschi et al., 2000a) leading to a progressive development of a chronic inflammatory status, known as "inflammaging", (Franceschi et al., 2000b) 2005; Ginaldi et al., 2005; De Martinis et al., 2006; Rudin and Barzilai, 2005)

1.3.2.4 Phenotype of peripheral T cells

The T cell population can be divided into distinct subsets based on their phenotype, i.e. the expression of diverse cell surface receptors. The most-commonly used markers are CD45RA (or CD45RO), CCR7, CD27, CD28 and CD95. Beyond the precise identification of naïve T cells ($CD45RA^+CCR7^+$ $CD27^+CD28^+$), the differential expression of these four molecules allows the distinction between numerous subsets of “resting” (referring to cells that are not involved in primary or acute infection phases) antigen-experienced T cells. However, it is essential to notice that the expression of a surprisingly large number of markers significantly overlap or associate with each other, as summarized in Figure III. The expression of markers, commonly used to

define CD8 T cell subsets, enables also the distinction between several CD4 T cell subpopulations, including CD4 cytotoxic T cells. Functionally distinct CD4 T cell populations can also be subdivided according to the expression of chemokine receptors (Figure IV A and IV B)

Figure III Phenotype and function of CD8⁺ T cells

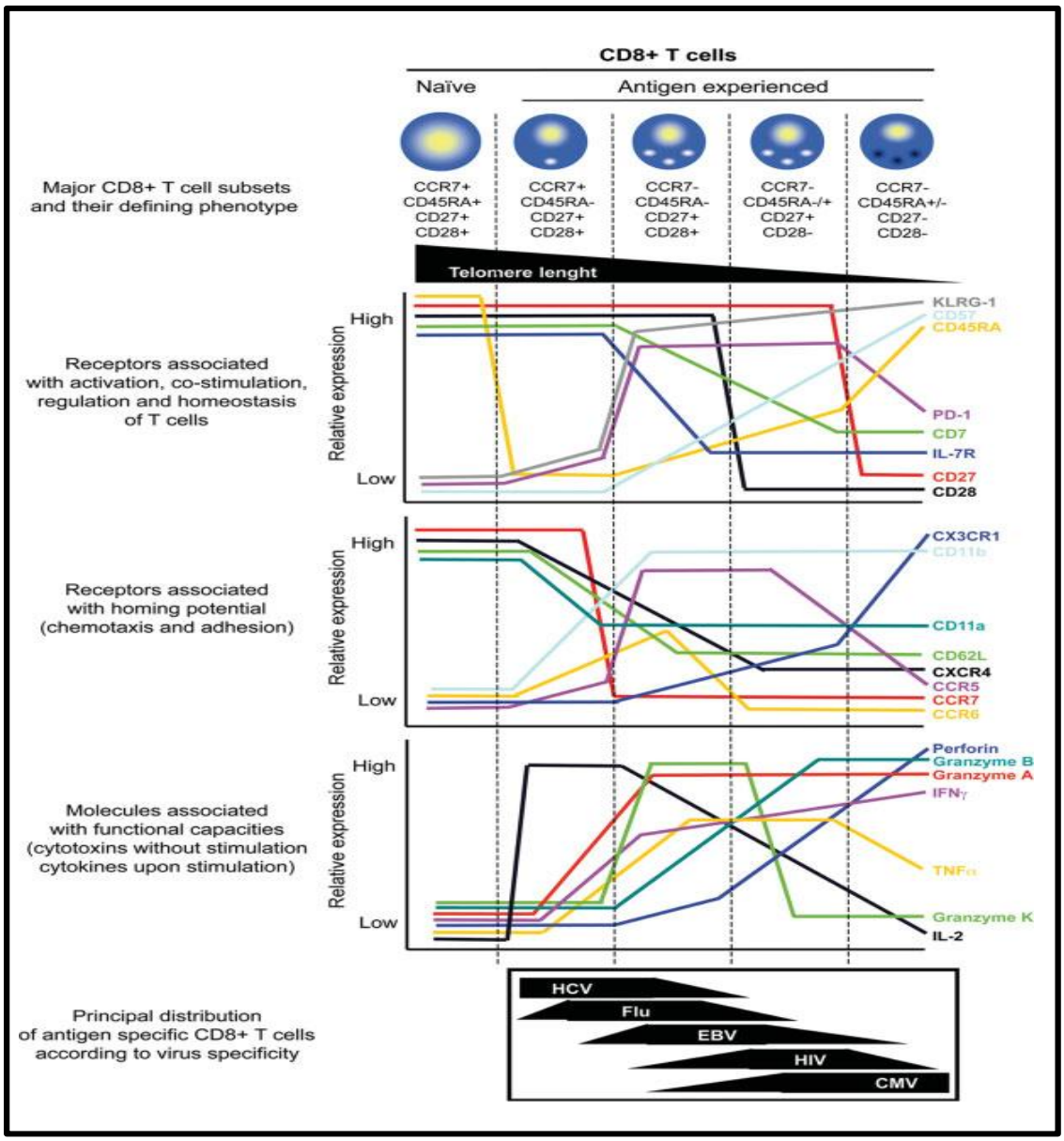

Figure III. Phenotypic associations within CD8 T cell subsets in humans and relationship with functional attributes. Five distinct subsets of circulating CD8 T cells are defined according to the expression of CD27, CD28, CCR7, and CD45RA. Relative telomere length and expression of a variety of cell surface receptors and intracellular molecules (related to T cell activation, costimulation, regulation, homeostasis, homing potential, and functional capacities) are illustrated in these subsets in a "resting" state according to data from the literature. The common phenotypic distribution of virus specific CD8 T cells is also depicted, after clearance of the virus (Flu) or in latent infection stages (for HCV, EBV, HIV, and CMV)**From Appay et al. 2008.**

It is important to note that these observations and relationships between T cell phenotype and functional attributes, as well as between T cell profile and pathogen have been established in the context of resting cells, that is to say, for cells that are not actively stimulated (e.g., by cognate antigen).
Many of these observations do not hold in a setting of T cell activation or inflammation. In fact, inflammatory settings, the T cell environment is also altered, which can result in further changes of T cell behavior.

Figure IV *Phenotype and function of CD4⁺ T cell*

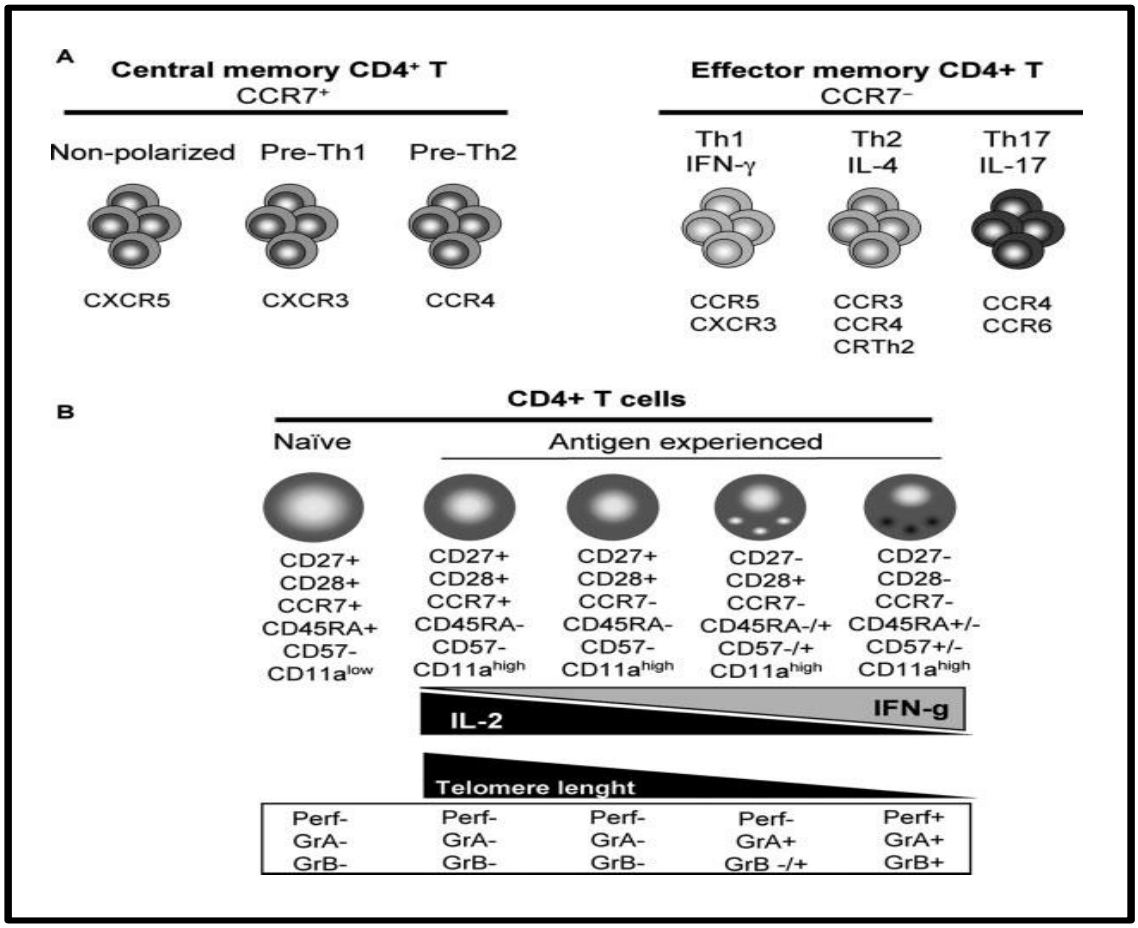

Figure IV. Phenotypic dissection of human CD4 T cells into functionally distinct subsets. (A) The expression of chemokine receptors is associated with CD4 T cell subpopulations presenting distinct TH cytokine profiles. (B) The expression of markers, commonly used to define CD8 T cell subsets, enables also the distinction between several CD4 T cell subpopulations, including CD4 cytotoxic T cells. **From Appay et al. 2008.**

For instance, T lymphocytes lacking the lymph node-homing receptors L-selectin and CCR7 do not migrate to lymph nodes in the steady state (Sallusto et al., 1999). Nonetheless, studies in the mouse have revealed that inflammatory or reactive lymph nodes (i.e., draining sites of mature dendritic cells) can recruit L-selectin-negative CCR7-T cells.

It is of utmost importance to note that there is no harmonization of nomenclature. Even basic terms like “memory,” “activated,” and “effector” have different meaning in the literature. **Memory T cells** may be defined as cells that have seen or been primed with an antigen (persistent or not). **Activated T cells** are sometimes referred to as cells that are simply no longer naïve (having been activated at some point by antigens), and are also referred as cells that have been very recently stimulated with an antigen (and, for example, may be entering mitosis). **Effector T cells** can be present during the primary or secondary phases of an infection, when fighting to halt active viral replication, or they can simply be cells which display effector functions ex vivo (like IFN-γ secretion or perforin content). In humans, longitudinal studies of T cell responses (e.g., from primary infection onwards) they are rare (for practical reasons) so that distinct subsets of resting cells are usually compared with each other at a given time point. It is also important to consider that infections with different pathogens represent different settings, for which comparing respective immune responses may not always be as straightforward as it seems. What is observed in one system is not necessarily valid in another system and different requirements in terms of T cell efficacy may potentially be involved. Last but not least, observations made in the human system do not necessarily apply to the mouse system, and vice versa. It is clear that undue extrapolation and generalization of findings across models and conditions is a problem, which can be a major source of confusion. Full understanding of T cell immunity will require making every effort to perform longitudinal studies in humans and to take in consideration changes of T cell attributes upon activation and pathogen-specific settings.

1.3.3. Cytomegalovirus (CMV) and immune aging

Cytomegalovirus (CMV) is one of the eight human herpes viruses and its seroprevalence ranges from 60 to >95% in different populations. In western countries infection rates increase gradually with age, such that approximately 70% of individuals over the age of 60 years are CMV-seropositive.

Epidemiological studies of elderly Scandinavian individuals associated CMV-seropositivity with the development of an "immune risk phenotype" which was predictive of early mortality (Strindhall J et al., 2007).

Functional studies suggest that the major structural protein pp65 and, to a lesser extent, the immediate early protein 1, IE-1 constitute major antigens against which **aged people** target **functionally efficient T cell effector responses** with massive production of Th1 cytokines and exhibition of CD107a degranulation marker. As a result, the production of IFN-gamma induced in T cells by both antigens was seven to eight times greater in very old than in young subjects. The comparative analysis of pp65-specific responses in these very long-term carriers revealed a reciprocal relationship between $CD4^+$ and $CD8^+$ producing IFN-gamma in the same individuals. Such a remarkable burden of effector $CD4^+$ and $CD8^+$ T cells may be necessary to protect the elderly from CMV endogenous reactivation, but can turn detrimental by giving a substantial contribution to the proinflammatory status that accompanies the main age-related diseases. Antibody responses to HCMV, but not to EBV, and anti-HCMV $CD4^+$, but not $CD8^+$, T cell responses were more intense in elderly subjects aged 85 years over in poor health and were inversely correlated with markers of functional activity and cognitive function. Therefore, humoral and $CD4^+$ T cell anti-HCMV responses were specifically intensified in advanced aging associated with comorbidity and cognitive and functional impairments. Such a distinctive pattern of adaptive immunity indicates that immune responses targeting the extracellular phase of HCMV are increased in these elderly subjects and could represent an indirect effect of localized and undetectable HCMV reactivation. These studies demonstrate that the oldest subjects in poor health with physical and cognitive impairment express intense functional immune responses to extracellular HCMV and suggest that they may be at risk for direct pathogenic effects by HCMV reactivation as well as indirect pathogenic effects linked to proinflammatory anti-HCMV effector responses. These results indicate that CMV represents an important pathogen responsible for a strong immune activation in human aging

(Vescovini et al., 2007; Vescovini et al., 2010). Moreover a recent paper indicated that mouse CMV, MCMV, significantly increased expression of pro-inflammatory cytokines such as IL-6, TNF-α, and MCP-1 and that CMV stimulated expression of renin in mouse and human cells in an infectious dose-dependent manner.

Consistent with the findings of the mouse trial, human CMV (HCMV) infection of blood vessel endothelial cells (EC) induced renin expression in a non-lytic infection manner. These results show that CMV infection is a risk factor for increased arterial blood pressure, and is a co-factor in aortic atherosclerosis. **Control of CMV infection** can be developed to **restrict hypertension** and **atherosclerosis in the cardiovascular system** (Cheng J et al., 2009).

2. Materials and Methods

2.1. The recruitment procedure

2.1.1. Preliminary and preparatory activities to the recruitment

Set up and standardization of the **Informed Consent Forms**, taking into account the Italian legislation in order to obtain the **approval of the local ethical committees**.

Set up of **a common introductory letter** to be presented to the people asked to participate in the study, in connection with a common informed consent form in order to give the participants a qualified basis for decision on participation. This introductory letter, written in a clear and understandable way for the recipients, explained the purpose and background of the study and that participating to the study would include a visit of approximately 90 minutes by a research nurse or interviewer and a blood sample to be taken. It also underlined that participation did not involve any risk for the participants, that participation was completely voluntary, and that the participant could resign from the study at any time.

Set up and standardization of a **Questionnaire** containing socio-demographic information (education); clinical and anamnestic data; functional activity (ADL and IADL); life style habits; physical performance (handgrip-function, chair stand); cognitive function (SMMSE); self-reported health. Then, the interviewer team **contacted the subjects and explained them the type of research envisaged by the project**, its aim and scope, and the role of the subjects in it. Particular attention was paid to illustrate the storage and use of the biological material derived from their blood donation (plasma, cells, etc). After obtaining the informed consent, the interviewer (a MD, preferably a geriatrician, or a specifically trained biologist, biotechnologist) **administered the questionnaire,** collected the clinical history using the standardized case sheet, performed a clinical and functional examination,

collected **blood sample** and stored the biological material (plasma, serum, DNA and blood cells).

Each sample was identified with an identification code named as **PID** for personal information. The PID is designed to be used during all phases of recruitment and for the material used by the recruiting teams. Questionnaires, tubes and work sheets are labelled with the PID. The PID is composed by 8 number specifying for:

1. The name of the recruiting unit (1 digit);
2. To which group subject belonging (3 digit), if they were centenarian offspring or subjects with both parents non long-lived;
3. Progressive number (3 digit) from 001 to 100 for centenarians offspring whose centenarian parent participate to the study and from 900-999 for subjects whose centenarian parents refused to participate to the study and from offspring of non long lived parents;
4. To discriminate how much siblings participate to the study (1 if only one subject participate and 2 and 3 if more siblings participate); offspring of non long-lived parents have 0.

2.1.2. Sample collection, processing, storing and shipment

A) Whole Blood

For each patient **3 tubes of EDTA (3ml and 9 ml), 2 tubes of NaCitr (3 ml each), 2 tubes** of **Clot Activator** (3 ml each) **whole blood** were collected.

The first tube of **EDTA blood** (3 ml) was used to perform the principal emato-chemical analysis (total number of leukocytes, red blood cells, hemoglobin, platelets and leukocyte differential count; blood concentration of glucose, blood concentration of insulin, cholesterol, HDL, LDL, bilirubin, albumin, triglycerides, transaminases, etc);

One ml of the **second EDTA tube (3 ml)** was put into a 96 deep-well and stored at -20°C for DNA extraction. The rest was store at -20°C as backup aliquots.

Approximately **1 ml of whole blood** from the **third tube** (9 ml) was used to perform immune phenotype analysis of the principal lymphocyte subpopulations.

The **third tube** of **EDTA blood (9 ml)** together with **2 tubes of NaCit blood** was centrifuged (1000g) to obtain plasma aliquots that were stored at -80°C. About ten aliquots of plasma EDTA were stored at -80°C until the time of the analysis and then used to determine cytokines and adipokines concentration. A part of NaCitr plasma aliquots was delivered to the University of Milan while the rest was stored at -80°C in local laboratory as backup.

Two tubes of Clot Activator whole blood were centrifuge at 769g for 20 minutes to obtain serum samples and then were stored at -80°C until the time of analysis. Insulin determination and CMV-serology were performed on serum samples.

B) Other biological samples

For each patient urine was collected. 4 aliquots of urine (1.5 ml each) were stored at –80°C.

Moreover, of a part of these subjects, faeces were collected and stored at –80°C.

2.1.3. Which threshold for longevity?

Subjects eligible to participate to the study were recruited following strict demographic criteria. Centenarians offspring and controls, that are individuals in their seventies, had to have both parents born in the same birth cohort, that is 1900-1908 and dead by non accidental cause.

But which age threshold would be chosen to classify parents in long-lived or not long-lived individuals?

They were classified in long-lived or non long-lived on the basis of the average life expectancy at 15 years for the birth cohort considered that is, for birth cohort 1900-1908, 67 years old for male and 72 years old for female. By asking directly to subjects to remember the date of birth and death of their parents and the cause of death it has been possible classify these individuals in long-lived and non long-lived, according to the average life expectancy.

Once obtained this classification subjects were divided in the following groups:

-**Centenarians Offspring** (having both parents born between 1900-1908 with at least one centenarian);

-**Offspring of both non long-lived parents,** (having **both parents** born between 1900-1908 and dead **at an age lower than the mean life expectancy**)

2.1.4. Population of the Study

A total of **374 subjects** were recruited in Northern Italy by the University of Milan, Parma, Bologna and Florence (Milan, Parma, Bologna, and Florence district). These subjects were divided into 2 groups:

- **centenarians offspring** (109 males and 158 females, mean age = 70.2 years)
- **offspring of non long-lived parents** (56 males and 51 females, mean age = 71.1)

The **local Ethical Committees**, Comitato Etico Indipendente Policlinico S.Orsola Malpighi (UNIBO) approved the study protocol.

Population registration took place at the municipal level through "Anagrafe" system. A letter was sent to all municipalities asking for list of long-lived persons and for controls and their family. More time was spent by MD, biologists and biotechnologists at the Anagraphe of Bologna to search through the paper register and import in electronic format a list of subjects complied with the design of the study. **This phase lasted more than two years.**

Eligible subjects were then contacted by an informative letter. After a week subjects were contacted by phone to obtain the consent and to fix an appointment for the home interview and blood withdrawal.

Subjects affected by malignant neoplasia and/or in therapy with immunosuppressor drugs (like cyclosporine, methotrexate, glucocorticoid, etc.) or anti-coagulant drugs were excluded from the study.

2.2. Variables assessed by the questionnaire and included in the analysis

2.2.1. Sociodemographic Factors

Questions about education and marital status. Education was reported both as mean of years and as prevalence of subjects obtained higher level of education. Higher education indicate subjects who successfully completed at least thirteen years of education.

2.2.2. Lifestyle Factors

Questions about smoking habits and alcohol consumption. Smoking was indicated as current and former smokers.

2.2.3. Disability assessment

The functional disability was assessed by the Katz Index of **activities of daily living (ADL)** and by the Lawton **Instrumental Activities of Daily Living (IADL)** scale.

The Katz Index of **ADL** including bathing, toileting, transfer, feeding and continence was used to classify subjects in "not disabled" as independent in all items, "moderately disabled" as dependent in one or two items, and "severely disabled" as dependent in three or more items in accordance with the definitions given in Katz'paper (Katz S., 1970). These categories define three sizable groups which ranged from a group capable of doing the most basic activities independently to a group that was dependent in the majority of the six basic activities. The mean value of the score was reported in the analysis.

The Lawton (Lawton, M.P., 1969) **IADL** scale assesses a person's ability to perform tasks and may provide an early warning of functional decline or signal the need for further assessment. It measures eight domains such as preparing food, housekeeping, doing laundry, handling medications, getting to places beyond walking distance, going shopping, handling finances and using a telephone. The Lawton IADL scale scores from 0 (low function) to 8 (high function).

Competence in skills such as shopping, cooking, and managing finances is required for independent living. Because IADL function is usually lost

before ADL function (such as bathing, eating, and using the toilet), assessment of IADLs may identify incipient decline—physical, cognitive, or both—in an older adult who might otherwise appear capable and healthy. The mean value of the score was reported in the analysis.

The disability was also assessed by the Nagi scheme, developed in the 1960s by sociologist Saad Nagi. To patients it was asked whether they were able to perform some activities with or without help (Nagi S. and 1965).

Handgrip strength and ability to perform a five times chair stand test were included in the study. Handgrip strength was measured using a hand-held dynamometer (SMEDLYS' dynamometer, Scandidact, Kvistgaard, Denmark) for two performances with each hand. The best performance of these four was used for the analysis (Nybo H et al., 2001; Jeune B et al., 2006).

The 'repeated chair stand' is a screening test to assess the overall performance of the lower limbs (Wolinsky et al., 2005). The use of arms or aids such as canes was not permitted. In the chair stand test, participants were divided in two groups (able to complete the test and unable to complete the test).

2.2.4. Cognitive function

Cognitive function was measured using the **mini-mental state examination (MMSE)** (Folstein MF, 1975). This test is a brief 30-point questionnaire that is used to screen for cognitive impairment. It is commonly used in medicine to screen for dementia. Cognitive impairment was graded as severe (0-17 points), mild (18-23 points), and not present (24-30 points). The score used in the analysis was corrected by age and years of educations according to Magni for old people (Magni et al., 1996). The mean value of the score was reported in the analysis.

Age-range	*65-69*	*70-74*	*75-79*	*80-84*	*85-89*
Years of education					
0-4 years	*+0,4*	*+0,7*	*+1,0*	*+1,5*	*+2,2*
5-7 years	*-1,1*	*-0,7*	*-0,3*	*+0,4*	*+1,4*
8-12 years	*-2,0*	*-1,6*	*-1,0*	*-0,3*	*+0,8*
13-17 years	*-2,8*	*-2,3*	*-1,7*	*-0,9*	*+0,3*

Presence of depression was evaluated through Geriatric Depression Scale, GDS and graded as absent (0-5 score) or present (>5 points), (Yesavage et al. 1982; Sheikh JI, et al., 1986). The mean value of the score was reported in the analysis.

2.2.5. Perceived health

Perceived health status was assessed by EQ-5D and EQ VAS to obtain health profile. The EQ-5D descriptive system comprises five dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has three levels: no problems, some problems, extreme problems. The subjects were asked to indicate their health state. In the analysis the EQ-5D levels were dichotomized into "no problems" and "problems". EQ-VAS records the subjects self-rated health on a vertical, visual analogue scale where the endpoints are labeled "best imaginable health state" and "worst imaginable health state". This information can be used as a quantitative measure of health outcome as judged by the individual respondents. Moreover perceived health status was assessed by asking participants how they considered their health status with 5 possible answers: very good, good, fair, poor and very poor. In the analysis these responses were clustered in very good/good, fair, poor/very poor. Finally it was asked to participants which was their attitude towards life with three possible answers: optimistic, pessimistic and neither one nor other.

2.2.6. Prevalence of diseases

Participants were questioned about a list of 31 possible diseases, 7 of which occurred in the past and the remaining still affecting the patients. Each participant was classified according to the presence of 0, 1-2 or 3 or more diseases to obtain a multimorbidity index.

2.2.7.Anthropometric features

Body mass index (hg/m^2) was calculated using measured height and weight. Mean value and the number of subjects having a BMI over 25, indicating overweight were used in the analysis. The height data used for the analysis

were measured by the interviewers using a common metre. The weight data were measured using a common scale (SECA Mod. 761).

2.3. Immunophenotype characterization

The immunophenotype characterization was performed using fluorochrome-conjugated monoclonal antibodies (mAbs) direct against the following lymphocyte surface markers:

CD3 a T-Cell Co-Receptor protein complex composed of four distinct chains involved in T lymphocytes activation;

CD4 a glycoprotein expressed on the surface of T helper cells;

CD8 a transmembrane glycoprotein and it is predominantly expressed on the surface of cytotoxic T cells;

CD5 a cluster of differentiation found on a subset of IgM-secreting B cells called B-1 cells, and also on T cells;

CD19 a protein expressed on follicular dendritic cells and B cells;

CD16 the Fc receptors FcγRIIIa and FcγRIIIb. It is found on the surface of natural killer cells, neutrophil polymorphonuclear leukocytes, monocytes and macrophages;

CD56 also called Neural Cell Adhesion Molecule, NCAM, is a homophilic binding glycoprotein expressed on the surface of neurons, glia, skeletal muscle and natural killer cells;

CD28 one of the molecules expressed on T cells that provide co-stimulatory signals, which are required for T cell activation. CD28 is the receptor for B7.1 (CD80) and B7.2 (CD86);

CD95 also known as Fas receptor, FasR, is a death receptor on the surface of cells that leads to programmed cell death (apoptosis).

CD45RA CD45RA is an isoform of CD45 antigen which was originally called leukocyte common antigen. CD45RA is located on naive T cells;

CCR7 or CD197 is a C-C chemokine receptor type 7. It controls the migration property of T cells to lymph node and inflamed tissues, as well as stimulate dendritic cell maturation.

The identification of the major lymphocyte subsets (NK cells, B and T lymphocytes, helper and cytotoxic T lymphocytes, virgin/naïve, memory and effector T lymphocytes), some of them considered important for the immunological risk phenotype (IRP) (Strindhall J, et al., 2007) or immunological markers predictors of mortality, were performed by flow cytometry analysis on fresh blood sample using the following combination of mAbs: CD16/CD56/CD3 to identify NK cells ($CD16^+CD56^+CD3^-$), CD5/CD19 to identify B ($CD5^-CD19^+$), CD4/CD8/CD3 to identify cytotoxic ($CD4^-CD8^+CD3^+$) and helper ($CD4^+CD8^-CD3^+$) T lymphocytes, CD28/CD95/CD4-CD8 to identify virgin ($CD28^+CD95^-$), memory/activated ($CD28^+CD95^+$) and effector ($CD28^-CD95^+$) T lymphocytes, CD45RA/CCR7/CD4-CD8 to identify virgin ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), effector memory ($CD45RA^-CCR7^-$), and terminally differentiated ($CD45RA^+CCR7^-$) T lymphocytes.

2.3.1 Method

Surface staining was performed on whole blood with EDTA as anticoagulant agent, using the following mAbs: FITC, PE or PE-Cy7-conjugated mAbs directed against CD3, CD4, CD8, CD95, CD28, CD16, CD56, CD45RA, CCR7 and isotype-matched irrelevant Abs (all from BD Biosciences, San Jose, CA). One hundred microliters of blood were incubated with saturating amounts of the mAbs for 20 minutes at room temperature and then were lysed with FACS Lysing Solution (BD Biosciences). Three-parameter flow cytometric acquisition was performed on a FACSCalibur instrument (BD Biosciences) using CellQuest software (BD Biosciences). A minimum of 30,000 cells for each tube were acquired. The data collected by flow cytometry were then exported and analyzed with Flowjo Software. The percentage of B, T and NK cells was calculated on lymphocyte gate in accordance to Forward (FSC) and Side (SSC) scatter. The percentage of helper and cytotoxic T lymphocytes was calculated inside the CD3 gate making an electronic gate on cells positive to this marker. The analysis of the combined expression of CD95 and CD28 as well as CD45RA and CCR7 was done inside the CD4 and CD8 gate.

2.4. CMV serology

Indirect chemiluminescence immunoassay (Liaison CMV IgG assay; DiaSorin) was used for the quantitative determination of serum-specific IgG antibodies, Abs, to human CMV. Samples were analyzed by a photomultiplier Liaison. All donors recruited were classified CMV positive if they had the IgG levels higher 0.6 IU/mL and CMV negative otherwise.

2.5. Evaluation of plasma cytokines and IGF-1 levels

Concentration of pro- and anti-inflammatory cytokines and adipokines (IL-6, TNF-α, IL-10, leptin, resistin, adiponectin and serum amyloid A) in plasma samples of enrolled subjects were measured by multiplex sandwich ELISA technology (SearchLight, Aushon Biosystems, Billerica, MA) according to the manufacturer's instructions. The concentration of each analyte in the array was detected by biotin-streptavidin reaction and quantified by a SearchLight CCD Imaging System. In particular, 3 multiplex kits were utilized: a custom 3-Plex Array able to measure at same time IL-6, IL-10 and TNF-α a custom 2-Plex Array able to detect simultaneously leptin and resistin and a custom 2-Plex Array able to detect at the same time adiponectin and serum amyloid A. Samples, standards, and reagents were dispensed in the plates by a standardized technique employing a robotic liquid handling system with 16 channels (Microlab® STAR, Hamilton Robotics, Reno, NV).

Plasmatic TGF-β1 concentration was determined by ELISA using a commercial kit (DRG Instruments GmbH, Marburg, Germany) according to the manufacturer's instructions. The analytical sensitivity was 1.2 pg/ml. The interassay coefficient of variation was 7.5%. Concentration of TGF-β1 was detected and quantified by a Synergy™ HT Multi-Detection Microplate Reader (Bio-Tek® Instruments, Winooski, VT). The serum level of IGF-1 was measured using an enzymatic immunoassay kit (mediagnost®, Germany) following the manufacture's instruction. Total IGF-I was assayed by one-step sandwich chemiluminescence immunoassay (CLIA) after prior separation of IGF-I from binding proteins on the Liaison autoanalyzer

(DiaSorin, Saluggia, Italy). Intraassay and interassay CV were 4.4 and 5.5%, respectively.

2.6. Statistical Analysis

Preliminary examination of the difference between centenarians offspring and controls were performed using chi square analyses for qualitative variables and t test or Mann-Whitney non parametric U test for quantitative variables. When appropriate variables were log transformed.

Logistic regression was used to examine the relationship between qualitative and quantitative variables and offspring status. Age, gender, smoking, education, marital status, BMI and waist circumference were entered in the regression model as covariates.

Subjects suffering for malignancies and those that have taken anti-inflammatory medication in the week before the blood withdrawal were excluded from the cytokines and immunophenotype analysis. The statistical analysis was performed using SPSS 15.0 for windows.

3. Aim of the Study

Data on centenarians, centenarians siblings and offspring of long lived parents are compatible with the hypothesis that **longevity** and **successful aging** have a **strong familiar component**. An increasing amount of data indicates that centenarians offspring undergoes a better aging process than that of subjects of the same age born from non long-lived parents. In particular it seems that these individuals had a lower prevalence of the major age-related pathologies such as cardiovascular diseases (CVD), diabetes and hypertension but data regarding functional, cognitive and physical abilities, i.e. MMSE, hand grip, chair stand, and others, are very scant or absent.

In humans, it is becoming recognized that the immune system declines with age, a process named **immunosenescence**, leading to a higher incidence of infections, neoplasia and autoimmune diseases and contributing to determine the overall health status. Immunosenescence is characterized by a progressive reduction of peripheral virgin T cells, by an increase of memory T cells and of $CD28^-$ T lymphocytes accompanied by a progressive increase of $CD8^+$ T lymphocytes specific for viral epitope (CMV, EBV) and by a progressive development of a low-grade chronic inflammatory status, known as "inflammaging". Data concerning the immunological aspects i.e. lymphocyte subpopulation and pro and anti-inflammatory cytokines are not present in literature in centenarians' offspring model.

However at present it is still unknown how much longevity and successful aging is due to genetics. This is a crucial issue from a theoretical (biology) and practical (biomedicine and public health) point of view, and this research project is aimed to contribute to its clarification.

In conclusion, the aim of the study was to determine if subjects with at least a centenarian parent could experience a better health status compared to offspring of non long-lived parents considering not only socio-demographic, life-style and functional aspects but also inflammatory, metabolic and immunological parameters known to impact on the aging process.

For this purpose a group of centenarians offspring and offspring of non long-lived parents have been recruited and characterized by a great number of parameters able to determine their healthy status.

Centenarian's offspring have been chosen because they **represent** one of **the best models** to study the different components contributing to human aging and longevity without the disadvantages inherent in the study of centenarians such as rarity, lack of a control group of the same age, presence of frailty due to extreme age. In fact, centenarian's offspring are on average seventy years old, are more numerous than centenarians and it is possible to compare them with a demographically-matched control group (subjects matched for age, sex, ethnicity, parent year of birth, but born from non long-lived parents) thus avoiding cohort effects.

4. Results

4.1. Study Population

A total of 267 centenarians offspring (109 male, mean age 70.2 ± 6.3 and 158 female, mean age 70.2 ± 6.8) and 107 offspring of non long-lived parents (56 male, mean age 70.2 ± 6.3 and 51 female, mean age 72.0 ± 5.6) were recruited. After obtaining the informed consent, a standardized questionnaire was administered to each participant. The strategy of enrolment and management of the data are described in details in Material and Methods. Moreover a blood sample was collected for hematochemical analysis and immunophenotype staining. All the data were entered in a database to perform statistical analyses. The main characteristics of the study population were reported in Table I. Age was not different between the two groups while the proportion of females is higher in the group of centenarians offspring compared to controls (p=.04). Hereafter the terms *offspring of non long-lived parents* and *controls* will be used interchangeably.

Table I. Study Population

Study Population	Centenarians offspring, n=267	Offspring of non long-lived parents, n=107	p-value
Age, mean (SD), y	70.2 (6.6)	71.1 (6.0)	ns
Male, n (%)	109 (40.8)	56 (52.3)	.04
Female, n (%)	158 (59.2)	51 (47.7)	

Table I. Study Population. SD=standard deviation; y=years.

4.2. Demographic, lifestyle characteristics and functional status of participants

A large number of studies showed that the aging process differently affects males and females. In light of this evidence a first analysis was conducted separately for both sexes. Results concerning socio-demographic, life-style and functional aspects are reported in Table II and Table III.

Considering socio-demographic and life-style characteristics no significant difference was found between male centenarians offspring and male

offspring of non long-lived parents, while female centenarians offspring had a significant higher level of education and a different distribution within marital status compared to female controls (Table II).

Regarding the anthropometric variables no difference was found between male centenarians offspring and male controls whereas female centenarians offspring showed a significantly lower weight, waist and hip circumferences than female controls. Furthermore, the mean value of body mass index, BMI, as well as the proportion of subjects with a BMI over 25 were lower in female centenarians offspring compared to female controls.

In addition, since the simultaneous presence of more pathologies or ailments is a common feature of the aged people a multimorbidity index based on the pathologies or conditions that affected the subjects was created. Each enrolled individual was classified into three levels: (0, 1-2 and >2 illnesses).

A lower prevalence of female centenarians offspring with more than two pathologies was found compared with female controls and a similar trend was observed for men although it was not significant (Table II). No difference was found between the two groups both in males and in females in the other parameters shown in Table II.

In Table III cognitive status and physical performances of participants were indicated. Even in this case no difference was found between male centenarians offspring and controls while female centenarians offspring had obtained a better performance in the hand grip test and also a significantly higher proportion of these subjects was capable of performing a chair stand test, walking 500 meters and climbing up and down the stairs without aid than female controls.

In order to determine which variables were associated with the centenarians offspring condition a logistic regression analysis, adjusted for age and gender, was performed in the studied population.

In Table IV the baseline characteristics of the study population were reported. Life-style habits and education were not different between centenarians offspring and controls.

Furthermore a statistically significant different distribution of subjects between groups was observed concerning the marital status, with a higher percentage of singles and divorced in the centenarians offspring group than in controls.

Table II. *Baseline Characteristics of Participant*

Sociodemographic and life-style characteristics	Centenarians offspring	Offspring of non long-lived parents	p-value	OR (95% CI)
Male, n	109	56		
Age, mean (SD)	70.2 (6.3)	70.2 (6.3)	*.06*	
Life-stlyle				
Current smokers, n (%)	13 (12.3)	9 (16.4)	ns	
Former smokers, n (%)	56 (58.9)	27 (60.0)	ns	
Alcohol consumption, n (%)				
Education				
Years, mean (SD)	11.3 (5.2)	10.8 (4.8)	ns	
Higher education, n (%)	55 (51.4)	27 (50.0)	ns	0.98 (0.79-1.22)
Marital status				
Never married, n (%)	12 (11.1)	0 (0.0)		
Married, n (%)	84 (77.8)	48 (87.3)	*.06*	
Divorced, n (%)	6 (5.6)	2 (3.6)		
Widow/widower, n (%)	6 (5.6)	5 (9.1)		
Anthropometric features				
Weight, kg, mean (SD)	77.0 (12.3)	79.1 (12.4)	ns	
Height, m, mean (SD)	1.7 (7.0)	1.7 (6.8)	ns	
BMI, kg/m², mean (SD)	26.9 (3.4)	27.8 (3.9)	ns	
BMI>25, n (%)	73 (68.9)	41 (74.5)	ns	1.10 (0.88-1.38)
Waist circumference, mean (SD)	97.4 (10.8)	97.8 (10.1)	ns	
Hip circumference, mean (SD)	103.7 (9.0)	101.8 (14.9)	ns	
Multimorbidity Index				
0 diseases, n (%)	19 (17.9)	8 (14.5)		
1-2, n (%)	60 (56.6)	24 (43.6)	ns	
>2, n (%)	27 (25.5)	23 (41.8)		
Female, n	158	51		
Age, mean (SD)	70.2 (6.8)	72.0 (5.6)	ns	
Life-stlyle				
Current smokers, n (%)	25 (15.9)	9 (17.6)	ns	
Former smokers, n (%)	46 (35.4)	12 (28.6)	ns	
Alcohol consumption, n (%)				
Education				
Years, mean (SD)	11.0 (4.9)	9.3 (3.7)	**.01**	
Higher education, n (%)	75 (47.5)	13 (26.5)	**.01**	**0.82 (0.71-0.95)**
Marital status				
Never married, n (%)	31 (19.6)	2 (4.0)		
Married, n (%)	74 (46.8)	33 (66.0)	**.02**	
Divorced, n (%)	8 (5.1)	1 (2.0)		
Widow/widower, n (%)	45 (28.5)	14 (28.0)		
Anthropometric features				
Weight, kg, mean (SD)	64.6 (11.4)	70.7 (12.0)	**.002**	
Height, m, mean (SD)	1.6 (6.8)	1.6 (6.6)	ns	
BMI, kg/m², mean (SD)	26.3 (5.0)	28.3 (4.4)	**.01**	
BMI>25, n (%)	85 (55.2)	38 (77.6)	**.005**	**1.25 (1.08-1.45)**
Waist circumference, mean (SD)	86.5 (12.6)	92.4 (12.1)	**.004**	
Hip circumference, mean (SD)	103.3 (10.8)	107.8 (9.2)	**.01**	
Multimorbidity Index				
0 diseases, n (%)	16 (10.5)	0 (0.0)		
1-2, n (%)	58 (37.9)	13 (27.7)	**.01**	
>2, n (%)	79 (51.6)	34 (72.3)		

Table II. *Baseline Characteristics of Participants. SD=standard deviation; OR=odds ratio; BMI=Body Mass Index.*

Regarding the anthropometric features it was observed that centenarians offspring were leaner than controls considering weight and waist

circumference. Moreover it was found that centenarians offspring had a significantly lower BMI and a lower proportion of individuals with a BMI over 25 in comparison to controls, suggesting that not only gender but also genetic, environmental and life-style factors could explain these differences.

Table III. *Health and functional status of Participants*

Health and function	Centenarians offspring	Offspring of non long-lived parents	p-value	OR (95% CI)
Male, n	109	56		
ADL score, mean (SEM)	5.9 (0.3)	5.9 (0.5)	ns	
IADL score, mean (SEM)	7.9 (0.4)	7.7 (0.9)	ns	
MMSE score, mean (SEM)	27.6 (1.2)	27.3 (1.5)	ns	
Handgrip strength, Kg, mean (SD)	38.1 (7.3)	37.0 (7.4)	ns	
Ability to perform chair stand, n (%)	103 (97.2)	46 (88.5)	*.06*	2.07 (0.82-5.26)
Walk about half a kilometer w/o walking aid, n (%)	107 (99.1)	53 (96.4)	ns	2.01 (0.40-9.98)
Go up and down the stairs w/o anyone's help, n (%)	101 (93.5)	47 (85.5)	ns	1.68 (0.99-2.85)
Do the patient use any aid? n (%)	2 (1.9)	4 (7.3)	ns	2.02 (0.65-6.29)
Female, n	158	51		
ADL score, mean (SEM)	5.8 (0.6)	5.5 (1.0)	*.07*	
IADL score, mean (SEM)	7.9 (0.6)	7.7 (1.3)	ns	
MMSE score, mean (SEM)	27.6 (1.5)	26.9 (3.2)	ns	
Handgrip strength, Kg, mean (SD)	23.9 (5.5)	21.2 (5.0)	**.002**	
Ability to perform chair stand, n (%)	146 (95.4)	40 (80.0)	**.001**	**1.91 (1.07-3.38)**
Walk about half a kilometer w/o walking aid, n (%)	145 (92.9)	41 (82.0)	**.02**	**1.42 (0.95-2.12)**
Go up and down the stairs w/o anyone's help, n (%)	125 (79.6)	28 (56.0)	**.001**	**2.26 (1.40-6.06)**
Do the patient use any aid? n (%)	7 (4.5)	6 (12.0)	*.06*	1.44 (0.86-2.39)

Table III. *Health and functional status of Participants. SD=standard deviation; OR=odds ratio; SEM=standard error of the mean; w/o=without; Kg=kilograms; ADL= activities of daily living; IADL=instrumental activities of daily living; MMSE=mini mental state examination.*

No difference between centenarians offspring and controls was found both in systolic and diastolic pressure but we have to keep in mind that a higher proportion of controls took anti-hypertensive drugs (see Table IX).
Regarding the multimorbidity index a significantly different distribution was observed for the two groups with a lower proportion of centenarians offspring with more than two pathologies compared with controls.

Table IV. *Baseline Characteristics of Participants*

Sociodemographic and life-style characteristics	Centenarians offspring, n=267	Offspring of non long-lived parents, n=107	p-value*	OR (95% CI)
Life-style				
Current smoker, n (%)	102 (45.3)	39 (44.8)	ns	
Former smoker, n (%)	38 (14.4)	18 (17.0)	ns	
Alcohol consumption, n (%)	136 (52.5)	57 (54.8)	ns	
Education				
Years, mean (SD), y	11.1 (5.3)	10.1 (4.3)	*.08*	
Higher education, n (%)	130 (49.1)	40 (38.8)	*.06*	*1.56 (0.97-2.50)*
Marital Status				
Never married, n (%)	43 (16.2)	2 (1.9)		
Married, n (%)	158 (59.4)	81 (77.1)	**.01**	
Divorced, n (%)	14 (5.3)	3 (2.9)		
Widow/widower, n (%)	51 (19.2)	19 (18.1)		
Anthropometric features				
Weight, Kg, mean (SD)	70.0 (0.7)	74.3 (1.2)	**.002**	0.97 (0.95-0.99)
Height, m, mean (SD)	1.6 (0.004)	1.6 (0.01)	ns	
Waist circumference, cm mean (SD)	91.3 (0.7)	94.3 (1.1)	**.03**	0.98 (0.96-0.99)
Hip circumference, cm mean (SD)	103.4 (0.7)	104.7 (1.1)	ns	
BMI score, Kg/m², mean (SD)	26.5 (0.3)	28.0 (0.4)	**.005**	0.93 (0.88-0.98)
BMI>25, n (%)	158 (60.8)	79 (76.0)	**.01**	0.51 (0.30-0.86)
SBP, mmHg mean (SD)	134.6 (1.1)	135.8 (1.7)	ns	
DBP, mmHg mean (SD)	77.9 (0.5)	78.8 (0.9)	ns	
Comorbidity Index				
0 diseases	35 (13.5)	8 (7.8)		1
1-2 diseases	118 (45.6)	37 (36.3)	.01	0.68 (0.29-1.62)
>2 diseases	106 (40.9)	57 (55.9)		0.33 (0.14-0.80)

Table IV. *Baseline characteristics of Participants. SD=standard deviation; y=years; BMI=Body mass index; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; * adjusted for age and gender; OR=Odds Ratio; CI=confidence interval.*

Cognitive and physical performances were reported in Table V. Autonomy and self sufficiency in conducting the activity of everyday life was measured by ADL and IADL tests in which, although both groups were self-sufficient, centenarians offspring obtained a significant higher score than controls. Cognitive function and symptoms of depression were assessed by MMSE and GDS tests. Even in the case of MMSE, although both groups were not cognitive impaired, centenarians offspring obtained a better score than controls while the GDS score was not statistically different between the two groups. Physical performances were assessed through handgrip strength and chair stand tests. Better performances were obtained by centenarians offspring in both tests, with a significantly higher value of hand strength and a greater proportion of subjects able to perform the chair stand test without some aid.

Table V. *Health and functional status of Participants*

Functional Status	Centenarians offspring, n=267	Offspring of non long-lived parents, n=107	p-value*	OR (95% CI)
Autonomy and self sufficiency				
ADL score, mean (SEM)	5.9 (0.4)	5.7 (0.6)	**.02**	1.52 (1.03-2.26)
IADL score, mean (SEM)	7.9 (0.4)	7.7 (0.7)	.05	1.32 (0.98-1.78)
Cognitive function				
GDS score, mean (SEM)	3.0 (0.2)	2.7 (0.3)	ns	1.04 (0.96-1.14)
MMSE score, mean (SEM)	27.6 (0.1)	27.1 (0.2)	**.02**	1.17 (1.02-1.35)
Physical Performance				
Handgrip strength, Kg, mean (SD)	30.1 (0.4)	28.6 (0.6)	**.03**	1.04 (1.00-1.09)
Ability to perform chair stand, n (%)	249 (96.1)	86 (84.3)	**<.001**	4.76 (2.01-11.30)

Table V. Health and functional status of Participants; *ADL=Activities of Daily Living, IADL=Instrumental Activities of Daily Living; GDS=Geriatric Depression Scale; MMSE=Mini Mental State Examination; SEM=standard error of the mean; SD=standard deviation; *adjusted for age and gender; OR=Odds Ratio; CI=confidence interval*

Moreover disability assessment was evaluated by a test developed by S. Nagi as described in Materials and Methods. This test allows to evaluate if a person was capable to do things of normal everyday life in an indipendent manner or by using several aids.

Table VI. *Disability assessment of Participants*

Disability Assessment	Centenarian's offspring, n=266	Offspring of non long-lived parents, n=107	p-value*	OR (95%CI)
Is the patient able to?				
• Read a newspaper w/o glasses, n (%)	75 (28.3)	27 (25.7)	ns	1.15(0.68-1.93)
• Clearly recognize the face of someone 4 meters away w/o glasses, n (%)	220 (83.3)	88 (83.8)	ns	0.95 (0.51-1.76)
• Distinctly hear what is being said in a conversation w/o hearing aid, n (%)	248 (94.3)	97 (92.4)	ns	1.31 (0.53-3.24)
• Walk about half a kilometer w/o walking aid, n (%)	252 (95.5)	94 (89.5)	**.02**	**2.85 (1.16-6.96)**
• Go up and down the stairs w/o anyone's help, n (%)	226 (85.3)	75 (73.5)	**.001**	**2.89 (1.56-5.16)**
Do the patient use any aid? n (%)	9 (3.4)	10 (9.5)	**.02**	**0.33 (0.12-0.87)**

Table VI. Disability assessment of Participants; ** adjusted for age and gender; OR=Odds Ratio; CI=confidence interval*

Results indicate that no difference was found between centenarians offspring and controls concerning the ability of reading, recognize a face and hearing without aid while a higher proportion of centenarians offspring was able to walk about 500 meters and to go up and down the stairs without aid in comparison to controls (p=.02 and p=.002 respectively). Furthermore, a

lower proportion of centenarians offspring usually were using aid such as cane, chair-wheel, and others (Table VI).

Summarizing these data it emerges that **lower weight**, **waist circumferences** and **BMI**, as well as **higher score** in the **ADL, IADL**, **MMSE** and **handgrip** tests, together with the ability to perform **chair stand**, **to walk 500 meters**, to **climb stairs without aid,** the **multi-morbidity index** and the **use of aids** were **associated** with **centenarians offspring** (Table IV, Table V and Table VI).

In light of these results it was examined whether the anthropometric variables (BMI and waist circumference) and life-style habits i.e., smoking, education, marital status, could affect the better functional condition ascertained in centenarians offspring. To clarify this topic a regression analysis adjusted for the above mentioned covariates was performed.

This analysis indicated that the only variables associated with the condition of centenarians offspring that persist after adjustment were those related to better physical performance (**hand grip strength, ability to perform chair stand** and **going up and down the stairs**) (table VII).

TableVII. *Functional assessment of Participants*

Health and function	Centenarians offspring, n=266	Offspring of non long-lived parents, n=107	p-value[2]	OR[2] (95% CI)
Autonomy and self-sufficiency				
ADL score, mean (SEM)	5.9 (0.4)	5.7 (0.6)	ns	1.39 (0.92-2.09)
Cognitive function				
MMSE score, mean (SEM)	27.6 (0.1)	27.1 (0.2)	*.08*	1.14 (0.98-1.33)
Functional status				
Handgrip strength, Kg mean (SD)	30.1 (0.4)	28.6 (0.6)	**.01**	**1.06 (1.01-1.10)**
Ability to perform chair stand, n (%)	249 (96.1)	86 (84.3)	**.02**	**3.13 (1.18-8.30)**
Walk about half a kilometer w/o walking aid, n (%)	252 (95.5)	94 (89.5)	.ns	2.13 (0.74-5.89)
Go up and down the stairs w/o anyone's help, n (%)	226 (85.3)	75 (73.5)	**.005**	**2.53 (1.32-4.86)**
Do the patient use any aid? n (%)	9 (3.4)	10 (9.5)	*.06*	0.34 (0.11-1.07)

TableVII. *Functional assessment of Participants; ADL=Activities of Daily Living; MMSE=Mini Mental State Examination; SEM=standard error of the mean; SD=standard deviation; [2] adjusted for BMI, waist circumference, smoking, education, marital status, age and gender; OR=Odds Ratio; CI=confidence interval.*

4.3. Perceived health Status

Perceived health status was assessed by EQ-5D, EQ-VAS, asking participants how they considered their health status and asking them which was their attitude towards life. For all asked questions to participants no differences were found between centenarians offspring and controls, only

the EQ-VAS was statistically different between the groups with centenarians offspring indicating higher self-rated health score than controls (p=.05) (Table VIII).

Table VIII. *Perceived health status of Participants*

Perceived Health	Centenarians offspring, n=266	Offspring of non long-lived parents, n=107	p-value*	OR (95% CI)
Mobility				
No problems, n (%)	217 (82.2)	78 (74.3)	*.06*	*0.58 (0.33-1.03)*
Self-Care				
No problems, n (%)	252 (95.5)	100 (95.2)	ns	0.91 (0.30-2.80)
Usual Activities				
No problems, n (%)	244 (92.4)	92 (87.6)	ns	0.53 (0.24-1.17)
Pain/Discomfort				
No problems, n (%)	140 (53.0)	49 (46.7)	ns	0.74 (0.46-1.18)
Anxiety/Depression				
No problems, n (%)	154 (58.3)	57 (54.3)	ns	0.81 (0.51-1.28)
EQ-VAS				
Mean (SEM)	76.3 (1.0)	72.8 (1.5)	**.05**	**1.01 (1.00-1.03)**
How was their health in general				
Good/Very good, n (%)	165 (62.5)	52 (50.0)		1
Fair, n (%)	69 (26.1)	36 (34.6)	*.08*	0.64 (0.37-1.05)
Poor/Very poor, n (%)	30 (11.4)	16 (15.4)		0.51 (0.25-1.04)
How was their attitude towards life				
Optimistic, n (%)	129 (49.0)	59 (56.7)		1
Neither optimistic nor pessimistic, n (%)	84 (31.9)	30 (28.8)	ns	1.30 (0.79-2.25)
Pessimistic, n (%)	50 (19.0)	15 (14.4)		1.52 (0.78-2.95)

Table VIII Perceived health status of Participants; *SEM=standard error of the mean; * adjusted for age and gender; OR=Odds Ratio; CI=confidence interval*

4.4. Pharmacological therapy

Health status was also assessed evaluating both **pharmacological therapy** and **prevalence of diseases** in both groups.

Regarding **pharmacological therapy** a significant lower proportion of centenarians offspring was under medications than controls, and moreover among those taking drugs, centenarians offspring took a significantly lower number of medicines. Additionally the kind of drug used by participants was evaluated in order to know the most common diseases affecting the studied population. Results showed that a significant lower prevalence of centenarians offspring were under both cardiovascular, hypotensive and lipid-lowering therapy compared with controls. It is interesting to highlight that the percentage of centenarians offspring taking lipid-lowering drugs was half of that of controls. After adjusting for covariates all the considered parameters continued to be associated with offspring status except for cardiovascular therapy (data not shown). These differences were observed

both in males and in females (data not shown). No statistical significant differences between the two groups were found concerning the antidiabetic therapy even if a lower prevalence was observed in centenarian's offspring compared to controls (5.6 *vs* 9.4%). (Table IX)

Table IX. *Pharmacological status of Participants*

Pharmacological Therapy	Centenarian's offspring, n=266	Offspring of non long-lived parents, n=107	OR (95% CI)	p-value*
Number of prescribed medicines, mean (SD)	2.7 (0.1)	4.3 (0.2)	0.75 (0.67-0.84)	**<.001**
Subjects taking drugs, n (%)	208 (78.5)	98 (93.3)	0.25 (0.11-0.58)	**.001**
Cardiovascular Therapy, n (%)	54 (20.2)	36 (34.0)	0.52 (0.31-0.87)	**.01**
Hypotensive Therapy, n (%)	107 (40.1)	68 (64.2)	0.37 (0.23-0.61)	**<.001**
Antidiabetic Therapy, n (%)	15 (5.6)	10 (9.4)	0.57 (0.24-1.33)	.19
Lipid-lowering Therapy, n (%)	47 (17.6)	41 (38.7)	0.33 (0.20-0.55)	**<.001**

Table IX. Pharmacological status of Participants; *SD=standard deviation; * adjusted for age and gender; OR=Odds Ratio; CI=confidence interval.*

4.5. Prevalence of past and current diseases

Table X and table XI provide the number of prevalence case of past and present age-related diseases for centenarian's offspring and offspring of non long-lived parents. With regard to pathologies occurred in the past, it was found that centenarian's offspring had a **58% lower prevalence** of **myocardial infarction** (p=.049), a **80% lower prevalence of stroke**, **cerebral thrombosis/haemorrhage** (p=.011), and a **49 % lower prevalence of cancer** (p=.046) than offspring of non long lived parents. Centenarian's offspring also displayed **lower prevalence** of **benign tumour** even if it did not reach statistical significance (Table X).

Table X. *Prevalence of past pathologies*

Disease or Condition	Centenarians Offspring		Offspring of non long-lived parents		Prevalence Odds Ratio (95% C.I.)	p-value*
	N	n (%)	N	n (%)		
Past Pathologies						
Pneumonia	265	65 (24.5)	105	22 (21.0)	1.21 (0.70-2.10)	.500
Myocardial Infarction	265	12 (4.5)	105	12 (11.4)	0.42 (0.18-0.99)	**.049**
Stroke, cerebral thrombosis, hemorrhage	264	4 (1.5)	104	8 (7.7)	0.20 (0.06-0.70)	**.011**
Cancer	265	26 (9.8)	105	18 (17.1)	0.51 (0.27-0.99)	**.046**
Benign Tumor	266	11 (4.1)	105	10 (9.5)	0.42 (0.17-1.03)	*.057*
Hip fracture	265	5 (1.9)	105	4 (3.8)	0.48 (0.12-1.85)	.287
Spinal collapse	265	8 (3.0)	105	6 (5.7)	0.47 (0.16-1.41)	.178

** adjusted for age and gender; CI=confidence interval.*

With regard to pathologies that affected the patients at the time of the interview, was found that **centenarian's offspring** had a **46% lower prevalence of irregular heart rhythm** (p=.05), a **62% lower prevalence of hypertension** (p=.001) and a **51% lower prevalence of osteoporosis** (p=.03) than offspring of non long lived parents.

A lower prevalence of heart failure, COPD, hyperthyroidism, hypercholesterolemia, chronic renal insufficiency and cancer were observed in centenarian's offspring compared with offspring of non long lived parents but these differences did not reach statistical significance. No significant differences between centenarian's offspring and controls were found regarding the other pathologies (both past and present) including diabetes.

Table XI. *Prevalence of pathologies*

Disease or Condition	Centenarians Offspring		Offspring of non long-lived parents		Prevalence Odds Ratio (95% CI)	p-value*
	N	n (%)	N	n (%)		
Current Pathologies						
Heart disease						
Heart failure	265	2 (0.8)	105	4 (3.8)	0.23 (0.04-1.28)	*.092*
Irregular heart rhythm	265	29 (10.9)	106	20 (18.9)	0.54 (0.23-1.00)	**.050**
Angina Pectoris	264	8 (3.0)	105	4 (3.8)	0.95 (0.28-3.28)	.937
Vascular System						
Hypertension	265	107 (40.4)	106	67 (63.2)	0.38 (0.23-0.62)	**.001**
Venus insufficiency in legs	264	56 (21.2)	106	23 (31.7)	0.89 (0.51-1.57)	.701
Obstructive Arteriopathy	265	7 (2.6)	105	3 (2.9)	0.82 (0.20-3.30)	.782
Respiratory system						
COPD	265	9 (3.4)	105	9 (8.6)	0.38 (0.14-1.00)	*.052*
Asthma	265	9 (3.4)	105	7 (6.7)	0.58 (0.20-1.64)	.303
Nervous System						
Depression/Anxiety	265	41 (15.5)	105	22 (21.0)	0.66 (0.37-1.20)	.173
Endocrine System						
Diabetes	265	26 (9.8)	105	12 (11.4)	0.91 (0.44-1.90)	.800
Hyperthyroidism	265	3 (1.1)	105	4 (3.8)	0.23 (0.05-1.06)	*.060*
Hypothyroidism	265	25 (9.4)	105	8 (7.6)	1.06 (0.45-2.49)	.894
Osteoporosis	265	44(16.6)	105	24 (22.9)	0.49 (0.27-0.92)	**.026**
Hyperurichemia	265	19 (7.2)	105	5 (4.8)	1.55 (0.56-4.34)	.401
Hypercholesterolemia	265	91 (34.3)	105	44 (41.9)	0.63 (0.39-1.02)	*.062*
Artrosis	265	120 (45.3)	105	51 (48.6)	0.82 (0.51-1.30)	.395
Chronic renal insufficiency	265	3 (1.1)	105	5 (4.8)	0.24 (0.05-1.06)	*.059*
Cancer	265	1 (0.4)	105	3 (2.9)	0.13 (0.01-1.28)	*.081*
Other diseases#	263	85 (32.3)	106	52 (49.1)	0.51 (0.32-0.82)	**.005**

Table XI. Prevalence of pathologies; ** adjusted for age and gender; COPD= chronic obstructive pulmonary disease; CI=confidence interval; #comprised the following conditions: prostatic hypertrophy; cataract and glaucoma; gastritis; hiatus hernia and cholelithiasis; allergies; hemicrania, haemorrhoids, etc.*

4.6. Assessment of biochemical parameters and pro and anti-inflammatory mediators

The principal bio-hematochemical parameters were determined in centenarian's offspring and offspring of non long-lived parents. Although lower levels of **uric acid,** of **calcium,** of **IgA** and **IgG** were found in centenarians offspring compared to offspring of non long-lived parents all the values were within the reference values (table VII). Regarding the lipid profile it was observed that even adjusting for lipid-lowering therapy centenarians offspring showed a higher level of triglycerides and a lower level of HDL cholesterol different from expected.

Table XII. *Hematochemical parameters of the study population*

Parameters (reference values)	**Centenarians offspring, n=248**	**Offspring of non long-lived parents, n=91**	**p-value***
Glucose Profile			
Glycemia, mg/dl (65-110), mean (SD)	93.4 (29.9)	95.7 (27.4)	ns
Insulin, μIU/ml, mean (SD)	12.0 (9.6)	11.5 (6.5)	ns
HOMA-IR, mean (SD)	2.9 (2.8)	2.8 (2.2)	ns
Lipid Profile**			
Total Cholesterol, mg/dl (120-220), mean (SD)	203.9 (40.3)	194.5 (33.4)	ns
Triglycerides, mg/dl (40-170), mean (SD)	128.3 (66.2)	120.0 (57.3)	**.05**
HDL-Cholesterol, mg/dl (40-80), mean (SD)	55.4 (21.1)	60.0 (20.0)	**.02**
Urea, mg/dl (16-60), mean (SD)	40.8 (15.3)	39.0 (11.9)	ns
Creatinine, mg/dl (0.6-1.2), mean (SD)	0.8 (0.2)	0.9 (0.2)	ns
Uric Acid, mg/dl, (2.5-7.5), mean (SD)	4.9 (1.4)	5.3 (1.4)	**.05**
Albumine, g/dl (3.5-5.0), mean (SD)	4.0 (0.7)	4.0 (0.6)	ns
Direct Bilirubin, mg/dl (0-0.2), mean (SD)	0.1 (0.1)	0.1 (0.1)	ns
Total Bilirubin, mg/dl (0.2-1.2), mean (SD)	0.6 (0.3)	0.6 (0.4)	ns
Total Proteins, g/dl (6.6-8.7), mean (SD)	7.1 (0.8)	7.2 (0.7)	ns
AST, UI/l (10-45), mean (SD)	19.2 (7.8)	20.1 (7.0)	ns
ALT, UI/l (10-43), mean (SD)	13.9 (11.2)	14.7 (9.5)	ns
GGTS2, UI/l (7-33), mean (SD)	25.6 (25.0)	28.2 (32.3)	ns
ALP, UI/l (<170), mean (SD)	71.7 (45.6)	72.5 (31.7)	ns
Calcium, mg/dl (8-10.4), mean (SD)	8.7 (0.9)	9.0 (0.8)	**.001**
Ferrum, μg/dl (70-175), mean (SD)	97.8 (30.4)	99.0 (37.0)	ns
IgA, mg/dl (90-400), mean (SD)	251.2 (123.6)	305.6 (164.3)	**.002**
IgG, mg/dl (800-1800), mean (SD)	1,133.0 (658.5)	1,256.9 (444.4)	**<.001**
IgM, mg/dl (60-280), mean (SD)	123.1 (177.7)	115.5 (103.2)	ns

Table XII. *Data were expressed as mean with standatd deviation (SD) in parenthesis.*adjusted for age and gender;** adjusted for age, gender and lipid-lowering therapy;* HOMA-*IR= Homeostasis Model of Assessment - Insulin Resistance; HDL=high density lipoprotein AST=aspartate aminotransferase; ALT=alanine aminotransferase; GGTS=gamma glutamyl transferase; ALP=alkaline phosphatase;*

The inflammatory status was also determined in both groups by the determination of the most important pro and anti-inflammatory mediators.

Surprisingly no differences were found between the two groups regarding both pro and anti-inflammatory mediators whereas significantly lower IGF-1 levels were found in centenarians offspring compared to offspring of non long lived parents (Table XIII).

Table XIII. *Pro and anti-inflammatory mediators of the studied population*

Pro and anti-inflammatory mediators (reference value)	**Centenarians offspring, n=266** mean (SEM)	**Offspring of non long-lived parents, n=107** mean (SEM)	**p-value***
Pro-inflammatory mediators			
hs-CRP, (<1 mg/L)	2.6 (0.2)	2.8 (0.3)	ns
A-SAA, μg/ml#	4.2 (0.1)	4.0 (0.2)	ns
TNF-α, pg/ml#	2.1 (0.1)	2.2 (0.2)	ns
IL-6, pg/ml#	2.9 (0.06)	3.0 (0.09)	ns
Resistin, ng/ml#	2.2 (0.04)	2.2 (0.06)	ns
IGF-1, ng/ml	115.7 (2.7)	134.0 (4.5)	**.001**
Leptin, ng/ml#	2.5 (0.08)	2.7 (0.1)	ns
Anti-inflammatory mediators			
IL-10, pg/ml#	0.9 (.08)	1.0 (0.1)	ns
TGF-β1, ng/ml#	1.5 (0.4)	1.5 (0.7)	ns
Adiponectin, μg/ml#	3.3 (0.08)	3.4 (0.1)	ns

Table XIII. *Data were expressed as mean with standard error of the mean (SEM) in parenthesis; *adjusted for age and gender; #log transformed; hs-CRP=high sensitivity C reactive protein; A-SAA=serum amyloid A; TNF-alpha=Tumor necrosis factor alpha; IL-6=interleukin 6; IGF-1=insulin growth factor-1; IL-10=interleukin 10; TGF-beta1=Transforming growth factor beta 1; SEM=standard error of the mean.*

4.7. White blood cell count and immunophenotype analysis

Blood cell count was determined in centenarians offspring and offspring of non long-lived parents. As reported in Table IX all the values were in the normal range although an increase of monocytes (percentage) and of basophils (cells, 10^3/mmc) was found in control group compared with centenarians offspring.

Table XIV. Blood cell count in centenarians offspring and offspring of non long-lived parents

Blood count (reference values)	Centenarians offspring, n=248	Offspring of non long-lived parents, n=91	p-value
	Mean (SD)	Mean (SD)	
Leucocytes (4.2-9.0)			
cells, 10^3/mmc,	6.2 (1.4)	6.2 (1.9)	*ns*
Neutrophils			
% (40.0-74.0)	56.4 (7.7)	57.2 (8.4)	*ns*
cells, 10^3/mmc, (1.7-6.7)	3.6 (1.0)	3.7 (1.7)	*ns*
Lymphocytes			
% (19.0-48.0)	31.6 (7.6)	30.0 (7.0)	*ns*
cells, 10^3/mmc, (0.8-4.3)	2.0 (0.9)	2.2 (3.6)	*ns*
Monocytes			
% (3.0-9.0)	6.0 (1.5)	6.5 (1.7)	***.03***
cells, 10^3/mmc, (0.1-0.8)	0.4 (0.1)	0.4 (0.5)	*ns*
Eosinophils			
% (0.0-6.0)	3.0 (1.9)	3.3 (1.7)	*ns*
cells, 10^3/mmc, (0.0-0.6)	0.2 (0.1)	0.2 (0.1)	*ns*
Basophils			
% (0.0-1.5)	0.5 (0.3)	0.6 (0.3)	*ns*
cells, 10^3/mmc, (0.0-0.1)	0.03 (0.02)	0.04 (0.03)	***.04***

SD = Standard deviation

Moreover the principal lymphocyte subsets were analyzed in centenarians offspring and offspring of non long lived parents. All data were age and gender adjusted.

The percentage and absolute number of T and B lymphocytes were not different between centenarians offspring and controls while **$CD16^+CD56^+$ natural killer** cells were **significantly higher** in centenarian's offspring compared to offspring of non long lived parents (both percentage and absolute number) (Table XV).

Table XV. *Percentage and absolute number of T, B lymphocytes and of natural killer cells in centenarian's offspring and offspring of non long lived parents*

	Centenarian's Offspring, n=248	**Offspring of non long lived parents, n=91**	*p*-value
Lymphocyte subpopulations	median (25^{th}-75^{th})	median (25^{th}-75^{th})	
T lymphocytes, $CD3^+$			
percentage, %	67.20 (60.69-73.16)	68.99 (61.51-75.76)	**.013**
absolute number, cells/μl	1208.14 (969.33-1502.40)	1138.95 (916.00-1453.17)	.427
B lymphocytes, $CD19^+$			
percentage, %	7.42 (5.49-9.91)	7.68 (5.72-10.23)	.488
absolute number, cells/μl	135.02 (97.47-194.66)	132.75 (87.89-190.22)	.553
Natural Killer cells, $CD16^+CD56^+$			
percentage, %	12.00 (7.98-17.74)	11.80 (6.76-16.03)	**.041**
absolute number, cells/μl	219.80 (136.25-345.73)	190.47 (110.58-306.18)	**.013**

Table XV. Data were expressed as median of percentage and absolute number (/μl blood) with 25^{th}-75^{th} percentiles in parenthesis. P-value were obtained by regression analysis. Significant differences are highlighted in bold.

Helper and **cytotoxic T lymphocytes** (both percentage and absolute number) as well as the **CD4/CD8 ratio** did not change between centenarians offspring and controls (as reported in Table XVI).

Table XVI. *Percentage and absolute number of helper and cytotoxic T lymphocytes and the CD4/CD8 ratio in centenarian's offspring and offspring of non long lived parents*

	Centenarian's Offspring, n=248	**Offspring of non long lived parents, n=91**	*p*-value
Lymphocyte subpopulations	median (25^{th}-75^{th})	median (25^{th}-75^{th})	
Helper T lymphocytes, $CD4^+$			
percentage, %	63.37 (54.44-72.11)	62.86 (55.83-70.21)	.803
absolute number, cells/μl	729.44 (549.54-983.98)	735.24 (549.47-870.53)	.534
Cytotoxic T lymphocytes, $CD8^+$			
percentage, %	23.07 (19.05-31.90)	26.50 (20.10-33.66)	.248
absolute number, cells/μl	279.82 (197.31-422.83)	302.20 (210.48-414.40)	.930
CD4:CD8 ratio	2.70 (1.79-3.67)	2.44 (1.73-3.43)	.509

Table XVI. Data were expressed as median of percentage and absolute number (/μl blood) with 25^{th}-75^{th} percentiles in parenthesis. P-value were obtained by regression analysis.

Using a combination of surface markers such as CD28 in combination with CD95 and CCR7 in combination with CD45RA it has been possible to distinguish different lymphocyte subpopulations both inside the *helper* and cytotoxic T lymphocytes compartment. In particular CD28 and CD95 allowed us to identify cells with naïve, activated/memory, and effector phenotype while CCR7 and CD45RA enabled us to distinguish cells with naïve, central memory, effector memory and terminally differentiated phenotype.

Table XVII. *Percentage and absolute number of lymphocyte subsets within the helper compartment in centenarian's offspring and offspring of non long lived parents*

	Centenarian's Offspring, n=248	**Offspring of non long lived parents, n=91**	*p*-value
Lymphocyte subpopulations	median (25^{th}-75^{th})	median (25^{th}-75^{th})	
Naïve, $CD28^+/CD95^-$			
percentage, %	39.59 (27.79-49.75)	35.40 (24.09-48.28)	.473
absolute number, cells/μl	267.37 (171.00-453.10)	250.70 (154.62-400.01)	.469
Memory/Activated, $CD28^+/CD95^+$			
percentage, %	52.00 (41.98-62.31)	51.32 (43.56-66.13)	.412
absolute number, cells/μl	368.82 (274.48-488.56)	379.15 (269.09-504.56)	.648
Effector memory, $CD28^-/CD95^+$			
percentage, %	4.72 (1.70-9.93)	3.36 (1.18-9.96)	.845
absolute number, cells/μl	35.96 (12.07-78.40)	22.21 (9.63-65.40)	.287
Total $CD28^+$			
percentage, %	94.23 (88.09-97.23)	95.36 (89.06-97.63)	.839
absolute number, cells/μl	697.56 (485.70-888.60)	663.66 (494.97-834.38)	.808
Total $CD28^-$			
percentage, %	5.61 (2.31-12.19)	4.09 (1.47-10.08)	.489
absolute number, cells/μl	43.20 (16.00-91.26)	26.48 (11.75-68.29)	.195

Table *XVII*. Data were expressed as median of percentage and absolute number (/μl blood) with 25^{th}-75^{th} percentiles in parenthesis. P-value were obtained by regression analysis. Significant differences are highlighted in bold.

Both in the **$CD4^+$** and **$CD8^+$ T cells compartment** the lymphocyte subsets identified by CD28 and CD95 did not change between the two groups (table XVII and table XVIII). The only interesting observation was that the naïve $CD8^+CD28^+CD95^-$ T cells were higher in centenarians offspring than in control subjects although not statistically significant.

Table XVIII. *Percentage and absolute number of lymphocyte subsets within the cytotoxic compartment in centenarian's offspring and offspring of non long lived parents*

	Centenarian's Offspring, n=248	**Offspring of non long lived parents, n=91**	*p*-value
Lymphocyte subpopulations	median (25^{th}-75^{th})	median (25^{th}-75^{th})	
Naïve, $CD28^+/CD95^-$			
percentage, %	16.60 (9.18-26.82)	11.00 (5.52-21.65)	*.094*
absolute number, cells/μl	43.27 (24.50-78.40)	35.37 (18.77-63.39)	*.088*
Memory/Activated, $CD28^+/CD95^+$			
percentage, %	36.07 (26.79-50.51)	37.00 (22.37-50.56)	.710
absolute number, cells/μl	101.62 (65.70-151.94)	100.20 (64.76-141.21)	.854
Effector memory, $CD28^-/CD95^+$			
percentage, %	37.10 (23.16-53.91)	41.09 (25.33-58.71)	.304
absolute number, cells/μl	95.57 (54.31-184.09)	111.63 (61.80-205.03)	.954
Total $CD28^+$			
percentage, %	57.93 (41.10-74.10)	52.90 (37.06-72.03)	.177
absolute number, cells/μl	157.54 (108.56-214.62)	141.75 (107.95-201.70)	.453
Total $CD28^-$			
percentage, %	42.68 (28.12-58.65)	47.10 (31.90-64.55)	.254
absolute number, cells/μl	112.11 (59.78-203.22)	134.46 (70.16-229.84)	.798

Table XVIII. Data were expressed as median of percentage and absolute number (/μl blood) with 25^{th}-75^{th} percentiles in parenthesis. P-value were obtained by regression analysis. Significant differences are highlighted in bold.

When other markers such as CCR7 and CD45RA were considered statistical significant differences between the two groups appeared.

In particular, in the ***helper*** **compartment** an **increase** of **central memory** and concomitantly a **decrease** of **terminally differentiated** *helper* T lymphocytes was observed in **centenarian's offspring** compared to controls while naïve T cells did not change (Table XIX).

Table XIX. *Percentage and absolute number of lymphocyte subsets within the helper compartment in centenarian's offspring and offspring of non long lived parents*

	Centenarian's Offspring, n=248	**Offspring of non long lived parents, n=91**	*p*-value
Lymphocyte subpopulations	median (25^{th}-75^{th})	median (25^{th}-75^{th})	
Naïve, CD45RA$^+$/CCR7$^+$			
percentage, %	24.91 (17.89-34.62)	22.30 (15.92-32.40)	.153
absolute number, cells/µl	185.65 (106.08-310.50)	160.90 (100.50-248.50)	.119
Central Memory, CD45RA$^-$/CCR7$^+$			
percentage, %	16.98 (9.68-25.72)	13.10 (6.31-19.78)	**<.001**
absolute number, cells/µl	127.45 (62.13-204.45)	92.18 (37.76-153.15)	**<.001**
Effector memory, CD45RA$^-$/CCR7$^-$			
percentage, %	41.58 (29.62-50.23)	45.07 (34.46-56.74)	**.004**
absolute number, cells/µl	287.62 (204.60-385.65)	320.81 (234.07-402.88)	.184
Terminally differentiated, CD45RA$^+$/CCR7$^-$			
percentage, %	11.07 (6.03-16.79)	13.25 (7.08-18.90)	**.006**
absolute number, cells/µl	74.20 (40.20-131.34)	86.18 (47.81-151.29)	**.046**

Table XIX. Data were expressed as median of percentage and absolute number (/µl blood) with 25^{th}-75^{th} percentiles in parenthesis. P-value were obtained by regression analysis. Significant differences are highlighted in bold.

In the **cytotoxic compartment** an **increase** of **central memory** (only absolute number) and **naïve** cytotoxic T lymphocytes were observed in **centenarian's offspring** compared with offspring of non long lived parents while effector memory and terminally differentiated CD8$^+$ T lymphocytes did not change between the two groups as reported in Table XX.

Table XX. *Percentage and absolute number of lymphocyte subsets within the cytotoxic compartment in centenarian's offspring and offspring of non long lived parents*

	Centenarian's Offspring, n=248	**Offspring of non long lived parents, n=91**	*p*-value
Lymphocyte subpopulations	median (25^{th}-75^{th})	median (25^{th}-75^{th})	
Naïve, CD45RA$^+$/CCR7$^+$			
percentage, %	15.27 (9.78-23.04)	12.14 (7.28-18.73)	**.021**
absolute number, cells/μl	45.76 (23.35-70.10)	36.34 (19.15-54.74)	**.037**
Central Memory, CD45RA$^-$/CCR7$^+$			
percentage, %	6.49 (3.06-10.98)	5.12 (2.61-10.37)	.088
absolute number, cells/μl	17.88 (8.46-33.75)	13.48 (8.00-28.29)	**.033**
Effector memory, CD45RA$^-$/CCR7$^-$			
percentage, %	38.99 (30.13-51.72)	40.51 (29.82-50.05)	.905
absolute number, cells/μl	104.12 (72.31-172.47)	117.67 (76.42-170.99)	.927
Terminally differentiated, CD45RA$^+$/CCR7$^-$			
percentage, %	33.23 (18.96-46.22)	38.12 (22.25-50.79)	.133
absolute number, cells/μl	88.48 (45.52-159.16)	107.99 (46.82-189.08)	.398

Table XX. Data were expressed as median of percentage and absolute number (/μl blood) with 25^{th}-75^{th} percentiles in parenthesis. P-value were obtained by regression analysis. Significant differences are highlighted in bold.

4.8. Immunophenotype analysis in relation to the CMV seropositivity

Human cytomegalovirus (HCMV) is a member of a distinct, widely distributed subgroup of β herpesviruses. A large portion of humanity harbors this virus and its seroprevalence increases with age, ranging from 40–50% in 18–24-years old to 90% in 75–80-years old subjects. The human CMV induces a widespread infection whose effects depend on the efficacy of immune surveillance; in immunocompetent host the infection usually develops without morbid symptoms resulting in a latent infection.

HCMV infections are characterized by a dynamic, life-long interaction in which host immune responses, **particularly of T cells**, restrain viral replication and prevent disease but do not eliminate the virus or preclude transmission. It was estimated that the total of the HCMV-specific T cell responses in seropositive subjects were enormous, 10% on average of both the CD4 and CD8 memory compartments in blood and it is possible to speculate that it may increase in aged people. Human aging is characterized by expanded and altered adaptive immune responses to human CMV (HCMV) particularly of cytotoxic CD28- T cells. The age-dependent expansions of $CD8^{+}CD28^{-}$ T-cells, mostly positive for pro-inflammatory cytokines and including the majority of CMV-epitope-specific cells indicate that a major force able to drive a chronic pro-inflammatory state observed during aging may be represented by persistent viral infections like CMV. In light of these observations and because of HCMV infection can cause substantial perturbations in T cell subsets particularly in the elderly population, the **seropositivity** to the **human Cytomegalovirus (HCMV)** was evaluated in all the subjects recruited. It was found that 91.0% of the centenarian's offspring and 94.1% of offspring of non long-lived parents were CMV seropositive (not significant) (Table XXI).

Table XXI. CMV seropositivity

	Centenarians offspring, n=245	Offspring of non long-lived parents, n=101	p-value
CMV positivity, n (%)	223 (91.0)	95 (94.1)	ns

CMV=Cytomegalovirus

Despite this we wanted to answer the question *if the CMV infection could modify the lymphocyte subsets in a different manner in centenarians offspring and in controls.*

Results indicate that **T cell count** was significantly higher within **CMV-seropositive centenarian's offspring** compared with the CMV negative centenarians offspring, but it was not different within offspring of non long-lived parents. **CMV infection** seems **not** to be **associated** with a major alteration in the size of the **CD4+ T** cell pool both in centenarian's offspring and in offspring of non long-lived parents, but clear effects were observed on the **CD8+ T** cells. In fact, **CD8+ T** cell count increased both in centenarian's offspring and in offspring of non long-lived parents. This increase was of 47% and 80% higher in centenarian's offspring and in offspring of non long-lived parents respectively, compared with CMV uninfected subjects.

Additionally this finding have **implications** for the **CD4:CD8 ratio**. Indeed, the CD4:CD8 ratio was about **27%** (in **centenarian's offspring)** and about **56%** (in **offspring of non long lived-parents) lower** within **CMV-seropositive** subject compared with uninfected groups (Table XXII).

Table XXII. Absolute number of lymphocytes, T lymphocyte subsets in centenarian's offspring and offspring of non long lived parents

	Centenarians Offspring			Offspring of non long-lived parents		
	CMV- (n=22)	CMV+ (n=223)	p value	CMV- (n=6)	CMV+ (n=95)	p value
Lymphocyte	1.60 (1.26-2.19)	1.80 (1.58-2.20)	ns	1.05 (1.30-1.60)	1.70 (1.44-2.09)	ns
T cells, $CD3^+$	0.90 (0.73-1.49)	1.21 (0.97-1.23)	**.002**	1.13 (1.08-1.14)	1.14 (0.93-1.40)	ns
$CD4^+$ T cells	0.63 (0.50-0.86)	0.74 (0.55-0.97)	.27	0.88 (0.70-1.16)	0.73 (0.54-0.86)	.16
$CD8^+$ T cells	0.19 (0.11-0.25)	0.28 (0.20-0.39)	**<.001**	0.17 (0.15-0.25)	0.31 (0.21-0.41)	**.03**
CD4/CD8 ratio	3.59 (2.59-6.63)	2.61 (1.80-3.64)	**.005**	5.55 (5.47-5.95)	2.43 (1.71-3.43)	**.005**

Table *XXII* Data were expressed as median of absolute number ($X10^3/\mu l$) with 25^{th}-75^{th} percentiles in parenthesis. Significant differences are highlighted in bold. Statistical analysis was performed by the Mann-Whitney U nonparametric test.

Regarding the *helper* compartment, **CMV infection** is **strongly associated** with the expansion of **$CD4^+CD28^-$ T cells** in **centenarian's offspring** but not in offspring of non long lived parents compared with uninfected subjects.
On the contrary, **CMV infection** is **associated** with about a **50% decrease** of **$CD45RA^+CCR7^+$ naïve $CD4^+$ T cells** in **offspring of non long-lived parents** but not in centenarian's offspring (Table XXIII). Moreover CMV positive centenarians offspring showed a higher number of naïve $CD4^+$ T cells ($CD45RA^+CCR7^+$) compared to CMV positive controls (p=0.04) (figure V).

Table XXIII. Absolute number of T lymphocyte subsets within the helper compartment ($CD4^+$) in centenarian's offspring and offspring of non long lived parents

	Centenarian's Offspring			Offspring of non long lived parents		
	CMV- (n=22)	CMV+ (n=223)	p value	CMV- (n=6)	CMV+ (n=95)	p value
$CD28^+$	0.62 (0.51-0.81)	0.70 (0.47-0.87)	ns	0.86 (0.71-0.89)	0.66 (0.49-0.82)	ns
$CD28^-$	0.006 (0.004-0.01)	0.05 (0.02-0.09)	**<.001**	0.01 (0.005-0.02)	0.03 (0.01-0.07)	ns
$CD45RA^+CCR7^+$	0.17 (0.10-0.27)	0.18 (0.10-0.30)	ns	0.28 (0.28-0.30)	0.15 (0.09-0.22)	**.01**
$CD45RA^+CCR7^-$	0.06 (0.04-0.11)	0.08 (0.04-0.13)	ns	0.13 (0.11-0.15)	0.08 (0.04-0.15)	ns

Table *XXIII* Data were expressed as median of absolute number ($X10^3/\mu l$) with 25^{th}-75^{th} percentiles in parenthesis. Absolute number of *helper* T lymphocyte subsets i.e., naïve ($CD28^+$ and $CD45RA^+CCR7^+$) effector ($CD28^-$) and terminally differentiated ($CD45RA^+CCR7^-$). Significant differences are highlighted in bold. Statistical analysis was performed by the Mann-Whitney U nonparametric test.

Considering the **cytotoxic** compartment **CMV seropositive subjects** resulted **strongly associated** with the **$CD8^+CD28^-$ T cells** expansion (quite similar in magnitude) both in centenarian's offspring and in offspring of non long-lived parents compared with uninfected subjects. CMV seropositivity seemed **not to** modify the **$CD28^+$** and **$CD45RA^+CCR7^+$ naïve $CD8^+$ T cells** both in centenarian's offspring and in offspring of non long lived parents. However CMV positive centenarians offspring showed a higher number of naïve ($CD45RA^+CCR7^+$) $CD8^+$ T cells compared with CMV positive controls.
Furthermore, **CMV presence** increased **$CD45RA^+CCR7^-$ $CD8^+$ T cells (terminally differentiated effector cells)** more than **two fold** in **centenarians offspring** and of about **four fold** in **offspring of non long lived parents** compared with CMV negative subjects (Table XXIV).

Table XXIV. Absolute number of T lymphocyte subsets within the cytotoxic compartment ($CD8^+$) in centenarian's offspring and offspring of non long lived parents

	Centenarian's Offspring			Offspring of non long lived parents		
	CMV-	**CMV+**		**CMV-**	**CMV+**	
	(n=22)	**(n=223)**	**p value**	**(n=6)**	**(n=95)**	**p value**
$CD28^+$	0.13 (0.08-0.16)	0.16 (0.11-0.21)	ns	0.14 (0.14-0.15)	0.14 (0.10-0.20)	ns
CD28-	0.03 (0.01-0.07)	0.12 (0.06-0.20)	**<.001**	0.03 (0.02-0.06)	0.15 (0.08-0.23)	**.01**
$CD45RA^+CCR7^+$	0.03 (0.02-0.05)	0.04 (0.02-0.07)	ns	0.02 (0.01-0.04)	0.03 (0.02-0.05)	ns
$CD45RA^+CCR7^-$	0.04 (0.02-0.06)	0.09 (0.05-0.14)	**<.001**	0.02 (0.02-0.04)	0.11 (0.05-0.19)	**.02**

Table XXIV Data were expressed as median of absolute number ($X10^3/\mu l$) with 25^{th}-75^{th} percentiles in parenthesis. Absolute number of *cytotoxic* T lymphocyte subsets i.e. naïve ($CD28^+$ and $CD45RA^+CCR7^+$) effector ($CD28^-$) and terminally differentiated ($CD45RA^+CCR7^-$). Significant differences are highlighted in bold. Statistical analysis was performed by the Mann-Whitney U nonparametric test.

The lymphocytes subsets that were differently altered in the two groups in the presence of the CMV infection are reported in Figure V.

Figure V. Lymphocyte subsets and CMV status in the studied population

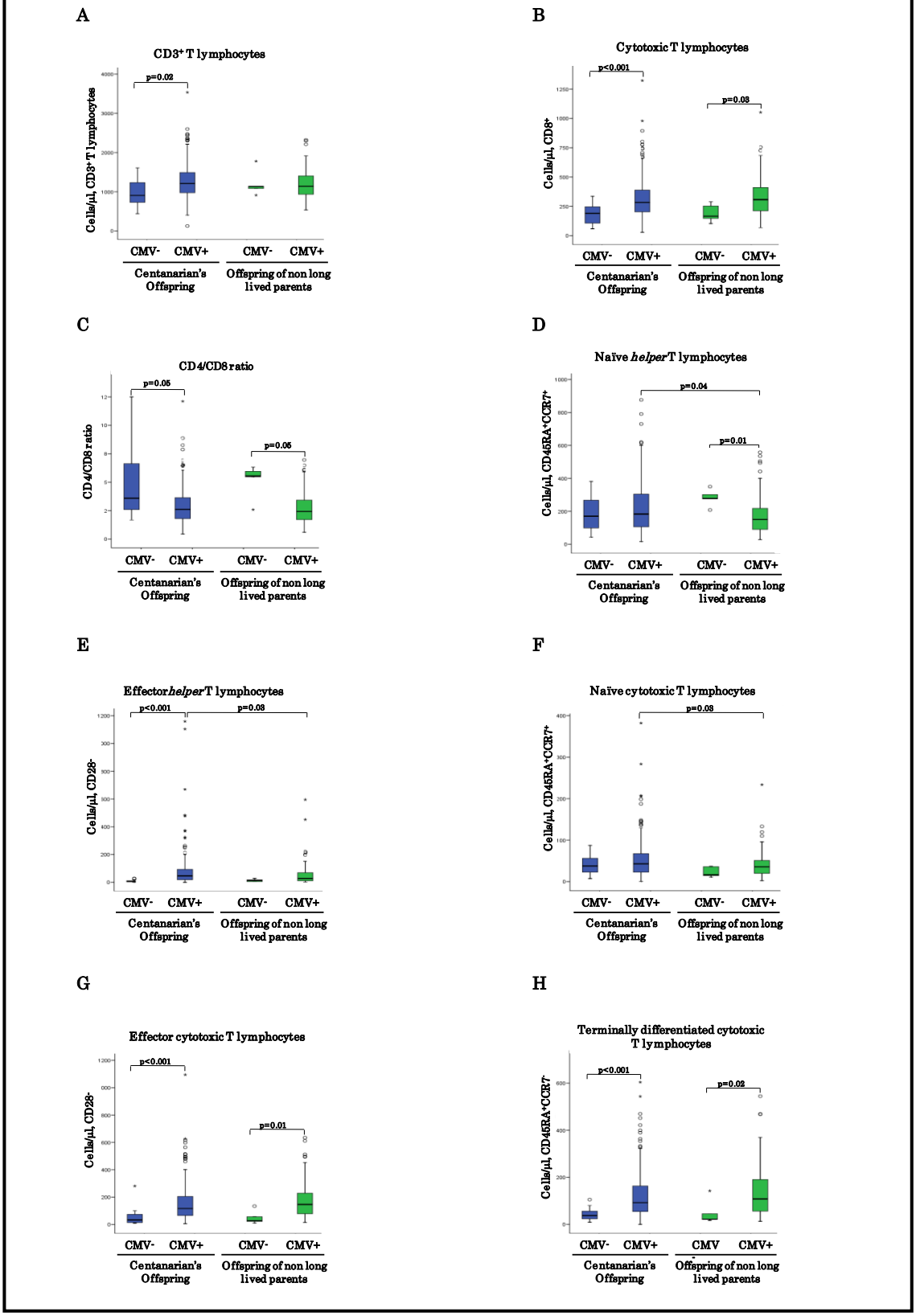

Figure V. Effect of CMV seropositivity on the frequency of $CD3^+$ T lymphocytes, cytotoxic and helper lymphocyte subsets as well as CD4/CD8 ratio. Box plots show the absolute count (median, 25th and 75th percentiles, range) of the lymphocyte subsets. $CD3^+$ T lymphocytes (A), cytotoxic $CD8^+$ T lymphocytes (B), CD4/CD8 ratio (C); naïve (D) ($CD45RA^+CCR7^+$) and effector (E) ($CD28^-$) T *helper* lymphocytes; naïve (F), effector (G) ($CD28^-$) and terminally differentiated (H) ($CD45RA^+CCR7^-$) cytotoxic T lymphocytes are shown.

5. Discussion

The aim of this work was to study the health, functional, inflammatory and immunological status of a group of centenarians offspring and offspring of non long-lived parents because of a plethora of literature data indicated that offspring of long-lived parents undergoes a better aging in terms of a lower prevalence of age-associated diseases (hypertension, cardiovascular disease and diabetes) and of a survival advantage regarding all causes, cancer and coronary heart disease-specific mortality.

It is of great importance to remember that the two groups of subjects studied show the same age, they were in their seventies and therefore they do not represent an extreme aging phenotype. Moreover the most of the recruited subjects could be considered healthy as evidenced by the hematochemical analysis in the ranges of normality (Table XII and Table XIV).

5.1. Anthropometric and functional status

With aging significant quantitative and qualitative changes occur in the body composition: intra abdominal fat tends to increase and subcutaneous fat on the limbs tends to decrease. In this study some **intriguing findings** emerge from the analysis of anthropometric features. Centenarian offspring showed a **significantly lower BMI**, **weight** and **waist circumference** compared with controls (**Table IV**). These results are of great importance because the decrease of body fat, particularly visceral adiposity, has been shown to extend life span and slow age-associated changes in several animal species including rodents and non human primates (Hadley et al., 2001). On the contrary an increase in body fat and BMI have been associated with insulin resistance (Paolisso et al., 2000) and increased risk for diabetes (Chan et al., 1994), heart disease (Willett et al., 1995) and metabolic disorders. The metabolic disorders including dyslipidemias, hypertension, and insulin resistance (Vega, 2001), could be less prevalent among the offspring of centenarians. In offspring of non long-lived parents the

contemporary presence of higher weight and BMI may worsen or highlight a hidden metabolic problem that could emerge with increasing age.

A great number of cross-sectional and longitudinal studies also indicate that weight gain and increased BMI, particularly obesity, contribute to decline in **walking** and **body transfer ability**. In light of this another exciting finding emerging from this study is that centenarians offspring appear to be more functionally fit than their age-matched controls (Table V and Table VI). Particularly **hand grip strength**, **ability to perform chair stand** and **go up and down the stairs** are associated with centenarians offspring status even after adjusting for age, gender, smoking, education, marital status, BMI and waist circumference (Table VII) indicating that not only life-style and anthropometric features but also genetic factors or multiple comorbidities may play a role in increasing the susceptibility to mobility loss. Evidence indicates that a poor performance in chair stand tests and loss of muscle strength are associated with adverse health outcomes in older persons (Cooper R et al., 2010 Cesari et al., 2006).

5.2. Health status

Considering the pharmacological therapy and prevalence of diseases it is interesting to note that centenarians offspring used fewer medications than controls, particularly those for cardiovascular diseases, hypertension and hypercholesterolemia (Table IX). Data about the lipid lowering therapy and those about the prevalence of hypercholesterolemia seems to indicate that about a half of centenarians offspring suffering of hypercholesterolemia is not medically treated. The discrepancy could be explained by the fact that these subjects suffer of a type of hypercholesterolemia that may be treated modifying life-style and nutrition habits. Moreover the data regarding the lipid profile indicate that centenarians offspring have a higher triglycerides and lower HDL cholesterol levels compared with controls (Table XII) . This apparent discrepancy could be explained by the fact that controls take a significantly higher number of lipid-lowering agents (Table IX). On the contrary there were no statistical significant differences regarding the use of

anti-diabetic medicine in line with the fact that also the proportion of subjects with diabetes was not different between the groups (Table IX and Table XI).

The decreased prevalence for age-related diseases in the offspring of centenarians is consistent with other research suggesting that long-lived parents have long-lived children. Prior studies suggest that both parents contribute to the heritability of life expectancy (Brand et al., 2006).

Intriguingly this study showed that centenarians offspring have had in their past fewer episodes of myocardial infarction, stroke, cerebral thrombosis and of cancer than offspring of non long-lived parents. Furthermore a lower prevalence of irregular heart rhythm, hypertension and osteoporosis was found in centenarians offspring compared with controls as already observed in other populations (Dellara et al., 2003; Dellara et al 2004a; Dellara et al., 2004b). The lower prevalence of hypertension, a recognized risk factor for stroke and heart attack, observed in centenarians offspring may protect them from the development of cardiovascular diseases. Weight loss, smoking cessation, increased physical activity and stress management may also help in controlling the blood pressure. The lack of differences for benign tumor, heart failure, diabetes, COPD, thyroid diseases, hypercholesterolemia and chronic renal insufficiency may be a function of the sample size, the choice of the controls, or it may be that families with exceptional longevity do not have differential susceptibility to these diseases.

Finally environmental factors, such as diet, were not taken into account in this analysis and might explain an important component of the observed differences.

5.3. Inflammatory status

A chronic low grade inflammatory status is a major feature of the aging process contributing to the development of a different pathological conditions (De Martinis et al., 2005; De Martinis et al., 2006). This peculiar inflammatory activity, leading to long-term tissue damage has been found to

be related to mortality risk from all causes in older persons (Bruunsgaard et al., 2001).

Plasma levels of some pro and anti inflammatory mediators were evaluated in centenarians offspring and offspring of non long-lived parents. Interestingly parameters related to systemic inflammation are not different between centenarians offspring and controls. The higher proportion of controls taking medication may explain this result or more simply it is plausible that differences between the two groups regarding the parameters measured could appear later in life with increasing age. Moreover the level of IGF-1 was also assessed. Intriguingly centenarians offspring show lower levels of IGF-1 compared with controls.

Low IGF-1 concentrations have been associated with sarcopenia and seem to predict cardiovascular mortality. Remarkably centenarians offspring, although having lower levels of IGF1 compared with controls, do not show the typical negative aspects related to a low IGF-1 such as loss of mass muscle power and cardiovascular diseases but, on the contrary they appeared to be more functionally fit (Table V, VI and VII) and seem to have a lower or postponed onset of cardiovascular diseases in comparison to controls (Table X and XI). This finding could be explained by the fact that low concentration of IGF-1 in offspring of centenarians could represent a constitutive trait inherited from the centenarian parent, known to have this peculiar characteristic but able to postpone the onset of main age related diseases reaching extreme lifespan. In humans there is evidence that subjects with decreased plasma IGF-I levels preserved insulin action compared with aged subjects, thus indicating that an efficient insulin response has an impact on human longevity (Paolisso G, et al., 1997 Paolisso et al., 2000). Moreover, it could be hypothesized that the decrease in plasma IGF-I observed in long-lived subjects might minimize the generalized mitogenic stimulus to tissues and contribute to the reduction in age-related pathologies due to the effect of IGF-I on cellular replication. This hormone is in fact linked to the development of several diseases, such as cancer (Wang et al., 1998), but the local expression (bioavailability) of IGF-I

may be an important factor contributing to the maintenance of normal tissue function. The inherently lower IGF-1 found in centenarians offspring could also explain, at least in part, the lower prevalence of cancer observed in centenarians offspring compared to controls.

In conclusion ccentenarians offspring appeared to be more fit and healthier than controls as could be inferred by the fact that they were leaner, more functionally active, unless pharmacologically treated and with a lower number of diseases affecting them as summarized in Table XXV.

Table XXV. Summary of the significant findings

Principal significant findings	Centenarians offspring	Offspring of non long-lived parents
Anthropometric features		
Weight, Kg	Lower	Higher
Waist circumference, cm	Lower	Higher
Body Mass Index, Kg/m^2	Lower	Higher
Functional status		
Handgrip strength, kg	Higher	Lower
Subjects able to perform chair stand	Higher	Lower
Subjects able to go up and down the stairs w/o aid	Higher	Lower
Pharmacological Therapy		
Mean number of medicines assumed	Lower	Higher
Number of subjects taking drugs	Lower	Higher
Subjects taking hypotensive drugs	Lower	Higher
Subjects taking lipid-lowering drugs	Lower	Higher
Diseases		
Past		
Myocardial Infarction	Lower	Higher
Stroke, cerebral thrombosis	Lower	Higher
Cancer	Lower	Higher
Present		
Irregular heart failure	Lower	Higher
Hypertension	Lower	Higher
Osteoporosis		
Presence of >2 pathologies or conditions	Lower	Higher
Cytokines and Hormones		
IGF-1, ng/ml	Lower	Higher

IGF-1=insulin like growth factor.

5.4. Immune System

An Immunophenotype staining was performed to evaluate the principal lymphocyte subsets in centenarians offspring and controls. Most of the lymphocyte subset does not change between the two groups. This is an expected result because of the main alterations in the B and T cell pool have been observed with increasing age. However some interesting findings emerge from this study. Significant results are summarized in Table XXVI.

Centenarians offspring have higher $CD16^{+}CD56^{+}$ NK cells (both percentage and absolute number). This subset of NK cells is highly cytotoxic compared with CD56- cells. Moreover through the expression of CD16 on their surface this subset is able to mediate the antibody-dependent cellular cytotoxicity (ADCC). Natural Killer cells represent the first line of defense against infections and developing malignancies. In humans it has been shown that NK cell cytotoxicity is well preserved in centenarians and that an increase in the actual number of NK cells can be observed in healthy aging (Franceschi et al., 1995; Sansoni et al., 1993). In disease states however, age-associated alterations in NK cell kinetics and function have been reported and include diminished proliferation rates (Zhang et al., 2007), association with higher incidences of infection (Ogata et al., 1997), onset of atherosclerosis (Bruunsgaard et al., 2001), and increased susceptibility to nutritional deficiencies (Ravaglia et al., 2000). Hence, the increase of NK cells found in centenarians offspring could protect them against a variety of infection and tumors.

Considering the acquired immunity, helper and cytotoxic T lymphocytes are not different between centenarians offspring and controls. An increase of central memory ($CD45RA^{-}CCR7^{+}$) helper (both percentage and absolute number) and cytotoxic (only absolute number) T lymphocytes was found in centenarians offspring compared with controls. These memory T helper and cytotoxic cells respond faster and with enhanced effector function compared to naïve cells and carry out their effector function by early production of cytokines such as IFNγ and IL4 or by helping B cells enhancing germinal

center reactions. Additionally by activating DC, memory CD4 T cells could enhance priming of CD8 T cells and, by the release of granzyme B and perforin, they could kill infected cells (MacLeod, 2009). The rise of central memory T cells in centenarians offspring is an interesting aspect that will require further and more in depth studies. On the contrary terminally differentiated helper T lymphocytes ($CD45RA^+CCR7^-$) are lower in centenarians offspring compared to offspring of non long lived parents. These cells are able to mediate effector functions, they are at their late stage of differentiation and had a poor proliferative capability. The presence of these cells is usually observed in particular settings like CMV or HIV infections although their role remains unknown to date.

Moreover another remarkable finding is the increase of naïve cytotoxic T lymphocytes in centenarians offspring in comparison with controls rendering the former more capable to respond to new antigens. It is likely that age-related morphological changes occurring in the thymus together with other factors, could be less pronounced in centenarians offspring with respect to controls (Aspinall et al., 2000). The age-associated reduction in naïve T cells and accumulation of $CD45RA^+$ terminally-differentiated effector memory T cells, are a hallmark features of immunosenescence, believed to contribute to the weakened immune status observed in the elderly.

5.4.1. Immune system and CMV

Cytomegalovirus is a ubiquitous herpesvirus and infection is so common that studies of lymphocyte phenotype and function are commonly undertaken in predominantly CMV^- seropositive cohorts. In our population the prevalence of CMV reaches about 95% in control subjects.

Comparing CMV positive centenarians offspring and offspring of non long-lived parents with their CMV negative donors, a different effects of the CMV positivity on lymphocyte subsets were observed in the two groups.

Particularly our data showed a marked effect of CMV infection in increasing $CD8^+$ T cells of greater extent in offspring of non long lived parents

reflecting in a more pronounced decrease of the CD4/CD8 ratio in the control group compared to centenarians offspring.

It is remarkable to note that, unexpectedly, the presence of CMV induces a great expansion of $CD4^+CD28^-$ T cells in centenarians offspring but not in controls on the contrary it decreased naïve helper T cells count in controls but not in centenarians offspring. Moreover CMV enormously increased $CD8^+CD28^-$ T cells in a similar manner in the two groups while it increased to a greater degree the effector $CD45RA^+CCR7^-$ $CD8^+$ T lymphocytes in controls compared with centenarians offspring. Surprisingly a change of naive $CD8^+$ T cells was not observed between CMV negative and positive donors in both groups. The differences observed in the magnitude of the change of lymphocyte subsets between the two groups may have different reasons/origins such as viral reactivation or, for example, the time of primary infection. In disagreement with findings observed by Derhovanessian et al., in the population studied, changes driven by CMV seropositivity occur both in centenarians offspring and in offspring of non long-lived subjects but they are different in magnitude.

Due to a higher prevalence of CMV in the population, a major limit of this part of the study is that numerically different groups were compared.

Table XXVI. Summary of the immunological significant findings

Immunological features	**Centenarians offspring**		**Offspring of non long-lived parents**	
Natural killer cells	Increase		Decrease	
T Cytotoxic compartment				
Central memory cytotoxic T lymphocytes, ($CD45RA^-CCR7^+$)	Increase		Decrease	
Naïve cytotoxic T lymphocytes, ($CD45RA^-CCR7^+$)	Increase		Decrease	
T *Helper* compartment				
Central memory helper T lymphocytes, ($CD45RA^-CCR7^+$)	Increase		Decrease	
Terminally differentiated helper T lymphocytes, ($CD45RA^+CCR7^-$)	Decrease		Increase	
Prevalence of CMV	not change		not change	
	CMV-	**CMV+**	**CMV-**	**CMV+**
$CD3^+$ T lymphocytes	↓	↑	not change	not change
$CD8^+$ T lymphocytes	↓↓	↑↑	↓↓↓	↑↑↑
CD4/CD8 ratio	↑	↓	↑↑	↓↓
Effector *helper* T lymphocytes, $CD28^-$	↓↓↓↓	↑↑↑↑	not change	not change
Naïve *helper* T lymphocytes, $CD4^+CD45RA^+$	not change	not change	↑↑	↓↓
Effector cytotoxic T lymphocytes, $CD28^-$	↓↓↓↓	↑↑↑↑	↓↓↓↓	↑↑↑↑
Terminally differentiated cytotoxic T lymphocytes, $CD45RA^+CCR7^-$	↓↓↓	↑↑↑	↓↓↓↓	↑↑↑↑

CMV=Cytomegalovirus

6. Conclusions and Perspectives

Data collected and analyzed in this work indicate that centenarians offspring seem to be healthier and more functionally fit than offspring of non long-lived parents.

It would be desirable to perform longitudinal studies on this highly selected population in order to evaluate the possible changes in the inflammatory settings that could emerge later in life. Moreover it would be of great interest to follow these subjects over time to verify whether centenarians offspring have a survival advantage for all-causes, cancer and cardiovascular-specific mortality compared with controls as shown for other populations.

7. References

Vaupel JW, Carey JR, Christensen K, Johnson TE, Yashin AI, Holm NV, Iachine IA, Kannisto V, Khazaeli AA, Liedo P, Longo VD, Zeng Y, Manton KG, Curtsinger JW. Biodemographic trajectories of longevity. Science. 1998 May 8;280(5365):855-60. Review

Brian T. Weinert and Poala S. Timiras. Theories of aging. Journal of Appiedl Physioogyl 95: 1706–1716, 2003 Invited Review

Baudisch, A. Inevitable Aging? Contributions to Evolutionary-Demographic Theory (Springer, 2008).

Finch, C. E. Longevity, Senescence and the Genome (Univ. Chicago Press, 1990)

Rose, M. R. Evolutionary Biology of Aging (Oxford Univ. Press, 1994).

Christensen, K., Doblhammer, G., Rau, R. & Vaupel, J. W. Aging populations: the challenges ahead. Lancet 374, 1196–1208 (2009)

Jagger C, Gillies C, Moscone F, Cambois E, Van Oyen H, Nusselder W, Robine JM; EHLEIS team. Inequalities in healthy life years in the 25 countries of the European Union in 2005: a cross-national meta-regression analysis. Lancet. 2008 Dec 20;372(9656):2124-31. Epub 2008 Nov 17. Erratum in: Lancet. 2009 Dec 20;372(9656):2114.

Parker, M. G., Schön, P., Lagergren, M. & Thorslund, M. Functional ability in the elderly Swedish population from 1980 to 2005. Eur. J. Aging 5, 299–309 (2008)

G. Passarino, C. Calignanoa, A. Vallonea, C. Franceschib, B. Jeunec, J.M. Robined, A.I. Yashine, L.L. Cavalli Sforzaf, G. De Benedictisa Male/female ratio in centenarians: a possible role played by population genetic structure, Experimental Gerontology 37 (2002) 1283–1289)

Oksuzyan, A., Juel, K., Vaupel, J. W. & Christensen, K. Men: good health and high mortality; sex differences in health and aging. Aging Clin. Exp. Res. 20, 91–102 (2008)

Austad, S. Why women live longer than men. Gend. Med. 3, 79–92 (2006)

Bonduriansky, R., Maklakov, A., Zajitschek, F. & Brooks, R. Sexual selection, sexual conflict and the evolution of aging and life span. Funct. Ecol. 22, 443–453 (2008)

Mair, W., Goymer, P., Pletcher, S. D. & Partridge, L. Demography of dietary restriction and death in Drosophila. Science 301, 1731–1733 (2003).

Vaupel JW. Biodemography of human aging. Nature. 2010 Mar 25;464(7288):536-42. Review.

Corder, E. H. et al. Apolipoprotein E genotype determines survival in the oldest old (85 years or older) who have good cognition. Arch. Neurol. 53, 418–422 (1996).

Walker, D. W., McColl, G., Jenkins, N. L., Harris, J. & Lithgow, G. J. Natural selection: evolution of lifespan in C. elegans. Nature 405, 296–297 (2000)

Friedman, D. B. & Johnson, T. E. A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. Genetics 118, 75–86 (1988)

Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. Nature. 1997 Oct 30;389(6654):994-9

McElwee, J., Bubb, K. & Thomas, J. H. Transcriptional outputs of the Caenorhabditis elegans forkhead protein DAF-16. Aging Cell 2, 111–121 (2003)

Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, Kenyon C. Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature. 2003 Jul 17;424(6946):277-83.

Tatar, M., Bartke, A. & Antebi, A. The endocrine regulation of aging by insulin-like signals. Science 299, 1346–1351 (2003).

Johnson TE, de Castro E, Hegi de Castro S, Cypser J, Henderson S, Tedesco P. Relationship between increased longevity and stress resistance as assessed through gerontogene mutations in Caenorhabditis elegans. Exp Gerontol. 2001 Nov;36(10):1609-17. Review

Herskind AM, McGue M, Holm NV, Sørensen TI, Harvald B, Vaupel JW. The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870-1900. Hum Genet. 1996 Mar;97(3):319-23

Hjelmborg, J. V. et al. Genetic influence on human lifespan and longevity. Human Genet. 119, 312–321 (2006

Iachine IA, Holm NV, Harris JR, Begun AZ, Iachina MK, Laitinen M, Kaprio J, Yashin AI. How heritable is individual susceptibility to death? The results of an analysis of survival data on Danish, Swedish and Finnish twins. Twin Res. 1998 Dec;1(4):196-205.

Vaupel JW, Carey JR, Christensen K, Johnson TE, Yashin AI, Holm NV, Iachine IA, Kannisto V, Khazaeli AA, Liedo P, Longo VD, Zeng Y, Manton KG, Curtsinger JW. Biodemographic trajectories of longevity. Science. 1998 May 8;280(5365):855-60. Review.

Perls TT, Wilmoth J, Levenson R, Drinkwater M, Cohen M, Bogan H, Joyce E, Brewster S, Kunkel L, Puca A. Life-long sustained mortality advantage of siblings of centenarians. Proc Natl Acad Sci U S A. 2002 Jun 11;99(12):8442-7.

Willcox BJ, Willcox DC, He Q, Curb JD, Suzuki M. Siblings of Okinawan centenarians share lifelong mortality advantages. J Gerontol A Biol Sci Med Sci. 2006 Apr;61(4):345-54.

Schoenmaker M, de Craen AJ, de Meijer PH, Beekman M, Blauw GJ, Slagboom PE, Westendorp RG. Evidence of genetic enrichment for exceptional survival using a family approach: the Leiden Longevity Study. Eur J Hum Genet. 2006 Jan;14(1):79-84.

Dellara F. Terry, Marsha Wilcox, Maegan A. McCormick, Elizabeth Lawler, and Thomas T. Perls Cardiovascular Advantages Among the Offspring of Centenarians Journal of Gerontology: MEDICAL SCIENCES Copyright 2003 by The Gerontological Society of America 2003, Vol. 58A, No. 5, 425–431;

Dellara F. Terry, Marsha A. Wilcox, Maegan A. McCormick, and Thomas T. Perls Cardiovascular Disease Delay in Centenarian Offspring Journal of Gerontology: MEDICAL SCIENCES Copyright 2004 by The Gerontological Society of America 2004, Vol. 59A, No. 4, 385–389. a

Dellara F. Terry, MD, MPH, Marsha A. Wilcox, EdD, ScD,w Maegan A. McCormick, BS,JaeMi Y. Pennington, BS, Emily A. Schoenhofen, BA, Stacy L. Andersen, BS, and Thomas T. Perls, MD, MPH Lower All-Cause, Cardiovascular, and Cancer Mortality in Centenarians' Offspring JAGS 52:2074–2076, 2004. b

IKEDA1, H. ISO , H. TOYOSHIMA, T. KONDO, T. MIZOUE, A. KOIZUMI, Y. INABA, A. TAMAKOSHI & JACC STUDY GROUP Parental longevity and mortality amongst Japanese men and women: the JACC Study Journal of Internal Medicine 2006; 259: 285–295.

Barzilai N, Atzmon G, Schechter C, Schaefer EJ, Cupples AL, Lipton R, Cheng S, Shuldiner AR. Unique lipoprotein phenotype and genotype associated with exceptional longevity. JAMA. 2003 Oct 15;290(15):2030-40

Gil Atzmon, PhD, Clyde Schechter, MD, William Greiner, RN, Deborah Davidson, MBA, Gad Rennert, MD, PhD, and Nir Barzilai, MD. Clinical Phenotype of Families with Longevity JAGS 52:274–277, 2004;

Emily R. Adams, BA, Vikki G. Nolan, MPH, Stacy L. Andersen, BS, Thomas T. Perls, MD, MPH, and Dellara F. Terry, MD, MPH Centenarian Offspring: Start Healthier and Stay Healthier. JAGS 2008).

De Martinis M, Franceschi C, Monti D, Ginaldi L. Inflammaging and lifelong antigenic load as major determinants of aging rate and longevity. FEBS Lett 2005; 579:2035–9;

Licastro F, Candore G, Lio D, Porcellini E, Colonna-Romano G, Franceschi C, Caruso C. Innate immunity and inflammation in aging: a key for understanding age-related diseases. Immun Aging 2005; 2:8)

van den Biggelaar AH, Huizinga TW, de Craen AJ, Gussekloo J, Heijmans BT, Frolich M, Westendorp RG. Impaired innate immunity predicts frailty in old age. The Leiden 85-plus study. Exp Gerontol 2004; 39:1407–14.

Penninx BW, Abbas H, Ambrosius W, Nicklas BJ, Davis C, Messier SP, Pahor M. Inflammatory markers and physical function among older adults with knee osteoarthritis. J Rheumatol. 2004 Oct;31(10):2027-31.

Ferrucci L, Penninx BW, Volpato S, Harris TB, Bandeen-Roche K, Balfour J, Leveille SG, Fried LP, Md JM. Change in muscle strength explains accelerated decline of physical function in older women with high interleukin-6 serum levels. J Am Geriatr Soc. 2002 Dec;50(12):1947-54.

Tracy RP, Lemaitre RN, Psaty BM, Ives DG, Evans RW, Cushman M, Meilahn EN, Kuller LH. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly. Results from the Cardiovascular Health Study and the Rural Health Promotion Project. Arterioscler Thromb Vasc Biol. 1997 Jun;17(6):1121-7.

Cesari M, Penninx BW, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K, Rubin SM, Ding J, Simonsick EM, Harris TB, Pahor M. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. Circulation. 2003 Nov 11;108(19):2317-22.

Kalogeropoulos A, Georgiopoulou V, Psaty BM, Rodondi N, Smith AL, Harrison DG, Liu Y, Hoffmann U, Bauer DC, Newman AB, Kritchevsky SB, Harris TB, Butler J; Health ABC Study Investigators. Inflammatory markers and incident heart failure
risk in older adults: the Health ABC (Health, Aging, and Body Composition) study. J Am Coll Cardiol. 2010 May 11;55(19):2129-37

Aviles RJ, Martin DO, Apperson-Hansen C, Houghtaling PL, Rautaharju P, Kronmal RA, Tracy RP, Van Wagoner DR, Psaty BM, Lauer MS, Chung MK. Inflammation as a risk factor for atrial fibrillation. Circulation. 2003 Dec 16;108(24):3006-10.

Ferrucci L, Corsi A, Lauretani F, Bandinelli S, Bartali B, Taub DD, Guralnik JM, Longo DL. The origins of age-related proinflammatory state. Blood. 2005 Mar 15;105(6):2294-9. Epub 2004 Nov 30.

Yaffe K, Lindquist K, Penninx BW, Simonsick EM, Pahor M, Kritchevsky S, Launer L, Kuller L, Rubin S, Harris T. Inflammatory markers and cognition in well-functioning African-American and white elders. Neurology. 2003 Jul 8;61(1):76-80.

Axelsson J, Bergsten A, Qureshi AR, Heimbürger O, Bárány P, Lönnqvist F, Lindholm B, Nordfors L, Alvestrand A, Stenvinkel P. Elevated resistin levels in chronic kidney disease are associated with decreased glomerular filtration rate
and inflammation, but not with insulin resistance. Kidney Int. 2006 Feb;69(3):596-604.

Zhang N, Ahsan MH, Purchio AF, West DB (2005). "Serum amyloid A-luciferase transgenic mice: response to sepsis, acute arthritis, and contact hypersensitivity and the effects of proteasome inhibition". J. Immunol. 174 (12): 8125–34)

Trayhurn P, Wood IS. Signalling role of adipose tissue: adipokines and inflammation in obesity. Biochem Soc Trans. 2005 Nov;33(Pt 5):1078-81. Review.

Fantuzzi G. Adiponectin and inflammation: consensus and controversy. J Allergy Clin Immunol. 2008 Feb;121(2):326-30. Epub 2007 Dec 3. Review.

Ukkola O, Santaniemi M (November 2002). "Adiponectin: a link between excess adiposity and associated comorbidities?". J. Mol. Med. 80 (11): 696–702

Matarese G, Moschos S, Mantzoros CS. Leptin in immunology. J Immunol. 2005 Mar 15;174(6):3137-42. Review.

Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. Resistin, an adipokine with potent proinflammatory properties. J Immunol. 2005;174:5789-5795.

Gomez CR, Boehmer ED, Kovacs EJ. The aging innate immune system. Curr Opin Immunol 2005; 17:457–62

Plowden J, Renshaw-Hoelscher M, Engleman C, Katz J, Sambhara S. Innate immunity in aging: impact on macrophage function. Aging Cell 2004; 3:161–7

Plackett TP, Boehmer ED, Faunce DE, Kovacs EJ. Aging and innate immune cells. J Leukoc Biol 2004; 76:291–9.

Mocchegiani E, Malavolta M. NK and NKT cell functions in immunosenescence. Aging Cell 2004; 3:177–84

Paolo Sansoni, Andrea Cossarizza, Vincenzo Brianti, Francesco Fagnoni, Gianluca Snelli, Daniela Monti, Antonio Marcato, Giovanni Passeri, Claudio Ortolani, Ermenegildo Forti, Umberto Fagiolo, Mario Passeri, and Claudio Franceschi. Lymphocyte Subsets and Natural Killer Cell Activity in Healthy Old People and Centenarians. Blood, Vol 82, No 9 (November l), 1993: pp 2767-2773

Borrego F, Alonso MC, Galiani MD, Carracedo J, Ramirez R, Ostos B, Pena J, Solana R. NK phenotypic markers and IL2 response in NK cells from elderly people. Exp Gerontol 1999; 34:253–65.

Cancro MP, Smith SH. Peripheral B cell selection and homeostasis. Immunol Res 2003; 27:141–8

Eaton-Bassiri AS, Mandik-Nayak L, Seo SJ, Madaio MP, Cancro MP, Erikson J. Alterations in splenic architecture and the localization of anti-double-stranded DNA B cells in aged mice. Int Immunol 2000; 12:915–26)

Zheng B, Han S, Takahashi Y, Kelsoe G. Immunosenescenceand germinal center reaction. Immunol Rev 1997; 160:63–77

Whisler RL, Grants IS. Age-related alterations in the activation and expression of phosphotyrosine kinases and protein kinase C (PKC) among human B cells. Mech Aging Dev 1993; 71:31– 46

Lazuardi L, Jenewein B, Wolf AM, Pfister G, Tzankov A, Grubeck-Loebenstein B. Age-related loss of naive T cells and dysregulation of T-cell/B-cell interactions in human lymph nodes. Immunology 2005; 114:37–43

Rink L, Cakman I, Kirchner H. Altered cytokine production in the elderly. Mech Aging Dev 1998; 102:199–209)

Miller JF. Immunological function of the thymus. Lancet 1961; 2:748–9

Li L, Hsu HC, Grizzle WE, Stockard CR, Ho KJ, Lott P, Yang PA, Zhang HG, Mountz JD. Cellular mechanism of thymic involution. Scand J Immunol. 2003 May;57(5):410-22. Erratum in: Scand J Immunol. 2003 Jul;58(1):118. William, GE [corrected to Grizzle, WE]

Flores KG, Li J, Sempowski GD, Haynes BF, Hale LP. Analysis of the human thymic perivascular space during aging. J Clin Invest 1999; 104:1031–9

Ostan R, Bucci L, Capri M, Salvioli S, Scurti M, Pini E, Monti D, Franceschi C. Immunosenescence and immunogenetics of human longevity. Neuroimmunomodulation. 2008;15(4-6):224-40. Review

Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM, Haynes BF, Polis MA, Haase AT, Feinberg MB, Sullivan JL, Jamieson BD, Zack JA, Picker LJ, Koup RA.
Changes in thymic function with age and during the treatment of HIV infection.Nature. 1998 Dec 17;396(6712):690-5

Jamieson BD, Douek DC, Killian S, Hultin LE, Scripture-Adams DD, Giorgi JV, Marelli D, Koup RA, Zack JA. Generation of functional thymocytes in the human adult. Immunity. 1999 May;10(5):569-75

Nasi M, Troiano L, Lugli E, Pinti M, Ferraresi R, Monterastelli E, Mussi C, Salvioli G, Franceschi C, Cossarizza A. Thymic output and functionality of the IL-7/IL-7 receptor system in centenarians: implications for the neolymphogenesis at the limit of human life. Aging Cell. 2006 Apr;5(2):167-75

Hulstaert F, Hannet I, Deneys V, Munhyeshuli V, Reichert T, De Bruyere M, Strauss K. Age-related changes in human blood lymphocyte subpopulations. II. Varying kinetics of percentage and absolute count measurements. Clin Immunol Immunopathol 1994; 70:152–8.

Goronzy JJ, Weyand CM. T cell development and receptor diversity during aging. Curr Opin Immunol 2005; 17:468–75

Tanchot C, Lemonnier FA, Perarnau B, Freitas AA, Rocha B. Differential requirements for survival and proliferation of CD8 naive or memory T cells. Science 1997; 276:2057–62

Messaoudi I, Lemaoult J, Guevara-Patino JA, Metzner BM, Nikolich-Zugich J. Age-related CD8 T cell clonal expansions constrict CD8 T cell repertoire and have the potential to impair immune defense. J Exp Med 2004; 200:1347–58

Weng NP. Aging of the immune system: how much can the adaptive immune system adapt? Immunity 2006; 24:495–9

Naylor K, Li G, Vallejo AN, Lee WW, Koetz K, Bryl E, Witkowski J, Fulbright J, Weyand CM, Goronzy JJ. The influence of age on T cell generation and TCR diversity. J Immunol. 2005 Jun 1;174(11):7446-52

Pawelec G, Sansom D, Rehbein A, Adibzadeh M, Beckman I. Decreased proliferative capacity and increased susceptibility to activation-induced cell death in late-passage human CD4+ TCR2+ cultured T cell clones. Exp Gerontol 1996; 31:655–68

Perillo NL, Naeim F, Walford RL, Effros RB. In vitro cellular aging in T-lymphocyte cultures. analysis of DNA content and cell size. Exp Cell Res 1993; 207:131–5

Akbar AN, Beverley PC, Salmon M. Will telomere erosion lead to a loss of T-cell memory? Nat Rev Immunol 2004; 4:737–43

Akbar AN, Fletcher JM. Memory T cell homeostasis and senescence during aging. Curr Opin Immunol 2005; 17:480–5

Fagnoni FF, Vescovini R, Passeri G, Bologna G, Pedrazzoni M, Lavagetto G, Casti A, Franceschi C, Passeri M, Sansoni P. Shortage of circulating naive CD8(+) T cells provides new insights on immunodeficiency in aging. Blood. 2000 May 1;95(9):2860-8.

Cossarizza A, Ortolani C, Paganelli R, Barbieri D, Monti D, Sansoni P, Fagiolo U, Castellani G, Bersani F, Londei M, Franceschi C. CD45 isoforms expression on CD4+ and CD8+ T cells throughout life, from newborns to centenarians: implications for T cell memory. Mech Aging Dev. 1996 Mar 29;86(3):173-95.

Fagnoni FF, Vescovini R, Mazzola M, Bologna G, Nigro E, Lavagetto G, Franceschi C, Passeri M, Sansoni P. Expansion of cytotoxic CD8+ CD28- T cells in healthy aging people, including centenarians. Immunology. 1996 Aug;88(4):501-7.

Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci. 2000 Jun;908:244-54. Review.

Franceschi C, Valensin S, Bonafè M, Paolisso G, Yashin AI, Monti D, De Benedictis G. The network and the remodeling theories of aging: historical background and new perspectives. Exp Gerontol. 2000 Sep;35(6-7):879-96. Review.

Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions [see comments]. Nature 1999; 401:708–712.

Strindhall J, Nilsson BO, Löfgren S, Ernerudh J, Pawelec G, Johansson B, Wikby A. No Immune Risk Profile among individuals who reach 100 years of age: findings from the Swedish NONA immune longitudinal study. Exp Gerontol. 2007 Aug;42(8):753-61. Epub 2007 May 21).

Vescovini R, Biasini C, Fagnoni FF, Telera AR, Zanlari L, Pedrazzoni M, Bucci L, Monti D, Medici MC, Chezzi C, Franceschi C, Sansoni P. Massive load of functional effector CD4+ and CD8+ T cells against cytomegalovirus in very old subjects. J Immunol. 2007 Sep 15;179(6):4283-91.

Vescovini R, Biasini C, Telera AR, Basaglia M, Stella A, Magalini F, Bucci L, Monti D, Lazzarotto T, Dal Monte P, Pedrazzoni M, Medici MC, Chezzi C, Franceschi C, Fagnoni FF, Sansoni P. Intense antiextracellular adaptive immune response to human cytomegalovirus in very old subjects with impaired health and cognitive and functional status. J Immunol. 2010 Mar 15;184(6):3242-9. Epub 2010 Feb 19.

Cheng J, Ke Q, Jin Z, Wang H, Kocher O, Morgan JP, Zhang J, Crumpacker CS. Cytomegalovirus infection causes an increase of arterial blood pressure. PLoS Pathog. 2009 May;5(5):e1000427. Epub 2009 May

Katz S, Downs TD, Cash HR, Grotz RC. Progress in development of the index of ADL. Gerontologist. 1970 Spring;10(1):20-30.

Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist. 1969 Autumn;9(3):179-86.

NAGI SZ, BURK RD, POTTER HR. BACK DISORDERS AND REHABILITATION ACHIEVEMENT. J Chronic Dis. 1965 Feb;18:181-97.

Nybo H, Gaist D, Jeune B, McGue M, Vaupel JW, Christensen K. Functional status and self-rated health in 2,262 nonagenarians: the Danish 1905 Cohort Survey. J Am Geriatr Soc. 2001 May;49(5):601-9.

Jeune B, Skytthe A, Cournil A, Greco V, Gampe J, Berardelli M, Andersen-Ranberg K, Passarino G, De Benedictis G, Robine JM. Handgrip strength among nonagenarians and centenarians in three European regions. J Gerontol A Biol Sci Med Sci. 2006 Jul;61(7):707-12

Wolinsky FD, Miller DK, Andresen EM, Malmstrom TK, Miller JP. Reproducibility of physical performance and physiologic assessments. J Aging Health. 2005 Apr;17(2):111-24.

Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975 Nov;12(3):189-98.

Magni E, Binetti G, Bianchetti A, Rozzini R, Trabucchi M: Mini-Mental state examination: a normative study in italian elderly population. Eur J Neurol 3:1-5, 1996

Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, Leirer VO. Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res. 1982-1983;17(1):37-49;

Sheikh JI, Yesavage JA: Geriatric Depression Scale (GDS): Recent evidence and development of a shorter version. Clinical Gerontology : A Guide to Assessment and Intervention 165-173, NY: The Haworth Press, 1986

Hadley EC, Dutta C, Finkelstein J, Harris TB, Lane MA, Roth GS, Sherman SS, Starke-Reed PE. Human implications of caloric restriction's effects on aging in laboratory animals: an overview of opportunities for research. J Gerontol A Biol Sci Med Sci. 2001 Mar;56 Spec No 1:5-6.

Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. Diabetes Care. 1994 Sep;17(9):961-9.

Willett WC, Manson JE, Stampfer MJ, Colditz GA, Rosner B, Speizer FE, Hennekens CH. Weight, weight change, and coronary heart disease in women. Risk within the 'normal' weight range. JAMA. 1995 Feb 8;273(6):461-5.

Vega GL. Obesity, the metabolic syndrome and cardiovascular disease. Am Heart J. 2001;142:1108–1116)

Brill PA, Probst JC, Greenhouse DL, Schell B, Macera CA. Clinical feasibility of a free-weight strength-training program for older adults. J Am Board Fam Pract. 1998 Nov-Dec;11(6):445-51

Cooper R, Kuh D, Hardy R; Mortality Review Group; FALCon and HALCyon Study Teams. Objectively measured physical capability levels and mortality: systematic review and meta-analysis. BMJ. 2010 Sep 9;341:c4467. doi: 10.1136/bmj.c4467. Review.

Cesari M, Leeuwenburgh C, Lauretani F, Onder G, Bandinelli S, Maraldi C, Guralnik JM, Pahor M, Ferrucci L. Frailty syndrome and skeletal muscle: results from the Invecchiare in Chianti study. Am J Clin Nutr. 2006 May;83(5):1142-8.

Brand FN, Kiely DK, Kannel WB, Myers RH. Family patterns of coronary heart disease mortality: the Framingham Longevity Study. J Clin Epidemiol. 1992;45:169–174

De Martinis M, Franceschi C, Monti D, Ginaldi L. Inflammation markers predicting frailty and mortality in the elderly. Exp Mol Pathol. 2006 Jun;80(3):219-27. Epub 2006 Feb 7. Review

Bruunsgaard H, Pedersen M, Pedersen BK. Aging and proinflammatory cytokines. Curr Opin Hematol. 2001 May;8(3):131-6. Review.

Paolisso G, Ammendola S, Del Buono A, Gambardella A, Riondino M, Tagliamonte MR, Rizzo MR, Carella C, Varricchio M 1997 Serum levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 in healthy centenarians: relationship with plasma leptin and lipid concentration, insulinaction and cognitive function. J Clin Endocrinol Metab 82, 2204–2209)

Paolisso G, Barbieri M, Bonafe` M, Franceschi C 2000 Metabolic age remodeling: the lessons from centenarians. Eur J Clin Invest 30:888–894

Wang YZ, Wong YC 1998 Sex hormone induced prostatic carcinogenesis in the noble rat: the role of insulin like growth factor-1 (IGF-I) and vascular endothelial growth factor (VEGF) in the development of prostate cancer. Prostate 35:165–177

Franceschi C, Monti D, Sansoni P, Cossarizza A. The immunology of exceptional individuals: the lesson of centenarians. Immunol Today. 1995 Jan;16(1):12-6. Review

Zhang Y, Wallace DL, de Lara CM, Ghattas H, Asquith B, Worth A, Griffin GE, Taylor GP, Tough DF, Beverley PC, Macallan DC. In vivo kinetics of human natural killer cells: the effects of aging and acute and chronic viral infection. Immunology. 2007 Jun;121(2):258-65. Epub 2007 Mar 7.

Bruunsgaard H, Pedersen AN, Schroll M, Skinhøj P, Pedersen BK. Decreased natural killer cell activity is associated with atherosclerosis in elderly humans. Exp Gerontol. 2001 Dec;37(1):127-36

Ravaglia G, Forti P, Maioli F, Bastagli L, Facchini A, Mariani E, Savarino L, Sassi S, Cucinotta D, Lenaz G. Effect of micronutrient status on natural killer cell immune function in healthy free-living subjects aged >/=90 y. Am J Clin Nutr. 2000 Feb;71(2):590-8.

MacLeod MK, Clambey ET, Kappler JW, Marrack P. CD4 memory T cells: what are they and what can they do? Semin Immunol. 2009 Apr;21(2):53-61. Epub 2009 Mar 9. Review.

Aspinall R, Andrew D: Thymic involution in aging. J Clin Immunol 2000; 20: 250–256

Derhovanessian E, Maier AB, Beck R, Jahn G, Hähnel K, Slagboom PE, de Craen AJ, Westendorp RG, Pawelec G. Hallmark features of immunosenescence are absent in familial longevity. J Immunol. 2010 Oct 15;185(8):4618-24. Epub 2010 Sep 20.

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