

Review

Bioactive Nutrients and Nutrigenomics in Age-Related Diseases

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Abstract: The increase in the average lifespan and the consequent proportional growth of the elderly segment of society has furthered the interest in studying ageing processes. Ageing may be considered a multifactorial process derived from the interaction between genetic and environmental factors including lifestyle. There is ample evidence in many species that the maximum age attainable (maximum lifespan potential, MLSP) is genetically determined and several mitochondrial DNA polymorphisms are associated with longevity. Many studies have shown that most of the phenotypic characteristics observed in the aging process are the result of the occurrence, with age, of a low grade chronic pro-inflammatory status called "inflammaging", partially under genetic control. The term indicate that aging is accompanied by a low degree of chronic inflammatory, an up-regulation of inflammatory response and that inflammatory changes are common to many age-related diseases. Therefore, the theory of oxidation-inflammation was proposed as the main cause of aging. Accordingly, the chronic oxidative stress, that appears with age, affects all cells and especially those of the regulatory systems, such as the nervous, endocrine, and immune systems and the communication between them. This prevents an adequate homeostasis and, therefore, the preservation of health. It was also proposed that the immune system plays a key role in the aging process, specifically in the rate of aging, since there is a relationship between the redox state and functional capacity of immune cells and longevity of individuals. Moreover, the role of the immune system in senescence could be of universal application. A confirmation of the central role of the immune system in oxi-inflamm-aging is that the administrationintake? of adequate amounts of antioxidants in the diet improves immune function, decreases their oxidative stress, and consequently increases longevity. The promotion of healthy lifestyles is one of the major goals of governments and international agencies all over the world. Human molecular processes are influenced by both physiological pathways and exogenous factors which include, for instance, those originating from diet. Dietary intake has substantive effects on molecular processes of metabolic health. Nutrients can directly regulate physiological changes in human body. In fact, in addition to have an energetic and structural value, nutritional intake provides bioactive molecules which are selectively able to modulate specific metabolic pathways, noticeably affecting cardiovascular and neoplastic diseases development or progress. Numerous bioactive nutrients are being progressively identified and their chemopreventive effects are being described at clinical and molecular mechanism levels. Systematic analyses comprise all "omics" technologies (such as transcriptomics, proteomics and metabolomics) and the goal is to investigate bioactive molecules effects derived from the diet. Nutrigenomic knowledge on physiologic status and disease risk will provide both developments of better diagnostic procedures and of new therapeutic strategies specifically targeted on nutritionally relevant processes. The present review was aimed to understand the molecular mechanisms underlying beneficial effects of bioactive nutrients and nutrigenomics on age-related diseases.

Key words: aging; bioactive nutrients; dietary; nutrigenomics; Oxiinflammaging

Introduction

Ageing may be considered a multifactorial process derived from the interaction between genetic and environmental factors including lifestyle. Old age in human beings is characterised by the onset of several diseases which, although not unique to old age, are nonetheless rather strictly correlated to it, the physiological decline over time being a pivotal factor in the increase of the risk the elderly have of developing said diseases [1, 2].

The view of ageing as a complex multifactorial process has superseded previous monofactorial theories which attributed the phenomenon of ageing to one single cause [3]. In fact, some of the phenomena which characterise physiological ageing can be explained by individual theories, but no one single theory is able to account for the ageing process in its entirety.

The leading theories which attempt to explain the phenomenon of ageing are: the genetic theory, the theory of cellular ageing, the neuroendocrine theory, the immunological theory, the free-radicals theory, and the network theory. Let us look at each of them in some detail.

The *genetic theory* suggest that ageing is the direct consequence of a genetic program. There are extensive data which support the theory that the lifespan of each animal species is regulated by genetic factors. In the case of the human species, for example, there are families whose members are fairly consistently long-lived, and diseases such as the Down's syndrome, Werner syndrome, Hutchinson Gilford syndrome (early ageing in children), which are characterised by an acceleration of the ageing process and a shortened lifespan, are hereditary [4,5]. Some studies do not so much point to the existence of "gerontogenes", genes, that is, which actively determine ageing, but rather to that of "longevity assurance genes", genes which guarantee a long lasting life. Extreme longevity appears to be related to a complex genetic pattern rather than a few isolated genes [4,5].

The *cellular ageing theory* hypothesises that cellular senescence is the process that limits the number of divisions normal human cells can accomplish in a culture [6]. Famous experiment by Hayflick has proven that embryo fibroblasts taken from different animal species can replicate a finite number of times, proportional to the maximum lifespan of the individuals of the species [6]. This limit in the cells' ability to replicate is reached after a characteristic number of cell divisions [7-10] (the Hayflick limit), but can also be brought about as a reaction to specific molecular events.

Replicative senescence can therefore be considered a cause of ageing in its own right, since it is correlated to the number of cell divisions as determined by the length of the telomeres, while stress-induced senescence is a response to stress, more specifically to a sudden modification of the genome and damage to the DNA. Cell senescence should therefore be considered the cell's response to age-related alterations, which in turn intensify or accelerate organic ageing. This view of senescence is compatible with the miscellaneous theories of damage build-up (such as the free-radicals one), which may explain the ultimate cause of cell senescence by means of ageing [11].

The *neuroendocrine theory* hypothesises that ageing is the consequence of a dysregulation of the neuroendocrine system [12-14]. During an organism's lifetime, chronic exposure to physical, biological, or emotional stress may overtax or damage its adaptive capacity, thus leading to the so called adaptation diseases and death [15, 16]. The amalgamating of reactions to external stimuli seems to be carried out by the hypothalamus via information provided by assorted brain structures mainly the cerebral cortex, the limbic lobe, and the reticular formation. In response to signals from the hypothalamus, the pituitary gland produces and secretes hormones which regulate crucial bodily functions and stimulates peripheral endocrine glands such as the adrenal gland, the thyroid, and the gonads [11]. These glands, in turn, elaborate and secrete hormones which carry their role remotely, via the blood flow. This is a closed-circuit system with mutual control and interference mechanisms which regulate hormone production according to the needs of the body, needs that vary greatly over time in response to the most disparate of circumstances (heat-cold, food-fast, activity-rest, sleep-vigil, stress in the broadest sense of the word). Such a sensitive biological mechanism may over time become at risk of functional alterations and trigger irreversible modifications in all organs and apparatuses [17-27].

The *Immunologic Theory* of ageing hypothesises that the normal ageing process in man is pathogenetically correlated to a dysregulation of the immune system [28]. The term

"immunosenescence" was coined to denote the ageing-related decreased immunocompetence which makes elderly individuals more susceptible to diseases and increases the morbidity and mortality due to diseases linked to infectious agents, compared to the young. The term immunosenescence denotes not only a decreased resistance to infections, but, according to some authors, an increased susceptibility to cancer and a diminished capacity of self-recognition, leading to autoimmune diseases. Indeed, the ageing-related changes to the immune system determine an impairment in the response to vaccines as well as to acute infections.

The most obvious changes ageing brings to the immune system are:

1. Thymus involution, which results in a decreased production of T cells, responsible for acquired and cell-mediated immunity [29];
2. Lymphocytic remodelling closely connected to the T-cell compartment [30-42];
3. A modification of the cytokines' pattern, related to the secretion of both pro- and anti-inflammatory cytokines [43-54].

The *Free Radicals Theory* is based on the evidence that living organisms (aerobes) produce oxygen-centered free radicals, inducing irreversible damage to biological structures. These are formed inside body cells when oxygen is used in metabolic processes in order to produce energy. Mitochondrial respiration produces Reactive Oxygen Species (ROS) by leakage of intermediates from the electron transport chain [55]. These molecules are highly unstable because they have an unpaired electron; they therefore seek to achieve a stable state by appropriating electrons from nearby molecules, these in turn become unstable and so on, thus creating an instability chain reaction.

The *Network Theory* was first proposed in 1989 by Franceschi et al. [56]; it is defined as "unifying" in that it is a modern synthesis of previous theories [57]. According to the network theory it is accepted a priori that ageing depends on genetic factors as well as environmental ones. The organism is exposed to both endogenous and exogenous damaging agents and it tries to eliminate them and maintain its homeostasis. These stress agents may be physical in nature (radiations, heat), chemical (toxic metabolites, free radicals), or biological (viruses and pathogenic microorganisms) and may trigger defence mechanisms, the most important among which are DNA repair processes, antioxidant systems, anti-inflammatory cytokines, Heat Shock Proteins (HSPs) production, activation of poly-ADP-ribose polymerase (PARP, a DNA repairing nuclear enzyme), and apoptosis, understood as the ancestral process by which damaged, mutated, infected, or transformed cells are eliminated [38, 40]. It is an actual network of integrated and interconnected mechanisms, a veritable defence network [38, 40].

These cellular and molecular processes have been layered one on top of the other throughout evolution [40].

Inflammaging

The term inflammaging was coined by Claudio Franceschi [45] to indicate that ageing is accompanied by a low degree of chronic inflammation and an up-regulation of the inflammatory response, and that inflammatory changes are common to many age-related diseases [58-63]. Multiple persistent weak stimuli (endogenous and exogenous toxins) cause a prolonged commitment of body's adaptive systems with multiple low-grade inflammatory responses, which tend to become chronic and often asymptomatic. Indeed, patients report vague and nonspecific signs and systemic order symptoms with complex diagnostic definition. Inflammaging is characterized by five conditions: low-grade, controlled, asymptomatic, chronic and systemic inflammation [64]. So inflammaging is the up-regulation of a variety of stress responses at the cellular and molecular levels. Inflammaging is the result of the body's ability to adapt to and counter the effects of a variety of stress factors that cause the accumulation of molecular and cellular damage.

Physiopathogenesis

The relationship between chronic systemic inflammation and ageing is now widely accepted. Many studies have shown that most of the phenotypic characteristics observed in the ageing process are the result of the occurrence, with age, of a low grade chronic pro-inflammatory state, called

"inflammaging", partially under genetic control. This state seems to be the result of continuous antigenic stimulation that continues beyond the reproductive age and therefore widely not expected by the evolution [52, 65, 66]. The resulting tissue damage appears to be related to death risk in elderly people and also appears to be deleterious to longevity [67]. Some studies have identified genetic, cellular, and serological markers of inflammaging, such as an immunophenotype characterised by a decrease of naive T cells and an accumulation of memory cells, increased levels of pro-inflammatory cytokines, and significant alterations in the frequency of functional or pro- or anti-inflammatory polymorphisms [54, 68, 69]. The inflammaging is characterised by the activation of macrophages and the expansion of specific T cell clones (megaclones) directed to common virus antigens such as the Cytomegalovirus (CMV) and the Epstein-Barr virus (EBV) [70-74]. In order to clarify the roles of CMV disease, 121 subjects have been recently studied. The subjects were between 25 and 100 years old: 18 subjects were serum negative and 103 serum positive for CMV infection [75]. It was also observed that the age-related reduction of CD8⁺ naive T cells was accelerated in CMV⁺ subjects. The reduction of naive CD8⁺ cells was accompanied by a progressive increase in CD8⁺ effector T cells, CD28⁻, in CMV⁺ subjects. Therefore, the CMV seropositivity seems to be associated with many phenotypic and functional alterations of T cell immunity considered biomarkers of ageing [75]. Another chronic infection that could play a key role in the immune-inflammatory response in the elderly is caused by Herpes Simplex Virus (HSV) that appears to play a role as cofactor in damages involved in Alzheimer's disease (AD) [76, 77]. A recent prospective study has shown an increased risk of AD in patients seropositive for IgM-HSV, probably responsible for progressive chronic damage [78]. Inflammaging is a complex inflammatory response to various endogenous and environmental stimuli due mainly to the increase in circulating levels of pro-inflammatory cytokines. Cytokines are a class of soluble proteins responsible for the communication between the different components of the immune system. Cytokines play an important role in inflammation by acting on the targeting, regulation, and termination of inflammatory processes. It has been determined that these molecules also play a central role in the ageing processes. During ageing there is a reversible decrease in the levels of IL2 cytokine, which is important in the development of Th1 and population in increased production of pro-inflammatory mediators such as IL1, IL6, and TNF α [40]. The increase in the age-related inflammatory markers (inflammaging) could be the basis of the reduced ability for elderly subjects to cope with various stressors. The inflammaging process may also generate reactive oxygen species (ROS) that cause oxidative damage and elicit an increase in the release of cytokines, perpetuating a vicious circle resulting in a chronic pro-inflammatory state where tissue damage and repair mechanisms proceed simultaneously. In this case the damage accumulates slowly and asymptotically over the years, resulting in ageing and in the development of age-related diseases [59-64]. Reactive oxygen species (ROS) are also capable of inducing profound effects on gene expression and are implicated in the pathogenesis of many age-related diseases such as atherosclerosis, type II diabetes, neurodegeneration, osteoporosis and osteoarthritis, all pathologies which share a strong inflammatory/immunological component. Moreover, oxidative stress and ROS are active inducers of apoptosis and may act as mediators influencing other transcription factors such as NF- κ B and AP-1 [68]. Alterations in apoptosis due to ageing may therefore explain some of the most important aspects of immunosenescence [79], such as the accumulation of memory cells, expanded megaclones, the restriction of the repertoire of T lymphocytes, and the increase in the incidence of autoimmune phenomena. The cellular and molecular mechanisms related to the body's ability to appropriately respond to a series of oxidative stresses and inflammations appear to play an important role in promoting human longevity and in avoiding/delaying the major age-related diseases. The participation of inflammatory cells and molecules in the pathogenesis of many age-related diseases such as atherosclerosis, Alzheimer, and Parkinson's disease is well documented. Conversely the control of inflammations may allow successful ageing that can be better achieved as is evident in centenarians. They are the living example of successful ageing, the desirable type of ageing, free from chronic debilitating diseases, and which preserves physical self-sufficiency and mental health. These subjects are a highly selected group who have lived for over a hundred years without the onset of any major age-related diseases; the study of these subjects could detect the

biological basis of ageing or the combination of genes and lifestyle that have allowed these people to avoid the aforementioned age-associated diseases. The "INCHIANTI Study", concluded in 2004 [80], showed that elderly subjects have high levels of IL-6, CRP, and IL-1 compared to young subjects in good health. A further study on a group of Italian centenarians showed that subjects genetically predisposed to produce IL-6 in old age have a reduced ability to reach the outer limits of human life.

Another study by Lyons et al. [62] on a group of centenarians assessed the levels of two cytokines, the anti-inflammatory protein IL-10 and the TNF-alpha, a protein that promotes inflammation. The study showed that centenarians express genes encoding high levels of IL-10 and low levels of TNF-alpha compared to the control group of younger subjects. Studies on the elderly and centenarians have shown that the frequency of variants (polymorphisms) of key genes involved in immune response and low-grade inflammation are present with different frequencies in centenarians by comparison to the young [74,81, 82]. The presence of an anti-inflammatory genotype in centenarians suggests that chronic inflammation is a key predictive marker of mortality and morbidity in senescence. Signs of inflammaging have also been found unexpectedly in healthy centenarians, together with an increase of inflammatory markers, such as IL10 and TGF-beta [83, 84]. It seems that beside the existent inflammatory phenomena, anti-inflammatory phenomena (antiflammaging) are also present and are equally important for longevity, and that longevity is the result of balancing these two conflicting responses [85]. A long life, which exceeds 90 years of age seems to have a strong genetic basis, which explains why the almost-one-hundred-years-old and centenarians are grouped into families. Longevity seems to be greatly influenced by a complex genetic pattern and not by a few isolated genes. It is easy to assume that genes and genetic variants associated with strong immune responses and inflammation have been selected and this has helped to ensure survival in reproductive age. It has been proposed that an effective inflammatory response useful for the resolution of infection in young age may become the cause of much pathology such as arthritis, diabetes, cardiovascular and neurodegenerative diseases in old age. The double biological role of inflammation, positive when young and negative when older, is consistent with the antagonistic pleiotropy theory that a gene can have opposite effects at different periods of life. Inflammation itself is not a negative phenomenon; indeed, in response to various stimuli, the immune system implements a complex series of local and systemic reactions that prevent tissue damage, isolate and destroy any infectious agents and activate the repair process. The damage is related to the increase in life expectancy not anticipated by evolution. When life expectancy is prolonged it means that immune system continues to react against external agents for decades longer than expected. The increased antigenic load eventually establishes a chronic inflammation that contributes to the deterioration of various organs, becoming a risk factor for all typical chronic diseases of old age. Indeed, the elderly that have higher blood levels of an acute phase protein, the PCR, are subject to chronic inflammatory diseases.

The systemic consequences determine a pattern of changes that goes by the name of "fragility" (chronic inflammatory disease or frailty) and epidemiological studies suggest that low grade inflammation observed in the ageing process promotes an atherogenic profile that is related to other chronic inflammatory diseases typical of old age. Other genetic and environmental factors that promote the diseases keep all their importance and even determinate what will be the principal organ affected; the differences in the inflammatory status help to explain why not all the subjects develop age-related disease, even under the same risk factors. The permanent exposure to a variety of infectious agents for a long period (chronic antigenic load) influences the ageing of the immune system (Immunosenescence) [58, 86].

So the potential of our immune system, genetically determined, is gradually depleted over time, in relation with the pathogenic antigen aggression. The improved hygienic conditions typical of industrialized countries may have reduced significantly the antigenic overload, preserving longer the immune system and avoiding a rapid depletion. This factor, in addition to reduced mortality due to acute infectious diseases, may have contributed to an increased life expectancy and to the increase in the number of subjects who reach the extreme limit of life.

Related Disorders

Inflammation is a chronic systemic cause of many age-related diseases such as atherosclerosis and cardiovascular illness, Alzheimer's disease and cancer.

Recent research has shown that chronic systemic inflammation contributes to anxiety, depression, cognitive decline, insulin resistance and adult-onset diabetes, obesity, and Parkinson's disease.

Cancer

Cancer is a disease that occurs mostly in elderly subjects. The average age for diagnosis in industrialized countries is approaching 70 years of age and it is expected to increase [87]. This may be due to various reasons such as increased duration of exposure to carcinogenic factors, stronger cell susceptibility to environmental carcinogens [88, 89] or to the immunosenescence in elderly [90]. Over the past 15 years a strong link between chronic inflammations, induced by chemical, biological, mechanical or physical lesions, and cancer has been discovered. For example, bowel inflammatory disease, ulcerative colitis and Crohn's disease are clinical conditions predisposing to the development of large intestine or terminal ileum cancer [91, 92]. *H. pylori* is a microorganism associated with atrophic gastritis, mucosal dysplasia, gastric adenocarcinoma. Inflammation plays an important role in the development of solid tumours such as colon cancer. This was clearly demonstrated by a prospective case-control study of 22887 adults followed for 11 years, where 172 cases of colon cancer were identified. In these patients plasma concentrations of CRP were higher compared to control cases [93]. Cancer susceptibility and severity may be associated with functional polymorphisms of cytokine genes involved in regulating inflammation. In particular, polymorphisms affecting IL6 and IL10 genes may influence susceptibility and in some cases the prognosis of cancer. Many different mechanisms may also link inflammation to cancer:

1. Induction of angiogenesis by inflammatory factors promotes cancer progression [94].
2. Increased release of pro inflammatory factors and certain cytokines such as IL1, TNF and interferon are both involved in inflammation and cancer development [94, 95].
3. Free radicals production promotes carcinogenesis [94].

Inflammatory state is the crucial mediator of the intermediate stages of tumour development. In cancer, genetic damage is the fuse that triggers the fire and inflammation is the fuel that feeds it. In 1978 Alberto Mantovani observed that innate immunity cells tend to cluster around some tumours [96]. Then Pollard et al. showed that cancer cells "reeducate" macrophages, turning them into cytokines and growth factors factories that stimulate cancer growth by acting as tumour promoters [96]. Macrophages produce tumour necrosis factor (TNF), which activates the nuclear factor NFkB in cancer cells. This nuclear factor triggers the production of proteins that stop apoptosis and activate cell proliferation. So the precancerous tissue in chronic inflammatory cells uses the innate immune system to help the tumour grow. How this process starts remains unanswered for the time being [96].

Atherosclerosis

Almost the 50% of all deaths in the developed world and 25% of the deaths in developing countries are attributable to cardiovascular diseases. Atherosclerosis is the leading cause of heart disease and stroke. Atherosclerosis, considered in the past a disease of lipid accumulation, is now considered a chronic inflammatory disease of large and medium size vessels [97]. The lesions begin in childhood as lipid streaks (reversible), which in old age tend to become plaques proper that can narrow the arterial lumen or ulcer and complicate in thrombosis which can occlude the lumen. The clinical manifestations depend on the vessels involved, so it will be angina pectoris and heart attack

during when the coronary arteries are involved, stroke if the arteries of the central nervous system are involved, and peripheral arterial disease if the peripheral circulation is concerned. It is clear that the seats that are more predisposed to the development of atherosclerotic lesions are the ramifications and the curvatures of the vessels of the blood stream due to friction on its surface (hemodynamic stress), an important factor for the intima thickening. The early lesion of atherosclerosis is identified in the functional/dysfunctional endothelial alteration by cardiovascular risk factors (smoking, hypercholesterolemia, hyperhomocysteinemia, hypertension, obesity and diabetes mellitus, and possibly infectious and immunological causes) and to the accumulation and subsequent oxidation of low density lipoprotein (LDL). The endothelial dysfunction is followed by adhesion and migration of monocytes and T cells to the intima in response to the surface expression of endothelial adhesion molecules such as selectins, VCAM-1, ICAM-1 and chemotactic signals (MCP-1). Recruited monocytes proliferate in the intima, and differentiate into macrophages that phagocytose infiltration and oxidized lipoproteins transforming them into foam cells that characterize striae lipid (fatty streaks). The secretion of cytokines and growth factors, mainly derived from macrophages, induces migration of smooth muscle cells from the media to the intima, where they proliferate, differentiate in "synthetic" phenotype and synthesize extracellular matrix, resulting in the transformation of lipid streaks in advanced lesions: fibrous plaques consisting of a fibrous cap that encloses a lipid core. The accumulation of LDLs is not only due to increased permeability of functionally damaged endothelium, but also its ability to bind the extracellular matrix constituents in the intima. The LDLs undergo oxidation and plays a key role in the development of the intima chronic inflammatory reaction. The LDLs are then trapped in the extracellular matrix of the subendothelial space. Oxidation of LDLs is due to enzymes and oxidative metabolites produced by the arterial wall cells, especially by monocytes-macrophages recruited in the intima as an endothelial damage result due to various causes. Initially, LDLs lipids peroxidation takes place, which interferes poorly with the binding between LDLs and the receptor ApoB-E (or LDL-R); MM-LDLs (minimally oxidized LDLs) are "Trojan horses", physically similar to LDLs, but with a bioactive macromolecular cargo, which is introduced into the cell by endocytosis of MM-LDLs. At later stages, lipid peroxides and aldehydic products are generated (malondialdehyde, MDA, 4-hydroxynonenal) that can covalently modify the LDL protein component; these OX-LDLs called "cellular saboteurs" are no longer recognized by LDL-R but bind to "scavenger receptors" (SR: SR-A, CD36 and CD68). Since SR are not subject to negative-feedback regulation, the OX-LDLs not only introduce active macromolecules into cells, but it also causes the accumulation of intracellular cholesterol esters, responsible for the transformation of macrophages into foam cells characteristic of atherosclerotic tissue.

The OX-LDLs activate some transcription factors (e.g. NF- κ B) in the cells (endothelium, macrophages, smooth muscle cells), which induce the expression of genes encoding adhesion molecules, cytokines, and growth factors and triggering the inflammatory response. Experimental studies have attested that oxidized LDLs have many biological activities on arterial wall cells, including cytotoxic action and direct mitogenic action on smooth muscle cells, macrophages, fibroblasts and endothelial cells. In the endothelium they induce the expression of adhesive molecules on leukocytes, stimulate the production of chemotactic substances (which are partly related to the endothelial surface and are partly released in subendothelium) and promote the synthesis of growth factors for monocytes/macrophages and smooth muscle cells, stimulate the synthesis of PAI-1 (plasminogen activator inhibitor-1) and tissue factor promoting coagulation, stimulate the production of endothelium and inhibit NO, inhibiting endothelium-dependent vasodilation. These species have a direct chemotactic effect on macrophages; determine the transformation into foam cells, stimulate the production of cytokines, growth factors and metalloproteases. In smooth muscle cells induce the synthesis of MCP-1. Finally, oxidized LDL activates platelets and cause aggregation. The fibrous plaques may experience more complications (ulceration, bleeding, thrombosis, calcification) determining the third and most serious atherosclerotic stage: the complicated lesions. There are many factors responsible for plaque fissuring, in particular the great importance of plaque inflammation and abundant lipid component that would make the plate less resistant to the blood component. Inflammatory cells and especially

macrophages produce hydrolytic enzymes such as metalloproteases able to lyse the collagen of the fibrous cap that becomes less resistant to hemodynamic stress.

Moreover elevated levels of some inflammatory markers predict the outcome of patients with acute coronary syndrome regardless of myocardial damage. The reactive C protein adds to the most common risk factors such as negative prognostic index for atherosclerotic cardiovascular events even in apparently healthy subjects as shown by Ridker. It is now commonly accepted that the cardiovascular risk increases for PCR levels in serum above 5 mg/l, but some authors have shown that subjects with PCR levels greater than 3.6 have a risk for cardiovascular events 2 times higher than the rest of general population.

Alzheimer's Disease

Alzheimer's disease is the most common neurodegenerative disease in Western Europe and an important public health problem as the number of cases is increasing with the aging of the population. It manifests with progressive decline in memory and intellectual abilities, impoverishment of language, disorientation and behavioral skills. The characteristic neuropathological aspects of Alzheimer's disease (AD) are senile plaques (SP), neurofibrillary tangles (NFT) and amyloid angiopathy. The NFT are accumulations of dystrophic neurites containing double helix filaments whose main component is the phosphorylated form of tau protein encoded by chromosome 17 and associated with microtubules. Amyloid beta deposits are observed both within the cerebral vessel wall and, more typically, as SP, represented by a central core of beta amyloid fibrils surrounded by a ring of dystrophic neurites, reactive astrocytes and microglia. The beta amyloid protein originates from the cleavage of a precursor consisting in two fragments of 40 (A β 1-40) and 42 amino acids (A β 1-42). There is a familial form of AD (FAD) characterized by autosomal dominant. In more than 50% of cases of this type have been identified mutations in the amyloid precursor protein (APP) on chromosome 21 [98], presenilin 1 gene (PS-1) on chromosome 14 [99] and presenilin 2 gene (PS-2) on chromosome 1 [100]. These changes are associated with increased production of amyloid beta fragment 1-42 (A β), highly toxic for neurons and main component of the plaques observed in AD. However, the role of genetic factors in the sporadic AD pathogenesis is not completely clear and is likely involved multiple risk factors. The presence of epsilon 4 allele (e4) in the gene for apolipoprotein E (APOE) is the major genetic risk factor associated to the development of sporadic AD. Subjects carrying respect to non-carrying e4 allele has a developing AD risk by three (in heterozygote's) to eight (in homozygote's) times greater. The e4 promotes the deposition of A β in extraneuronal plaques. The annual incidence rate is estimated about 1% in subjects over 65 years old to more than 3% in patients over eighty.

Immune responsiveness in AD appears to be altered [101, 102]. In the brain of these patients there are some acute phase proteins and elements of the immune system, although there are the classic inflammation signs such as oedema and neutrophil invasion. Alterations of this type found in the brains of patients affected by AD, but not in age-matched healthy controls, include a greater number of receptors for immunoglobulin and for the complement, increased microglial expression of major histocompatibility complex, increased production of cytokines (like IL-1 β , IL-6), increased acute phase proteins alpha-1-antichymotrypsin (ACT) and infiltration of T lymphocytes in the tissues [103-106]. A case-control study [103] showed that plasma levels of ACT are related to the degree of cognitive impairment in AD patients and that peripheral markers of inflammation or altered immune response could be used to monitor disease progression. The role of inflammation is further underlined by epidemiological studies showing that the long-term use of NSAIDs may protect against AD [107]. From the immunohistochemical prospective microglia is phenotypically related to monocytes, which replaces in phagocytic role in brain. It also expresses a number of important marker of immune function including major histocompatibility antigens type I and II, which facilitates interaction with T lymphocytes. It is shown by numerous studies the increased concentrations of some cytokines and their receptors that regulate and amplify immune responses, conversely lymphocytic infiltration does not seem to be an important component. Complement system also participate to these reactive processes [108]. When complement

system is activated prepares the cell to the phagocytosis and stimulates the cytolysis. Antibodies against complement components are demonstrated in brain tissue of patients with AD but they don't bind to age-matched controls tissues. These and other data imply that in this disease there is activation of the complement reactions cascade to the production of a lytic membrane complex; neurons exposed to the complex are defended increasing the synthesis of inhibitors. However in the disease status prevails the process of self-destruction and phagocytosis. These findings provide helpful tips for AD treating, such as assumptions regarding the use of inflammatory, and stimulate suggestive questions such as what is the relationship between beta-amyloid and inflammatory response. It seems that Beta-amyloid directly activate components of the inflammatory response as the complement cascade and microglia [109] and macrophages activity. The 1-40 and 1-42 forms of the peptide evoke neurotoxins release by microglia, including a newly-identified [105], and it seems also involved a "scavenger receptor" present on microglia that should bind beta-amyloid. According to some authors, the beta-amyloid may activate the cytotoxic response of mononuclear phagocytes and macrophages, causing a cytopathic response directed against neuronal elements. In terms of measurable parameters directly in the patient were observed high antibodies values against microglia in liquor, indirect evidence of abnormal activation system. Despite the progress made by research in this area and even though it was discovered the direct confirmation that brain tissue inflammatory cascade involving COX-2 is activated in patients with the this disease [110-112], clinical data, as opposed to the epidemiologicals, are disappointing [113]. The only recent trial using prednisone gave essentially negative results [114].

In conclusion, the brain lesions associated with AD such as neurofibrillary tangles and senile plaques, are characterized by the presence of a broad spectrum of inflammatory mediators produced by cells residing in the brain, including neurons. Although of secondary importance compared to the fundamental cause that determines the tangles and plaques, there are strong evidences that inflammation exacerbates the neuronal loss. Consequently, the AD risk is substantially influenced by several polymorphisms in the promoter region of genes, and other non-coding regions, coding for inflammatory mediators. Alleles that support the increased expression of inflammatory mediators or alleles that favour the reduced expression of anti-inflammatory mediators are more frequent in patients with AD compared to controls. The polymorphisms are fairly common in the general population, so there is a strong probability that everyone will inherit one or more high risk alleles [104,115,116-123]

Bioactive nutrients and Nutrigenomics in Inflammation and Related Diseases

The interaction among genomic and environmental factors is extremely determinant in development or progression of several human diseases. Particularly, nutritional intake is one of the most important environmental factors and has a prominent role in disease etiology [124].

Firstly, diet provides adequate metabolic and energetic requirements for body composition homeostasis, however it may contribute as well to the improvement of human health through regulation of specific processes [125]. Indeed, nutrients are considered as effective dietary signals, able to modify both metabolic programming and cell homeostasis. Bioactive nutrients or chemopreventive molecules as well, as part of diet, definitely affect human health reducing disease risk with specific molecular mechanisms [126-128]. For instance, many experimental and epidemiological studies have emphasized the potential of diets and dietary components, considering both macronutrients (carbohydrate, proteins, fat and fiber) and micronutrients (antioxidant vitamins and minerals), as a first-line intervention in the prevention or treatment of neoplastic and other several diseases [127,128].

Nutritional research studies span over many disciplines of biology, at the molecular and genetics level [129,130]. Either as preventive or therapeutical tool, the diet may be strictly connected to human genome in several diseases, and this kind of approach comprises two different branches, nutrigenomics and nutrigenetics.

Nutrigenomics and nutrigenetics are new research approaches that strive to optimize health by looking beyond the diet to understand the effects of food at genetic and epigenetic levels. Specially,

nutrigenetics focuses on understanding how the genetic composition (i.e., genetic variation) of an organism influences their response to a given diet, well providing recommendations about risks or benefits of specific diets or dietary components to each individual as “personalized nutrition”.

In parallel, nutrigenomics properly investigates how nutrients can affect gene expression and downstream processes as well.

Moreover nutrigenomics and nutrigenetics connect many fields: nutrition, bioinformatics, molecular biology, genomics, functional genomics, epidemiology and epigenomics.

To a certain extent, nutrigenomics approaches to pharmacogenomics, which systematically studies the effect of drugs on genome. However drugs are pure compounds, acting with specific affinity and selectivity for a limited number of biological targets, and administered in precise and low doses. By contrast, nutrigenomics handles the complexity and variability of diet. Moreover nutrients can reach high concentrations (μM to mM) without being toxic and can also bind targets with different affinities and specificities [131,132].

Eating patterns may be considered like endogenous cellular mediators, since they influence gene, protein expression and metabolism as direct ligand or co-factor. Once its absorption at cellular level, a nutrient can interact with specific signaling pathways and even a small change in its structure can differentially activate metabolic steps; fatty acids class and their level of carbon chain unsaturation can provide a proper example. The n-3 polyunsaturated fatty acids in fact promote anti-inflammatory pathways, whereas n-6 polyunsaturated fatty acids induce synthesis of pro-inflammatory molecules. Furthermore, *trans* fatty acids increase plasma levels of LDL-cholesterol [133-135], in contrast, n-3 polyunsaturated fatty acids do not.

The protective and restorative effects of several dietary compounds are likely to result from the modulation of distinct signal transduction pathways. Particularly, scientific literature confirmed that nutrients are able to affect gene expression as consequence of a direct interaction with transcription factors [136]. Fat-soluble ligands, such as vitamin A/D, activate their cognate nuclear receptors for ligand-dependent transcriptional regulation.

Since dietary habits strongly impact disease onset, population studies based on self-reported dietary questionnaires supply a consistent part of data relating dietary intake to phenotypes and disease risk [137]. It is also possible to indirectly monitor the features of nutritional intake by measuring specific nutrient molecules in blood, urine, fat or other tissues. This may also result in the identification of nutritional biomarkers providing a functionally connection between nutrition and health. Altered serum lipid profiles (eg, cholesterol, triglycerides), increased blood pressure or reduced insulin sensitivity are common predictors of diet related diseases. A wide biomarkers profile should then facilitate to characterize the health status better than of an individual biomarker.

Through “omics” technologies in nutrition, coordinated by bioinformatic tools, it is possible to integrate analyses of transcriptome, proteome and metabolome, all highly related each other, to identify specific differences in relation of nutritional habits and to investigate all likely mechanisms which caused the change [138-143].

Genetics contributes to determine individual’s risk of developing diseases. In particular, nutrigenetics, as a branch of genetics, examines genetic variations according to individual nutrient effects. For this reason it is significant relating SNPs, point of mutations in DNA sequence, to diet responsiveness [144,145]. Population differences in SNPs can have, for example, a predictive role in risk assessment and life style recommendations. Phenylketonuria (PKU) was the first evidence of a single-gene defect condition responding to dietary treatments, using a low-phenylalanine containing diet as nutrigenetic therapy [146].

Another significant approach is “Nutriproteomics”, as recent branch of proteomics, that systematically studies proteins structure and function, as well as protein-protein interactions, aiming to identify the molecular targets of dietary components [147-177]. Even in this case the goal is to identify differential elements in protein patterns under a certain condition, for example before and after a determinate dietary treatment.

Thus proteomics analyzes at various levels the effect of dietary components allowing to investigate peptides as bioactive markers. Concerning clinical applications, proteomic techniques can

investigate on nutritionally relevant biological pathways and on dietary interventions. Proteomics, also combined with gene expression analysis, was used in cancer prevention studies to identify innovative biomarkers [178-188].

A quantifiable change relating a normal or pathological condition with a modulation of an mRNA, a protein, or a metabolite concentration can be used as a molecular biomarker. In particular, a protein concentration or its modification status can easily become a diagnostic tools, being relatively easy to have precise and reproducible clinical diagnostic assays for these analytes [189]. Dietary dose levels of most nutrients are only weakly biologically active and may have several targets. Selecting a biomarker, the timing of its responses should be considered according to nutrient specific bioavailability and bioefficacy. Biomarkers may correlate with nutrient intake, but often their modulation is a combined result of intake, absorption, metabolism, and excretion. Also environmental factors and genetic predisposition may modulate the correlation between dietary intake and biomarkers. For all these reasons it can likely expected that best results will not come from a single proteomic biomarker but from their multiple combination also with the information available from the other “omics” technologies.

Individual metabolites levels can be considered as the last step of biological systems according to genetic or environmental factors and among these nutritional intake has a crucial role.

Metabolome, from the term “genome”, examines the complete set of metabolites in an organism, classifying and quantifying them individually in a biofluid, cell culture or tissue sample through analytical methods. Their measures give information on how the enzymes and all other functional proteins act in cellular homeostatic mechanisms [162]. Nutrients can directly interact with our body at organ, cellular and molecular levels. They usually come in complex mixtures, in which it is both important the amount of a single compound and its interaction with multiple components, since this squarely influences their bioavailability and bioefficacy.

Thus metabolomics allows to systematically investigate small organic molecules and within nutrigenomics its main interest is to recognize how those molecules can reflect the effects of different diets, indicating an interactive and regulatory role of nutrition [164].

The main idea of nutritional metabolomics is to detect and identify all endogenous human metabolites and exogenous components from food that coexist at least transiently in human biofluids. The multiple metabolites profile, corresponding to specific nutrition states, could help to characterize physiological or pathological conditions and certainly have more interest and possibility to be used as biomarkers better than a single molecule. Also the integration of these information with those from the other “omics” technologies will result in relevant information [165-167].

Another aspect to discuss is nutritional epigenetics, as a novel mechanism underlying gene–diet interactions, further elucidating the role of nutrition in aging and age-related disease development. Epigenetics is defined as a heritable modification to the DNA that regulates chromosome architecture and modulates gene expression without changes in the underlying bp sequence, ultimately determining phenotype from genotype. DNA methylation and post-translational histone modifications are classical levels of epigenetic regulation. Epigenetic phenomena are critical from embryonic development through the aging process, with aberrations in epigenetic patterns emerging as aetiological mechanisms in many age-related diseases such as cancer, CVD and neurodegenerative disorders. Nutrients can act as source of epigenetic modifications and can regulate the placement of these modifications. Nutrients involved in one carbon metabolism, namely folate, vitamin B12, vitamin B6, riboflavin, methionine, choline and betaine, are involved in DNA methylation by regulating levels of the universal methyl donor S-adenosylmethionine and methyltransferase inhibitor S-adenosylhomocysteine. Other nutrients and bioactive food components such as retinoic acid, resveratrol, curcumin, sulforaphane and tea polyphenols can modulate epigenetic patterns by altering the levels of S-adenosylmethionine and S-adenosylhomocysteine or directing the enzymes that catalyse DNA methylation and histone modifications. Aging and age-related diseases are associated with profound changes in epigenetic patterns, though it is not yet known whether these changes are programmatic or stochastic in nature. Future work in this field seeks to characterise the epigenetic pattern of healthy aging to ultimately identify nutritional measures to achieve this pattern.

The term "systems biology" was coined to indicate cross-disciplinary research in biology. Biochemical systems biology includes and put together several traditional disciplines, such as genomics, biochemistry and molecular biology, though mathematical and computational analysis, engineering practices and "omics" platform technologies, such as transcriptomics, proteomics and metabolomics [190].

The progress of high-throughput technologies has driven to a substantial raise of functional genomics data. Different experimental strategies combine quantitative measurements of cellular components (mRNA, protein and metabolite) with the development of mathematical and computational models as an equal partner [191-212].

Conclusions

Despite during recent years many theories about aging have been developed we are still far from a full explanation of the mechanisms underlying the aging process. It has not yet been found an answer to the question that always arises from the man : "why grow old? ", " what do to live longer? ". Great strides have been made by immunological-inflammatory research , but it is still waiting for effective and validated therapeutic strategies. It has great consensus the hypothesis that aging is multifactorial and complex process, produced by the interaction between genetic, environmental and lifestyle. According to the latest scientific understanding our genes are programmed to make us live 120 years. But we know that longevity is not only written in the genes, but for a good 70% must conquer day by day with healthy lifestyles. So from the earliest years of life is essential for healthy nutrition, physical exercise constant, constant mental activity, a limited use of tobacco and alcohol, a life away from polluted environments, with limited sun exposure and most importantly it is necessary to lead a life animated by many interests.

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