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Anti-inflammatory effects of natural compounds: focus on miRNA modulation

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Summary

Over past few years, the research on the aging has faced a great challenge to improve healthspan (Tchkonia et al. 2013) and to achieve "healthy aging" (Olivieri et al. 2017). A major issue in aging research is to understand how cellular phenomena affect aging at the systemic level by inducing inflammation, immune response and senescence (Dodig, Cepelak, and Pavic 2019).

One of the most important contributor of aging is the development of cellular senescence. The growing disproportion between young cells and senescent cells (SCs), caused by excessive intracellular or extracellular stress or damage, is believed to be a major risk factor for the most common age-related diseases (ARD) such as neurodegenerative disorders, cardiovascular disease, diabetes, osteoarthritis, and cancer (St Sauver et al. 2015). SCs can fuel the chronic, systemic and age-related proinflammatory status (named inflammaging) and impair the regenerative abilities of stem cells through the acquisition of a senescence-associated secretory phenotype (SASP) (Franceschi et al. 2000).

The identification of pathways that control human aging process, focusing on cellular senescence and the age related inflammation mechanisms may be beneficial to older people. Physical function, blood-based candidate markers, and molecular/DNA-based markers are several putative biomarkers of aging (Wagner et al. 2016). Therefore, some of these biomarkers have clinical relevance as diagnostic/prognostic factors.

Chemical substances that have been shown to increase longevity in animal/cellular models or to postpone some related feature of senescence are promoted as anti-aging or anti-senescence compounds.

In this framework, a number of natural compounds have been investigated for their anti-senescence and anti-aging potential effects through the modulation of senescence-associated inflammatory phenotype (SASP), including cytokines, non-coding RNA (miRNAs) and

epigenetic-related enzymes, i.e. SIRT1 (Vaiserman, Lushchak, and Koliada 2016). A common advantage of natural compounds deriving from fruit and vegetables is the fact that they are often current components of the human diet, devoid of important adverse effects and could elicit anti-senescence, anti-SASP and anti-aging effects.

Introduction

1. Aging, inflammaging and age-related diseases

Aging is a complex biological process and highly regulated by multiple mechanisms still not fully understood. A widespread feature of aging is a chronic and systemic pro-inflammatory status, defined "Inflammaging" (Franceschi et al. 2000). The source of this age-related systemic chronic inflammation was mainly attributed to the progressive activation of immune cells over time that is also a shared risk factor for the most common ARDs such as neurodegenerative disorders, cardiovascular disease (CVD), type 2 diabetes and cancer (Franceschi 2017). Somatic diseases and multiple chronic conditions, psychological, cognitive and social changes can be considered as age-associated hallmarks (Jaul and Barron 2017). In addition, there is a decrease in basal metabolism, a change in gastrointestinal system activity and several functional modifications of the respiratory system.

In the background of all the alteration that occur during aging, there are two key factors – inflammation/immune system activation and cellular senescence (Dodig, Cepelak, and Pavic 2019).

Inflammation plays an important role in damage healing response, and is essential in keeping the organism safe from bacterial and viral infections and noxious environmental agents. On the other hand, when inflammation persists, it can become damaging and destructive, triggering many age-related pathophysiologic processes and diseases (Franceschi et al. 2007).

The pathways of cytokine is a complex system belonging to immune system. It is regulated by the interaction with different kind of receptors and serum mediator (Abe et al. 2013). It has been extensively demonstrated that the immune system loses its functionality as the individual's age

increase. This impairment is to some extent triggered by an imbalance between pro- and anti-inflammatory cytokines that leads to inflammaging and consequently to ARDs (Franceschi 2007). In older age, the cytokine dysregulation is believed to play a key role in the remodeling of the immune system. The pro-inflammatory cytokines (IL-1, IL-2, IL-6, IL-8, IL-12, tumour necrosis factor alpha – TNF-α, and interferon-gamma – IFN-γ) are significantly increased in comparison with younger individuals. At the same time, in elderly people concentration of anti-inflammatory cytokines (interleukin-1 receptor antagonist –IL-1Ra, IL-4, IL-10, IL-37, transforming growth factor beta 1 – TGF-β1) are higher than in young individuals (Rea et al. 2018) (Ventura et al. 2017).

Several studies have demonstrated that immunological changes in elderly undergo on the decline of the functional capacity of the immune system, which related to an increased risk of infections increased appearance of neoplasia, and increased production of autoantibodies responsible for the occurrence of autoimmune diseases.

Different types of innate immune cells are involved in the activation and maintenances of the inflammatory process. Their functions are modulated during aging:

- the neutrophils - reduced capability to migrate towards a chemotactic signal, ROS production and reduced phagocytosis;

- the monocytes/macrophages - reduced phagocytosis, cytokine and chemokine secretion, reduced generation of NO and superoxide, reduced IFN-γ, inhibited response to growth factors;

- the dendritic cells - reduced phagocytosis and pinocytosis, increased IL-6 and TNF-α production, diminished TLR expression and function (Busse and Mathur 2010) (Poland et al. 2014).

Modulation of the functions of acquired immunity cells during aging was also described:

-B cells - decreased number, reduced proliferative capacity, reduced antibody avidity, increased concentration of IgG and IgA, and concentration of autoantibodies;

-T cells - reduced CD28 expression, reduced TCR diversity, reduced signal transduction, reduced response and proliferation, increased differentiation of CD4+ into Th17 cells (Jagger et al. 2014), (Montecino-Rodriguez, Berent-Maoz, and Dorshkind 2013).

Nevertheless, immunity and inflammation contribute to the development or maintenance of chronic disease such as cardiovascular disease (CVD), type 2 diabetes, and osteoporosis, which are commonly referred to as age-related diseases (Edwards et al. ; St Sauver et al. 2015)

Type 2 diabetes (T2DM) is a multifactorial chronic disease, characterized by several complications, such as CVD, peripheral vascular disease, stroke, diabetic neuropathy, amputations, renal failure, and blindness thus resulting in increasing disability, reduced life expectancy, and increased health costs (Chatterjee, Khunti, and Davies 2017). The prevalence of patients with CVD and T2DM is growing exponentially and most T2DM patients die from cardiovascular causes. Approximately 40% of patients hospitalized with heart failure suffer of T2DM (Low Wang et al. 2016).

Beyond chronic hyperglycemia, dyslipidemia is the other most common feature of T2DM. Hyperlipemia, the most common form of dyslipidemia (Hobbs 2003; Nelson 2013), is recognized as an independent risk factor in the development of atherosclerosis and CVD. Diabetic dyslipidemia is characterized by an increase in triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), and small-dense (atherogenic) particles, and by a decrease in low high-density lipoprotein cholesterol (HDL-C) and apolipoprotein (Apo) A1 that are strongly related to insulin resistance (Arca, Pigna, and Favoccia 2012). A detrimental outcome of excessive glucose exposure is the inhibition of AMPK and a consequent raise of lipogenesis (Cai and Lin 2009). The increased flux of free fatty acids from adipose tissue to the liver aggravates hepatic insulin resistance and promotes all of aspects of the dyslipidemic state. Hyperlipidemia, insulin resistance, and hyperinsulinemia all precede overt T2DM diagnosis and can each induce the other when tested experimentally (Czech 2017).

It is important to note that the risk of atherosclerotic cardiovascular disease, particularly coronary heart disease (CHD), is higher in T2DM patients at any given level of LDL-C (Schofield et al. 2016). This notion provides the rationale for adjusting the intervention strategies for dyslipidemia to the total cardiovascular risk. According to the most recent guidelines, individuals with T2DM are considered at high to very-high CV risk, according to the presence or not of target organ damage. For this reason, they should be treated in order to achieve an LDL-C target value of < 100 mg/dl or < 70 mg/dl, respectively (Catapano et al. 2016). Patients with LDL-C values slightly superior to the recommended targets could benefit from lifestyle interventions without the need of lipid lowering drugs (Piepoli et al. 2016). Moreover, statins have been shown to negatively impact glycemic control, an effect that increases the risk of T2DM in prediabetes subjects and which could be partially prevented by the concomitant use of metformin (van Stee, de Graaf, and Groen 2018). Given that dietary factors could impact on atherogenesis either directly or through the reduction of plasma lipids or glucose levels, the consumption of functional foods and selected dietary supplements is strongly encouraged in the clinical practice, in alternative or in combination with lipid-lowering drugs (Sirtori et al. 2009).

2. Senescence and SASP

Senescence is one of the key factor in the complex aging process. Consequently, aging and senescence research has undergone a significant progress, over the last decades. Senescent cells are characterized by morphological changes such as increased cell size and distinctive enlarged flat morphology and acquire a permanent growth arrest state. Their metabolic active state exhibits several features such as telomere-dysfunction-induced foci, senescence-associated heterochromatin foci (SAHF), increased activity of SA–β-galactosidase (β-gal), expression of

tumour suppressors and cell cycle inhibitors (Dimri et al. 1995), (Salminen, Kauppinen, and Kaarniranta 2012).

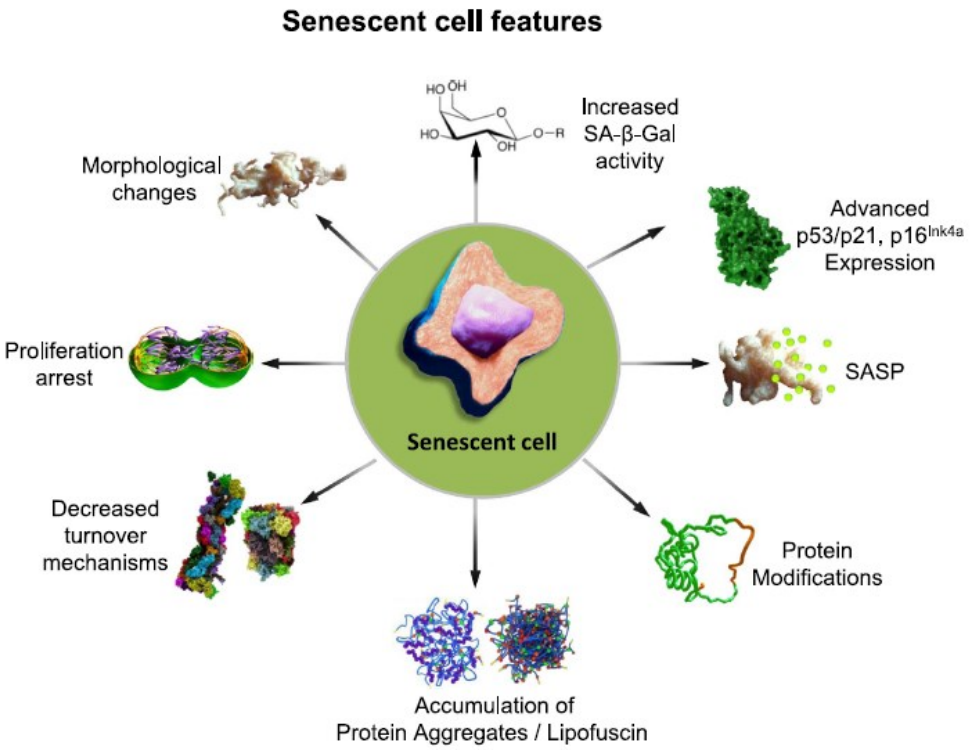


Figure 1. The typical features displayed by senescent cells (Hohn et al. 2017)

Based on kinetics of senescent cell pathways there are two main categories of senescence – acute (programmed, transient) and chronic (not programmed, persistent) senescence. Acute senescence leads wound healing and tissue repair, to embryonic development, of specific populations of cells and tissues through activation of senescence-associated secretory phenotype (SASP) factors and consequently activated immune clearance (Jun and Lau 2010). Chronic senescence is induced by a prolonged period of cellular stress or slow damage, due to large SASP heterogeneity involved in chronic processes and high resistance of senescent cells to immune clearance (Childs et al. 2015), (van Deursen 2014), (Watanabe et al. 2017).

Cells undergoing senescence display dramatic alterations as a result of the secretion of SASP factors, such as interleukins (the most prominent is interleukin-6, IL 6), chemokines, growth factors (e.g. insulin-like growth factor, IGF) and regulators, proteases (e.g. matrix metalloproteinases - MMPs, serine proteases), etc. (Coppe et al. 2010), (Freund et al. 2010), (Ozcan et al. 2016). Not all SASP factors are secreted at the same time. In fact, most of them are released in small amounts over several days following the induction of genotoxic stress (Coppe et al. 2008). For instance, pro-inflammatory cytokines IL-1α and IL-1β play a critical role in the early phase of the SASP. Indeed, IL-1α acts as upstream regulator of IL-6/IL-8 cytokine network (Orjalo et al. 2009). Moreover, IL-1α is implicated in the upregulation of microRNA-146a/b, which are responsible for the inhibition of interleukin-1 receptor- associated kinase 1 (IRAK-1) (Bhaumik et al. 2009). Another important pathway activated in response to cell stress is the mitogen-activated protein kinase p38 (p38MAPK). P38MAPK phosphorylation is important for the establishment of the senescence growth arrest due to its ability to activate both the p53 and pRb/p16 pathways (Freund, Patil, and Campisi 2011). GATA4 is another important factor that has recently been proposed as a senescence regulator activated by DNA damage response system (Lee et al. 2018).

These different signaling pathways (DDR, p38MAPK and IL-1 pathway) converge toward the activation of the nuclear transcription factor-κB (NF-κB), necessary for the expression of genes encoding SASP factors (Salminen, Kauppinen, and Kaarniranta 2012).

A group of cytoplasmic and nuclear factors, including Jak2/Stat3, the inflammasome, mTOR, HSP90, GATA4, macroH2A1, and ATM, have been shown to be involved in the development of SASP (Soto-Gamez and Demaria 2017).

Released SASP factors are involved in sensitizing non-senescent neighboring cells to senescent, cell proliferation, disruption of tissue structure and function, immunomodulation, angiogenesis, disabling or fostering of cancer growth (Lujambio 2016).

The SASP process is triggered by a variety of genotoxic senescence-inducing stress (e.g. telomere shortening or damage, cytotoxic drugs, radiation, oncogene activation, oxidative stress) (Coppe et al. 2008).

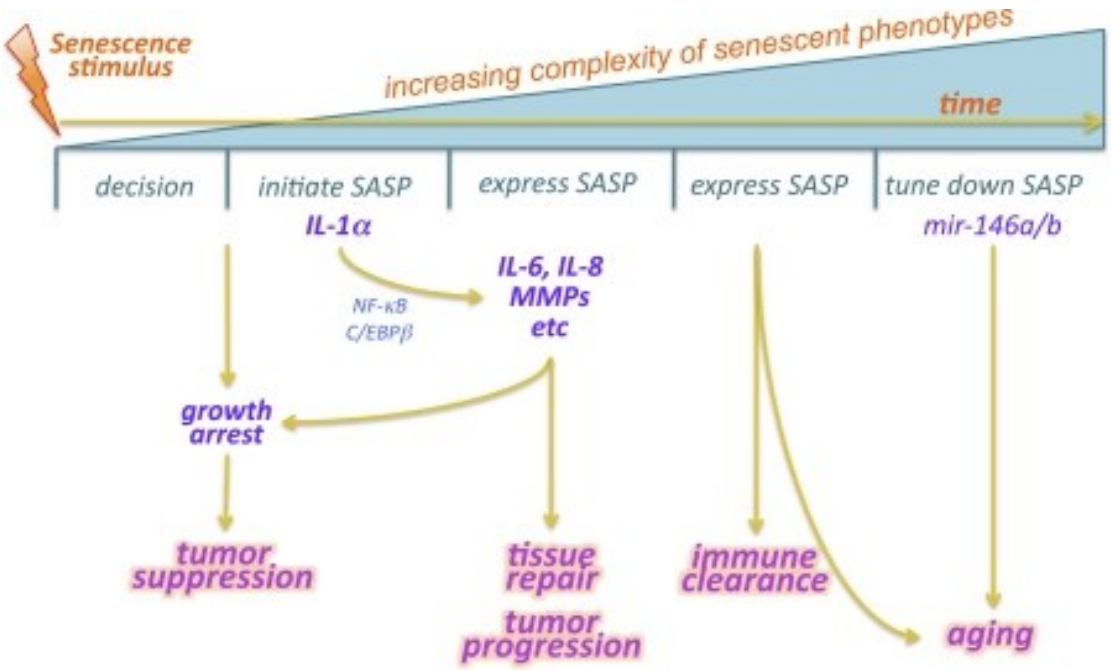


Figure 2. Timing of senescence and SASP regulation (Rodier and Campisi 2011).

Factors leading to senescence

Senescence can be triggered by different stimuli: oxidative stress, telomere damage/shortening, chromatin disorganization, DNA damage, mitochondrial dysfunction, oncogene activation, inflammation and epigenetic dysregulation (Campisi and d'Adda di Fagagna 2007; Arai et al. 2015; Collado, Blasco, and Serrano 2007).

Reactive oxygen species are a natural byproduct of the normal oxygen metabolism; it can regulate several physiological functions, like signal transduction, gene expression and proliferation. The cellular source of ROS are mitochondria as they provide most of the cellular energy through oxidative phosphorylation. It is considered that mitochondrial dysfunction (leads to an over-

production of ROS with concomitant ATP production decrease) is associated with senescence, and consequently with the aging process (Han, Williams, and Cadenas 2001) (Davalli et al. 2016). Response to DNA damage, epigenetic regulation and tumour suppression pathway activation e.g. cell cycle control related proteins: p53 (cellular tumour antigen p53), p21 (p21Cip1, cyclin-dependent kinase inhibitor 1), pRB (retinoblastoma protein) more specifically SASP factors of senescent cells, can stimulate positive feedback loop and result in increased ROS levels, especially mitochondrial ROS (mtROS) (Passos et al. 2010).

Telomeres are regions of repeated sequences, at the end of each linear chromosome, bound by multiple telomeric proteins. Telomere DNA contains double-stranded tandem repeats of TTAGGG and are located at the ends of human chromosomes. Telomere DNA probably adopt the T-loop structure, where the telomere end folds back on itself and the 3′ G strand overhang invades into the double-stranded DNA (the so-called D- loop) (Lu et al. 2013). The basic function of telomeres is to protect the chromosomes from degradation rearrangements, end-to-end fusions, and chromosome loss (Siderakis and Tarsounas 2007). The shortening of the telomeres that occurs during normal aging is controlled by the activity of enzyme telomerase (enzyme complex that maintains telomere length). Telomere length progressively shortens with replication of nuclear DNA during mitosis, or with oxidative stress or with senescence and aging (Sanders and Newman 2013).

The short telomeres and non telomeric-DNA damage trigger a DNA damage response (DDR) and represent a signaling cascade, which direct cell fate toward cell cycle progression, senescence or apoptosis (Galbiati, Beausejour, and d'Adda di Fagagna 2017). The DNA damage in senescence (which may be the result of telomeric and non-telomeric DNA damage or oxidative stress) strongly depend on the activation of the tumour suppression pathways p53 and p21 and the p16/retinoblastoma protein (Barnes 2015; Campisi and d'Adda di Fagagna 2007).

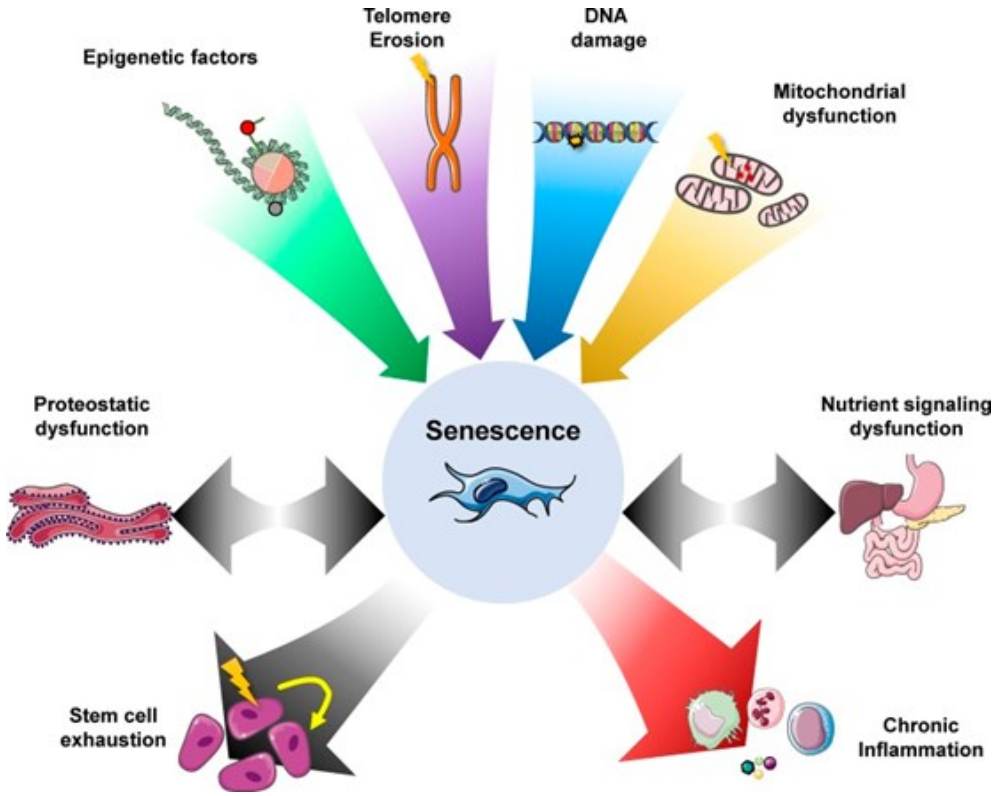


Figure 3. Senescence as a central hallmark of aging (McHugh and Gil 2018).

3. Biomarkers of cellular senescence and ARD

A biomarker, or biological marker is any substance, structure or process that can be objectively measured in the body and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to therapeutic intervention (Biomarkers Definitions Working 2001). Therefore, biomarkers might be a valuable measure in order to examine physiological/biological aging and when health is compromised. According to the American Federation for Aging Research (AFAR) recommendations, aging biomarkers should meet several criteria such as: 1. predict the rate of aging (correlate with aging); 2. monitor a basic process that underlies the aging process (determine "healthy aging", not the effects of disease); 3. be able to

be tested repeatedly without harming the person; 4. be applicable to humans and animals (Xia et al. 2017).

The term epigenetics includes several phenomena such as DNA methylation, histone tail modifications, and microRNAs mediated mechanisms, which are able to mould the chromatin structure and/or gene expression levels, without altering the primary DNA sequence (Stoccoro et al. 2013).

DNA methylation (achieved by the action of DNA methyltransferases) is often lost in repetitive sequences, while hyper-methylation is found in several specific promoter regions.

Epigenetic alterations are ostensibly reversible and embody promising pharmacologic targets such as DNA methyltransferase and histone deacetylate inhibitors for therapies designed to promote healthy aging, unlike cumulative DNA damage and many other hallmarks of aging (Wang et al. 2013; So et al. 2011).

DNA damage (independent of telomeres) and deregulation of the INK4/ARF locus induce cellular senescence and occur with advancing age. As a biomarker, protein levels of p16INK4a (a cyclin-dependent kinase inhibitor) and p19ARF are correlated with chronological age (Krishnamurthy et al. 2004; Ressler et al. 2006). Therapeutic removal of p16INK4a-positive cells could extend healthy lifespan (Baker et al. 2016).

The H2A.X is a variant of the H2A protein family, and phosphorylated H2A.X, γ-H2A.X, is an initial and essential component of DNA damage foci and, therefore, a reliable marker of the extent of DNA damage (Kuo and Yang 2008). Serum markers of DNA damage, including CRAMP, EF-1a, stathmin, N-acetyl-glucosaminidase, and chitinase, have also been detected (Song et al. 2010).

The microRNAs (miRNAs) are single-stranded and non-coding RNA molecules of 21–23 nucleotides that regulate a broad spectrum of biological activities and several pathways that are involved in aging and cellular senescence (Wessner et al. 2010).

The biogenesis of miRNAs involves multiple steps and specific cellular mechanism (Hastings and Krainer 2001). MiRNAs are encoded as short inverted repeats having a double-stranded RNA (dsRNA) stem loop and are found in both introns and intergenic clusters in the genome (Hastings and Krainer 2001). RNA polymerase II is responsible for the synthesis of the introns and exons of both protein-coding and non-coding transcripts from where miRNAs are derived (Morey and Avner 2004). In the nucleus, miRNAs are transcribed as primary pri-miRNA transcripts and then are processed to form the precursor pre-miRNA stem loop structure before transportation into the cytoplasm where they are cleaved by the Dicer RNAase III endonuclease and produce mature miRNA (Bilen, Liu, and Bonini 2006).

Among the many miRNAs expressed in the human genome, several miRs (such as miR-155, miR-21, and miR-146a) that appear to be modulated during cellular senescence and aging are found in a range of ARDs such as cardio vascular disease, diabetes mellitus and neurodegenerative diseases (Dimmeler and Nicotera 2013). MiR-126 was demonstrated to inhibit the expression of vascular cell adhesion molecule 1 (VCAM-1), which is required to mediate leukocyte adherence to endothelial cells (Harris et al. 2008) . In macrophages, miR-155 is known to be induced by cytokines such as TNFα and IFN-β (Tili et al. 2007) and contributes to physiological granulocyte/ monocyte expansion during inflammation (O'Connell et al. 2008).

Circulating miRNAs can exist in various types of body fluids, such as serum, plasma, and urine. They are secreted by donor cells via incorporation into vesicles including exosomes, macrovesicles, or apoptotic bodies. Increasing evidence has indicated the potential of circulating miRNAs as predicting markers for diabetes and its complications (Feng, Xing, and Xie 2016).

Zhang et al. found that miR-126 is the miRNA whose expression is significantly reduced in susceptible individuals and T2DM patients compared with normal individuals, suggesting that plasma miR-126 may serve as a potential marker for the early prediction of susceptible individuals to T2DM (Zhang et al. 2013). Similarly, by comparing expression profiles of miRNAs in the

plasma of patients with prediabetes and newly diagnosed T2DM, Yan et al. demonstrated plasma miR-1249, miR-320b, and miR-572 levels to be potential biomarkers for early diagnosis of T2DM (Yan et al. 2016)

Circulating miRNA levels could be used as biomarkers for the major age-related diseases with important diagnostic and prognostic implications, but further research needs to be conducted to evaluate their sensitivity, selectivity and potential as biomarkers for aging (Zampetaki et al. 2012; Cortez and Calin 2009; Olivieri et al. 2013).

Kumar and Reddy have mentioned five different ways of miRNA transportation into extracellular circulation: (1) bound with high-density lipoproteins (HDL) particle in nonvesicle form; (2) complex form with Ago2 protein; (3) packaged within exosomes; (4) encapsulated within MVs; and (5) accumulated in apoptotic bodies (Kumar and Reddy 2016).

The cytokines play a key role in molecular processes and pathways underpinning inflammation. A common finding in aging and age-related diseases is "inflammaging," a dysregulation of the cytokine network and its homeostasis.

As age increases and in age-related diseases, a chronic inflammatory state predominates, which is not properly contained or resolved and the anti-inflammatory side of the immune system seems to be similarly dysregulated, and unable to damp down the inflammatory episode in a timely effective manner. Specific cytokine receptors for IL-1, TNF-α, and IL-18, together with soluble receptor antagonists, chemokines, microRNA, siRNAs, also function as inhibitors for pro-inflammatory cytokines.

Where increases in anti-inflammatory cytokines have been reported, one interpretation would be that increases might reflect the immune system's attempt to suppress the persistent pro-inflammatory response and support a return to immune homeostasis (Rea et al. 2018).

Interleukin- 38 is a member of the IL-1 cytokine family (primarily proinflammatory cytokines) (Yuan et al. 2015). This recently discovered cytokine shares structural features with IL-1 receptor

antagonist (IL-1Ra) and IL-36Ra. These cytokines are involved in the regulation of inflammation and immune responses and might provide new insights for developing anti-inflammatory treatments in the near future.

IL-38 is strongly correlated with chronic inflammatory diseases such as rheumatoid arthritis (RA) (Boutet et al. 2016) (Takenaka et al. 2015), psoriasis (Li, Liu, et al. 2017), systemic lupus erythematosus (Ressler et al. 2006; Chu et al. 2017) (Rudloff et al. 2015), chronic obstructive pulmonary disease (COPD) (Xu and Huang 2018) and inflammatory bowel diseases (IBD) (Boutet et al. 2016). A recent study showed that IL-38 concentrations are elevated and correlate with C-reactive protein (CRP) during acute ST-Segment Elevation Myocardial Infarction (STEMI). IL-38 might play a role in modulating inflammatory responses during ischemic events (Zhong et al. 2015).

Moreover, IL-38 was found to be elevated in the umbilical arteries and veins of patients with gestational diabetes mellitus (GDM) that is associated with vascular dysfunction (Yu et al. 2017). Another study showed that serum levels of IL-38 increased in children with asthma, and this higher levels of IL-32 is correlate with the lower the circulating peripheral regulatory T-cell population (Chu et al. 2016).

Physical function and anthropometry remain important markers of current and future health. Functional assessments for physical performance such as handgrip strength, chair stand, gait speed, timed up and go for monitoring the biological aging process (Wagner et al. 2016). Therefore, the poorer these performances are and the higher is the risk for cardiovascular disease (CVD), dementia and institutionalization (as a marker for the loss of independence). Aging is also associated with body composition changes including high body mass index, doubled waist circumference and reduced muscle mass (Wagner et al. 2016). Different studies showed that older adults with a lower performance in tests of physical capability (by assessing activities of daily

living-ADL) are more likely to become functionally disabled (Vermeulen et al. 2011; Gobbens, van Assen, and Schalk 2014).

Protein metabolism is also involved in tissue aging. Proteins undergo nonenzymatic posttranslational modifications (NEPTMs), namely glycation, oxidation, carbonylation, or carbamylation (Gillery and Jaisson 2013). The latter is a more recently described NEPTM that may significantly contribute in the structural and functional tissue damages encountered during aging (Gorisse et al. 2016).

The glycation has generally been recognized to significantly contribute to aging processes. Advanced glycation end products (AGEs) are a heterogeneous group of bioactive molecules that are formed by non-enzymatic glycation that accumulate in organisms in an age-dependent manner and lead to inflammation, obesity, and other age-related disorders (Sayej et al. 2016; Scavello et al. 2019).

Lipids are important for a variety of biological processes, including aging and longevity. The plasma triglyceride levels and circulating lipid–protein complexes increase with age and could be a biomarker of aging (Gonzalez-Covarrubias 2013). Another biomarker could be sphingolipids (membrane lipids) that are in serum profiling of long-lived humans (Montoliu et al. 2014). In addition, the lipid metabolism could alter the regulation of physiological processes through changes in chromatin states (Sen et al. 2016).

The dietary restriction is able to slow aging and age-associated diseases that relive to an important role of metabolism in aging regulation and to the potential for metabolic factors to be biomarkers. The earliest discovered and the most well-known pathway to antagonize longevity is the insulin/insulin-like growth factor 1 (IGF-1) signaling (IIS) pathway, which participates in glucose sensing. Attenuating IIS activity extends life span in mouse models led to the potential inclusion of IIS pathway members (growth hormone and IGF-1) as biomarkers of aging. (Schumacher et al. 2008; Crimmins et al. 2008).

Others nutrient sensing systems that contribute to the aging process are the amino acid-sensing mammalian target of rapamycin (mTOR) and low-energy state detectors (AMP kinase and the sirtuins) (Houtkooper, Williams, and Auwerx 2010). AMPK stimulates ATP production and reduces its consumption, increasing glycolysis and fatty acid oxidation, halting cell growth, biosynthesis, and proliferation, and partially suppressing the mammalian target of rapamycin (mTOR) a serine/threonine protein kinase (Vaiserman, Lushchak, and Koliada 2016; Johnson et al. 2015).

The sirtuin family of NAD-dependent protein deacetylases may promote healthy aging through NAD + reduction and sirtuins downregulation (Massudi et al. 2012). SIRT1 is the most studied mammalian sirtuin, with many roles in several tissues and organs, including the ability to restrain SASP at the transcriptional level (Hayakawa et al. 2015); it also regulates the cellular response to stressors and delays vascular senescence by activating eNOS and suppressing the p53 and NF-kB pathways (Kida and Goligorsky 2016). Endothelial NF-κB inhibition by SIRT1 prevents expression of endothelial adhesion molecules such as intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 (Stein et al. 2010). Consequently, SIRT1 inhibits monocyte binding to endothelial cells, as well as monocyte transmigration into the arterial wall, indicating anti-inflammatory effects in endothelial cells. The activation of SIRT1 may inhibit NLRP3 inflammasome activation and subsequent caspase-1 cleavage as well as interleukin (IL)-1β secretion, whereas SIRT1 knockdown obviously enhances the activation of NLRP3 inflammasome as demonstrated in endothelial and monocyte cell (Lin et al. 2017); (Zhao et al. 2019).

4. Nutraceutical compounds

Numerous substances from the natural world can interact with biological processes and are therefore referred as “bioactive compounds”. Bioactive compounds contained in food can be defined as “nutraceuticals” (Biesalski et al. 2009). In recent years, the role of phytochemicals in preventing pathological conditions, especially ARDs, has been the subject of intense research. One of the most promising hypotheses on healthy lifestyles is that bioactive compounds contained in food could improve health span through the modulation of SASP, opening up new directions to revolutionize ways to slow down the onset and development of ARD (Pazoki-Toroudi et al. 2016).

A key objective of current gerontology research is to develop drugs capable of modulating the aging process (Vaiserman, Lushchak, and Koliada 2016). Compounds with anti-aging properties could be divided into those that are fully synthesized (because they are not found in nature), semi-synthesized (through chemical modification of substances from microorganisms or plants), or completely natural. Although some synthetic molecules developed to treat a variety of conditions, i.e. chemotherapeutics, have been seen to exert activity on the senescence process, they do carry significant adverse effects; this implies that their clinical development will be limited to conditions where the benefit/risk ratio is favourable. Since the field of senolytics is in its infant phase, the translation of pre-clinical data to human trials will require the identification of specific settings of subjects as well as the identification of measurable markers/outcomes (Kirkland et al. 2017). Notably, senolytic treatment not necessarily needs to be continuous, as demonstrated in mice models in which the periodic elimination of SCs was associated with a reduced progression of the major ARDs (Kirkland et al. 2017).

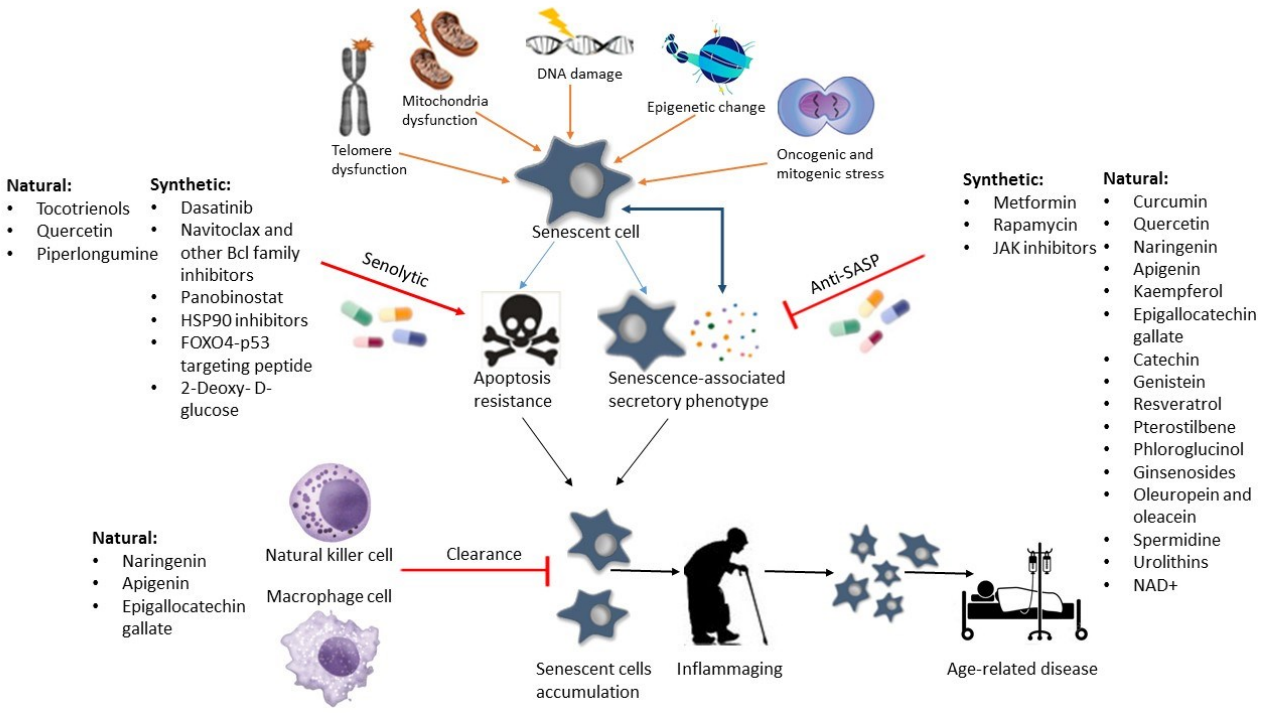


Figure 4. The main anti-SASP and senolytic effects of synthetic and natural compounds (Gurau et al. 2018).

Polyphenols are secondary metabolites of plants involved in the response against ultraviolet radiation or pathogens. They are responsible for the colour, flavour, and smell of food (Li et al. 2014). Their concentrations can be affected by a variety of factors like sun exposure, rainfall, degree of ripeness, processing, storage, and cooking process.

They are classified according to the number of phenol rings and the elements they bind and are grouped into flavonoids and non-flavonoids. Their fundamental chemical structure usually involves a linkage to one or more sugars, amines, carboxylic and organic acids, lipids, or other phenols (Durazzo et al. 2019).

A number of compounds contained in food can act as epigenetic modifiers, able to modulate gene expression, chromatin assembly status, DNA methylation pattern and non-coding RNA expression (miRNA, siRNA, piRNA) (Bacalini et al., 2014). A number of studies suggested that the administration of polyphenol–rich foods can modulate the activity of DNA writers/readers, such as DNA methyltrasferase (DNMT), histone deacetylases (HDACs), histone acetyl

transferases (HATs) and HDAC sirtuins (SIRTs), highlighting new molecular mechanisms that could contribute to a healthy aging (Szarc vel Szic et al. 2015).

Several studies show that polyphenols can act on various biological targets; they exert a strong anti-oxidant activity and can control several processes involved in inflammation. Due to these, polyphenols are considered the natural compounds with a strong effect on SASP phenotype (Gurau et al. 2018).

It has been widely proven that resveratrol is an activator of SIRT1, miming effects of calorie restriction, thus improving cells longevity (Schilder et al. 2009; Howitz et al. 2003) and leading to a suppression of SASP factors production such as IL-6 and IL-8 (Takenaka et al. 2015). Two studies have pointed out resveratrol beneficial effects on human umbilical cord fibroblasts (Yamashita et al. 2012) and on human mesenchymal stem cells (Peltz et al. 2012), preventing in the first case the senescence process and delaying it in the second one.

Resveratrol and curcumin have displayed the capacity to inhibit mTOR, suggesting their possible application in developing nutraceuticals to delay senescence (Malavolta et al. 2014).

In studies conducted on animals, curcumin has been able to enhance the lifespan of C. elegans, upregulating sirtuins and the antioxidant responses transcription factor SKN-1/NRF2, and that of Drosophila flies, countering oxidative stress and lipid peroxidation (Argyropoulou et al. 2013).

β-caryophyllene (BCP) is a naturally occurring phytocannabinoid with bicyclic sesquiterpene structure. It is present in a large number of plants such as cinnamon, black pepper, cannabis etc (Fidyt et al. 2016). Caryophyllen can modulates cytokine production and reverse age-associated impairments in memory on aged mice (Lindsey et al. 2019). β-caryophyllene is a selective cannabinoid receptor type 2 (CB2) agonist with many beneficial effects such as anti-inflammatory, analgesic, antioxidant and antimicrobial, also improved glycaemic parameters and dyslipidaemia (Baldissera et al. 2017; Basha and Sankaranarayanan 2016). It also decreases mRNA levels of IL-1β and TNF-α and inflammation in the hippocampus in mouse model (Cheng,

Dong, and Liu 2014). According on a study carried out on C. elegans, BCP was observed to increase the lifespan and to significantly reduced free radical levels (Pant et al. 2014). In another *in vivo* study, BCP downregulated proatherogenic adhesion molecule (VCAM-1) and restored vascular eNOS/iNOS expression balance (Youssef, El-Fayoumi, and Mahmoud 2019).

Increasing evidence support the hypothesis of potential additive effects, both agonistic or antagonistic, of mixtures of natural compounds. The synergistic effects of curcuma and resveratrol was demonstrated in human articular chondrocytes through the inhibition of inflammation (Csaki et al., 2009) and in animal models of neuroinflammation (Zaky et al., 2017). The anti-senescence effect of polyphenols could contribute to delay the development of human ARDs. Cardio- and neuro- protective functions were reported for a number of polyphenols (Sun et al., 2017), and increasing evidences suggest that polyphenols can reduce postprandial hyperlipemia, as well as insulin resistance (Meydani and Hasan 2010).

Accumulating evidences suggest a plethora of beneficial effects of PPs for human health. However, the real efficacy of PPs-enriched foods and PPs supplementation has been questioned due to the low oral bioavailability of PPs. This consideration raises doubts regarding the molecular underpinnings of PPs pharmacological activities. Recent evidence highlighted the production of bioactive molecules from PPs-enriched foods by the bacteria in the colon, thus suggesting the ability of PPs to interact with bacteria of gut microbiome (Gurau et al. 2018). Most of the ingested molecules from PPs-enriched foods enter the large intestine where they are digested to smaller phenolic acids that may be the key bioactive effectors (Edwards et al. 2017). These considerations support the hypothesis that PPs can exert pleiotropic effects both through their metabolites or through the modulation of human microbiota.

High-fiber and rich in fruit and vegetables diets have been associated with lower risk of human chronic disease, including type 2 diabetes (T2DM) and cardiovascular diseases (CVD) (Estruch et al. 2018). The effects of PPs on glucose and lipids metabolism is increasingly studied for their

potential glucose- and lipid-lowering activities. Evidence from prospective observational studies and clinical trials has converged to support the relevance of foods-dietary patterns and exercise in the prevention and management of T2DM (Palacios, Kramer, and Maki 2019). Diets moderate in alcohol consumption, lower sugar-sweetened beverages and rich in fruits, vegetables, legumes, nuts and whole grains, and therefore rich in PPs, have demonstrated to reduce diabetes risk and improve glycemic control and blood lipids in patients with T2DM (Ley et al. 2014).

4.1. Effects of polyphenols on glucose metabolism

Nutritional intervention is a key component of diabetes management and the importance of balancing macronutrients, lowering glycemic index, reducing carbohydrate load and implementing an overall healthy dietary pattern are emerging as therapeutical approaches for diabetes. The characterization of natural compounds that could improve the health status of diabetic patients or prevent diabetes onset is therefore an important challenge (Deepa et al. 2018; Tafesse et al. 2017).

Even if the consumption of PPs could be a good strategy to manage hyperglycemia, the bioavailability in human represents a challenge, given their rapid metabolism and excretion (Villa-Rodriguez et al. 2018). Although many dietary PPs may have a biological impact through direct effects on glucose transporters, PPs reaching the colon can be metabolized by the resident microbiota to bioactive products. However, the understanding of the microbial bioconversion processes is still limited (Murota, Nakamura, and Uehara 2018).

Studies related to this topic evidence that dietary PPs contribute to the maintenance of intestinal health by preserving the gut microbial balance through the stimulation of the growth of beneficial bacteria (*i.e.*, lactobacilli and bifidobacteria) and the inhibition of pathogenic bacteria, exerting prebiotic-like effects (Duenas et al. 2015). Therefore, individual differences in microbiota

populations may result in different capabilities for PPs bioconversion with potential implications for health.

PPs can affect glycemic homeostasis through different mechanisms, such as: 1) inhibition of α-amylase and α-glucosidase, 2) inhibition of glucose transporters, 3) stimulation of insulin secretion, 4) effects on hepatic glucose homeostasis, 5) protection against oxidative stress and inflammation associated with hyperglycemia (Kim, Keogh, and Clifton 2016), and 6) reshaping of the microbiota.

PPs effects on α-amylase and α-glucosidase activity

The most important enzymes involved in carbohydrate digestion are α-amylase and α-glucosidase (Kim, Keogh, and Clifton 2016), in association with sucrase and maltase that catalyze the hydrolysis of disaccharides (Williamson 2013). Increasing evidence suggest that PPs can inhibit both amylase/glucosidase and disaccharidases. Several studies have demonstrated the ability of dietary anthocyanins to suppress the activity of α-amylase and α-glucosidase (Belwal et al. 2017). Quercetagetin, scutellarein and fisetin, were able to inhibit salivary α-amylase (Lo Piparo et al. 2008). An effective inhibitory activity of both α-amylase and α-glucosidase was recently reported for rosmarinic acid, olivetol, curcumin, resveratrol, p-coumaric acid, 1,1,2,2-tetrakis (p-hydroxyphenyl) ethane, isoliquiritigenin, and caffeic acid phenethyl ester (Taslimi and Gulcin 2017).

Disaccharidases are targets of a number of flavonoids. For instance, quercetin reduced the effect of sucrase and maltase in both *in vivo* and *in vitro* treatments (Pereira et al. 2011).

PPs effects on inhibition of glucose transporter

Glucose is a primary energy source for each and every cell of the body. Increasing evidence suggested that PPs exploit the inhibition of metabolizing enzymes and transporters of glucose.

Only limited data are available on the inhibition of the sugar transporters GLUT2, GLUT5 and GLUT7 by flavonoids or other classes of bioactives compounds (Williamson 2013).

Glucose is absorbed by enterocytes trough SGLT and GLUT2. It was observed that tea polyphenols, namely (-)-epigallocatechin gallate, (-)-epigallocatechin and (-)-epicatechin gallate, were able to inhibit both SGLT1 and GLUT2. The same effects were observed for quercetin-3-O-rhamnoside, phlorizin, pelargonidin-3-O-glucoside and 5-caffeoylquinic acid, that have a major affinity for GLUT2 (Manzano and Williamson 2010).

PPs effects on stimulation of insulin secretion

Glycemic control is an accurate process regulated by the coordination between glucose sensing and endocrine activity. A number of evidence deriving from epidemiologic studies has demonstrated the support of food-rich PPs for the treatment of metabolic disorders associated with insulin resistance. However, the molecular mechanisms underpinning this effect have not been fully investigated (Munir et al. 2013).

A caffeine-free coffee polyphenols extract, whose main components are caffeoylquinic acid (CQA) and feruloylquinic acid (FQA) derivatives, has shown the capability to increase GLP-1 output both *in vitro* and *in vivo*, probably by increasing cAMP intracellular concentration (Fujii et al. 2015). Similarly, an anthocyanin-rich grape seed extract was observed to be as effective as vildagliptin in enhancing GLP-1 secretion and consequently insulin release, in a way dependent on the glucose load (Gonzalez-Abuin et al. 2014). The antidiabetic properties of CQA derivatives of sweet potato leaves through the improvement of GLP-1 secretion were observed *in vitro* studies (Nagamine et al. 2014).

An increased cAMP concentration is associated with increased insulin secretion. The phosphodiesterase enzymes (PDEs) degrade cAMP, thus reducing insulin secretion. Resveratrol and curcumin are able to inhibit PDE, enhancing insulin release in *in vitro* studies (Rouse,

Younes, and Egan 2014). Genistein was associated with an increased amount of intracellular cAMP, which, in turn, stimulates protein kinase A (PKA), thus amplifying glucose-induced insulin secretion (Liu et al. 2006).

Recently, the antidiabetic properties of natural chicoric acid extract (NCRAE) of chicory were explored. Its main components arechicoric acid and chlorogenic acid were tested in streptozotocin diabetic rats, demonstrating that NCRAE was able to improve hyperglycemia (Ferrare et al. 2018).

PPs effects on hepatic glucose homeostasis

The liver is highly involved in the regulation of glycemia. In the case of increasing blood glucose due to food consumption, it can store glucose as glycogen through the combined pathways of glucokinase (GK) and glycogen synthase (GS) (Hanhineva et al. 2010).

Cinnamon polyphenols are also known to have antidiabetic properties. A study aimed to evaluate the glucose lowering activity of PPs of an aqueous cinnamon extract (e.g. procyanidins, cinnamic acid, and catechin) demonstrated that these compounds effectively suppressed PEPCK and G6Pase gene expression *in vitro* and reduced fasting blood glucose levels in diet-induced obese hyperglycemic mice (Cheng et al. 2012).

Administration of EGCG to db/db mice induced a reduction of blood glucose levels in association with an increased glucokinase expression and a reduction of PEPCK expression (Wolfram et al. 2006). Ferulic acid and p-coumaric acid increase the activity of glucokinase in diabetic rats (Jung et al. 2007), while hesperidin and naringin up-regulate glucokinase activity via peroxisome proliferator-activated receptor gamma (PPAR-γ) (Jung et al. 2006).

PPs effects of protection against oxidative stress and inflammation associated with hyperglycemia

Chronic high glucose concentration leads to an increased intracellular production of reactive oxygen species (Dominguez Avila et al. 2017) that fuels inflammation, thus exerting harmful effects not only on pancreatic β-cells, impairing their normal functions, but also on endothelial and immune system cells. PPs are known to counter oxidative imbalance by scavenging free radicals and promoting endogenous antioxidant defense systems (Bahadoran, Mirmiran, and Azizi 2013). Both EGCG and quercetin exerted a protective action on INS-1 cells against oxidative injuries and prevented apoptosis. Both compounds improved detoxifying enzymes activity (i.e. SOD, catalase and GPx) and increased PI3K/Akt activity (Kim et al. 2010). Anthocyanins-rich Chinese bayberry extract exhibited a protective action on β-cells via enhancement of heme oxygenase-1, and modulation of ERK1/2 and PI3K/Akt pathways (Zhang et al. 2011).

Similarly to oxidative stress, the inflammatory process can impair the normal control of glucose homeostasis leading to diabetes onset (Keane et al. 2015). In several studies, cyanidin 3-glucoside, procyanidins, blueberry anthocyanin and quercetin reduced the expression of the typical inflammation markers (e.g. TNF-α, NF-kB, COX-2, CRP, IL-6 etc.) *in vivo*, by increasing insulin sensitivity in target tissues (Kim, Keogh, and Clifton 2016).

4.2. Effects of polyphenols on lipid metabolism

Oral supplementation with PPs has recently been suggested to improve cardioprotection in such patients (Mollace et al. 2019). PPs are known to be effective in reducing serum cholesterol by inhibiting cholesterol absorption, increasing bile flow and inhibiting the activities of HMGCR and acyl-CoA cholesterol acyltransferase (ACAT) (Gorinstein et al. 2005; Krecman et al. 1998). One of the most referenced studies about the correlation between PPs and lipid profile dates back 1980s (Richard, Cambien, and Ducimetiere 1981). A group of epidemiologists observed that French population had a lower coronary heart disease mortality risk despite a high consumption of cholesterol-rich foods. They described this phenomenon as "the French paradox" (Ferrieres 2004). However, several authors highlighted the paucity of clinical studies on human subjects on the evaluation of the activity of dietary polyphenols against hyperlipidemia once they are introduced into the organism, the effective dose, and their bioavailability, that could confirm or not the findings observed in *in vitro* and *in vivo* studies (Chen et al. 2014; Millar, Duclos, and Blesso 2017).

The potential effects of PPs on lipids metabolism can be summarized as follow: 1) inhibition/decrease of intestinal transport of cholesterol and triglycerides, 2) reduction of serum levels of cholesterol, triglycerides and lipoproteins and 3) interaction with synthesis and elimination of cholesterol and triglycerides.

PPs effects on intestinal absorption of cholesterol and triglycerides

The effects of luteolin and quercetin on lipid metabolism were observed both *in vivo* and *in vitro*. These PPs are able to inhibit cholesterol absorption by Caco-2 cells and human embryonic kidney 293T cells expressing NPC1L1 (Nekohashi et al. 2014). Silymarin and polyphenolic fraction (PF) such as silibinin, silicristin, taxifolin reduced cholesterol absorption in rats fed on high-cholesterol

diet (HCD) and caused significant decreases in plasma and VLDL cholesterol and in the hepatic content of cholesterol and triglycerides (Sobolova et al. 2006).

Tea is probably one of the best PPs mixture exerting the ability to reduce lipid absorption. The catechins and theaflavins present in green and black teas are the most effective polyphenolic compounds in reducing the micellar cholesterol solubility in the small intestine. This effect has been reported in various studies on animal and human models (Kobayashi et al. 2016; Ogawa et al. 2016; Raederstorff et al. 2003).

The PPs present in black chokeberry (Aronia melanocarpa), in particular proanthocyanidins and anthocyanins, cause the inhibition of NPC1L1, SR-BI and ABCA1 expression and an increase in ABCG5, ABCG8 and LDLR expressions *in vitro*. As a consequence, increased apical cholesterol efflux to the intestinal lumen and reduced lipid uptake was observed (Kim et al. 2013). Similarly, the polyphenol curcumin can suppress NPC1L1 expression in the intestinal cells (Feng, Ohlsson, and Duan 2010).

PPs effects on blood cholesterol, triglycerides and lipoproteins levels reduction

Reducing serum lipids, as a well as the concentration of circulating LDL, can prevent the atherosclerotic risk. In several studies, PPs were proven to exert their protective effects through different mechanisms.

The PPs contained in olive oil (hydroxytyrosol, oleocanthal and oleuropein) and thyme (luteolin, caffeic acid) decreased the atherogenic ratios small HDL/large HDL, HDL-cholesterol/HDL-P, and the lipoprotein insulin resistance index (Fernandez-Castillejo et al. 2016).

The flavonols in cocoa products, e.g. epicatechin, procyanidins, catechin, seem to be responsible for increased HDL-cholesterol. This effect may depend on different mechanisms, such as increased expression of scavenger receptor B type I (SR-BI), ATP-binding cassette transporters

ABCA1 and ABCG1 or apolipoprotein A1 (Martinez-Lopez et al. 2014). Curcumin treatment showed considerably lower levels of plasma TC, LDL-C, apoB, and TG in mice (Shin et al. 2011). Citrus flavonoids, including naringenin, hesperidin, nobiletin, and tangeretin have been reported to decrease plasma lipids and to reduce hepatic lipid content in *in vivo* and *in vitro* studies (Mulvihill, Burke, and Huff 2016).

PPs effects on cholesterol synthesis

PPs have been proved to interfere with cholesterol synthesis, thus limiting the contribution provided by this pathway to the total cholesterol amount in the organism.

Solanum nigrum polyphenol extract (SNPE), mainly containing catechin and gallic acid, suppresses HMGCR and sterol regulatory element-binding proteins (SREBPs) expression in HepG2 cells (Chang et al. 2017). The same inhibitory activity on HMGCR was exerted by curcumin in an *in vitro* study (Lin et al. 2015). The epigallocatechin gallate is able to increase cholesterol fecal elimination *in vivo* by displacing cholesterol from intestinal micelles (Kobayashi et al. 2014). In addition, this compound potently inhibits HMG-CoA reductase *in vitro* (Cuccioloni et al. 2011).

It is worth noting that PPs can also modulate other pathways involved in lipids synthesis beyond those specific for cholesterol. A polyphenolic extract obtained from wine lees was tested on a zebrafish animal model. The most abundant components were rutin and quercetin, whereas catechin and resveratrol were present in a lesser amount. Outcomes showed a reduction in fat reserve of zebrafish attributable to a decreased microsomal triglyceride transfer protein (MTP) activity, responsible for the assembly and secretion of VLDL (Caro et al. 2017).

Aim of the work

1. Explore the potential of miR-146a as functional biomarkers of healthy/unhealthy aging and explore the role of miR-155, miR-146a and miR-21 in the modulation of lysine degradation and lengthening of fatty acids pathways, which are involved in the modulation of microbiota composition.

In vitro and *in vivo* studies extensively reported that a diabetic microenvironment promotes premature cellular senescence through the SASP-mediated mechanism (Chang, Hsu, and Wu 2015) (Prattichizzo et al. 2018). The microRNAs (miRNAs), have been extensively investigated in relation to the aging process and ARDs, including T2DM (Huntzinger and Izaurralde 2011). The evidence that miRNAs are present not only in the intracellular compartment but also in a very stable circulating form in many biological fluids has opened the field to their potential application as diagnostic/prognostic biomarkers for a number of diseases. A large body of evidence suggest that several miRNAs are modulated during human aging and many of them can modulate target genes involved in age-related pathways (Huan et al. 2018; Olivieri et al. 2012). MiR-146a is one of the first miRNAs reported to restrain NF-κB activation and down-regulate IL-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6) (Taganov et al. 2006). MiR-146a has been named inflamma-miR because of its ability to fine tune NF-κB signalling (Olivieri et al. 2013).

The aim of this study is to evaluate the expression of plasma miR-146a in CTR and in T2DM group.

2. Could be IL-38, a recently identified anti-inflammatory cytokine, a innovative biomarkers for T2DM patients?

Interleukin (IL)-38, a novel member of the IL-1 cytokine family, is homologous to IL-1 receptor antagonist (IL-1Ra) and IL-36Ra, and has been reported to act as an antagonist (van de Veerdonk et al. 2012). Its expression has been observed in skin, tonsil, thymus, spleen, fetal liver, placenta, and salivary glands (Lin et al. 2001).

Recent findings suggests that expression of IL-38 is involved in chronic inflammatory diseases, especially rheumatic autoimmune diseases, such as rheumatoid arthritis (RA) (Boutet et al. 2016) (Takenaka et al. 2015), psoriasis (Boutet et al. 2016; Li, Liu, et al. 2017), systemic lupus erythematosus (Ressler et al.), (Chu et al. 2017; Rudloff et al. 2015), chronic obstructive pulmonary disease (COPD) (Xu and Huang 2018) and inflammatory bowel diseases (IBD) (Boutet et al. 2016).

Experiments *in vitro*, in human retinal endothelial cells (HRECs) and human umbilical vein endothelial cells (HUVECs), suggest that IL-38 is able to attenuate endothelial cell proliferation, migration, and suppress pathological angiogenesis (Zhang et al. 2017).

Another study has demonstrated that IL-38 overexpression attenuates the severity of experimental arthritis in two mice arthritis models. IL-38 may exert its anti-inflammatory effects by decreasing the production of pro-inflammatory cytokines by macrophages and synovial fibroblasts (Boutet et al. 2017). Moreover, in gestational diabetes mellitus (GDM) was observed an up-regulation of IL-38 in the chorionic villi and umbilical cords (Yu et al. 2017). This suggests that innovative biomarkers are required to manage patients with T2DM and its severe complications.

3. Evaluate the anti-inflammatory synergistic properties of marketed natural extracts, such as curcuma (CUR), resveratrol (RSV) and β-caryophyllene (BCP).

A wide range of natural molecules present in numerous foods have shown antioxidant and anti-inflammatory activity and could contribute to counter the mechanisms through which SCs spread inflammation at the systemic level. As a result, PP-rich foods could have "anti-aging" effects.

Moreover, inflammatory stimuli that last over time can induce a state of chronic inflammation, which is associated with a greater risk of developing age-related diseases (ARD), such as cardiovascular diseases, type 2 diabetes and neurodegenerative diseases.

Since a chronic inflammatory state may contribute to endothelial dysfunction, which is common to all ARDs, an *in vitro* model of human endothelial cells (HUVEC) was used in this study. Hence, the synergistic anti-inflammatory effect of natural extracts on senescent HUVEC and on inflammation-induced human monocytic cell line (THP-1) was evaluated.

To evaluate the anti-inflammatory synergistic effect of natural compounds, both classic and innovative molecular biomarkers were analyzed. The expression of IL-6, IL-1β and TNF-α was evaluated as a classic marker of inflammation.

The two most important inflamma-miRs, miR-146a and miR-21, and an angiomiR, the miR-126-5p, involved in the maintenance of endothelial function were analyzed as innovative markers.

Furthermore, expression of two important proteins involved in inflammation, namely SIRT-1 and Caspase 1, in both cell models were evaluated.

Results

1. Circulating miR-146a in healthy aging and type 2 diabetes: Age- and gender-specific trajectories

Mensà E, Giuliani A, Matacchione G, **Gurău F**, Bonfigli AR, Romagnoli F, De Luca M, Sabbatinelli J, Olivieri F. Mech Ageing Dev. 2019 Jun;180:1-10. doi: 10.1016/j.mad.2019.03.001. Epub 2019 Mar 14.

- MiR-146a plasma levels in healthy individuals
- MiR-146a plasma levels in diabetic patients

2. Inflamm-aging microRNAs may integrate signals from food and gut microbiota by modulating common signalling pathways

Teodori L, Petrignani I, Giuliani A, Prattichizzo F, **Gurău F,** Matacchione G, Olivieri F, Coppari S, Albertini MC. Inflamm-aging microRNAs may integrate signals from food and gut microbiota by modulating common signalling pathways. Mech Ageing Dev. 2019 Sep;182:111127. doi: 10.1016/j.mad.2019.111127. Epub 2019 Aug 8.

A trio of experimentally validated inflamma-miRs such as miR-155, miR-146a and miR-21, was in silico analysed, to support the hypothesis that they can modulate lysine degradation and lengthening of fatty acids pathways, which are involved in the modulation of microbiota composition, i.e. prevotella, ruminococcus and oscillibacter and vice versa. Food homologues to human miR-21, miR-155 and miR-146a were found in cow fat, cow milk, and eggs suggesting that they may be able of targeting, and probably exacerbating, inflammation related pathways.

3. IL-38 could be innovative biomarker in T2DM

3.1. Clinical data and correlation of IL-38 plasma levels in T2DM patients and healthy subjects

The clinical, anthropometric, and biochemical variables of healthy subject-CTR (57.8 yrs, n=95) and T2DM patients, after dividing them into two subgroups: uncomplicated T2DM patients-T2DM-NC (68.1 yrs, n=50), and T2DM patients with complications-T2DM-C (67.0 yrs, n=50) are reported in Table 1.

Clinical and biochemical characteristics of the T2DM patients (NC and C) and CTR subjects were compared. A significant increase of age, BMI, waist-hip ratio, glucose, HbA1C and HOMA-index were found in T2DM patients (NC and C) compared with healthy subjects (CTR). Moreover, a significant glucose, HbA1C, HOMA-index and creatinine increase were observed in T2DM-C compared with T2DM-NC and CTR. Hence, abnormalities in these biological parameters are positively associated with the onset of T2DM.

Table 1. Comparison of biochemical and anthropometric characteristics among healthy control subjects (CTR), uncomplicated T2DM patients (T2DM-NC), and T2DM patients with complications (T2DM-C). Variables are expressed as mean (standard deviation). p-value for one-way ANOVA. *, $p < 0.05$ vs. CTR; #, $p < 0.05$ vs. T2DM-NC.

Variables	**CTR N=95**	**T2DM-NC N=50**	**T2DM-C N=50**	**p-value**
Age (years)	57.8 (13.9)	68.1 (6.7) *	67.0 (7.5) *	< 0.001
Gender (Males)	48	25	24	0.940
BMI (Kg/m^2)	26.6 (5.1)	28.5 (4.4) *	28.6 (3.4) *	0.013
Weight (Catapano et al.)	72.7 (11.8)	75.6 (12.0)	78.1 (10.0) *	0.024
Waist-hip ratio	0.88 (0.08)	0.92 (0.06) *	0.93 (0.07) *	< 0.001
Total cholesterol (mg/dL)	215.0 (40.8)	215.4 (38.4)	212.9 (41.6)	0.943

HDL-C (mg/dL)	60.3 (15.3)	57.7 (16.4)	53.5 (17.7)	0.059
LDL-C (mg/dL)	126.0 (37.5)	127.7 (31.8)	121.2 (33.8)	0.626
Triglycerides (mg/dL)	97.0 (66.6)	131.6 (107.3)	158.8 (119.9) *	0.001
ApoA1 (mg/dL)	180.6 (34.2)	175.3 (36.8)	164.2 (39.6) *	0.037
ApoB (mg/dL)	104.9 (35.7)	108.7 (27.1)	104.6 (27.1)	0.752
Glucose (mg/dL)	94.1 (9.0)	144.0 (29.4) *	183.6 (56.0) *#	< 0.001
HbA1C (%)	5.7 (0.4)	7.0 (0.8) *	8.1 (1.3) *#	< 0.001
Insulin (UI/mL)	5.3 (3.4)	6.5 (4.3)	6.6 (3.8)	0.069
HOMA index	1.23 (0.81)	2.37 (1.67) *	3.05 (2.16) *#	< 0.001
WBC (n/mm^3)	6.2 (1.5)	6.6 (1.5)	6.6 (1.6)	0.135
Platelets (n/mm^3)	232.6 (52.4)	228.1 (123.6)	217.2 (69.8)	0.548
Hs-CRP (mg/L)	2.2 (2.6)	3.2 (3.2)	4.3 (3.9) *	0.001
PAI-1 (ng/mL)	20.1 (11.0)	21.6 (10.7)	18.6 (8.3)	0.350
Fibrinogen (mg/dL)	289.5 (70.5)	291.6 (87.4)	293.4 (85.1)	0.963
Iron (µg/dL)	83.8 (29.4)	83.6 (32.6)	73.7 (19.4)	0.096
Ferritin (ng/mL)	118.9 (95.7)	159.1 (168.0)	99.7 (76.4) #	0.031
Creatinine (mg/dL)	0.82 (0.21)	0.84 (0.17)	1.03 (0.50) *#	< 0.001
eGFR (mL/min)	90.6 (34.7)	81.3 (18.2)	72.7 (22.7) *	0.001
Azotemia (mg/dL)	37.6 (7.9)	39.5 (9.4)	44.2 (13.6) *	0.001
Uric acid (mg/dL)	4.7 (1.0)	5.0 (1.2)	5.0 (1.7)	0.193

Correlation analysis showed that plasma levels of IL-38 in diabetic patients (NC and C) and healthy subjects were strongly related with age, glucose, HbA1c, creatinine, HOMA and telomere length.

Significant positive correlations were found between IL-38 in T2DM patients (NC and C) and healthy subjects with age ($r^2 = 0.232$, $p = 0.001$), glucose ($r^2 = 0.212$, $p = 0.003$), HbA1c ($r^2 = 0.242$, $p = 0.001$), creatinine ($r^2 = 0.157$, $p = 0.028$), HOMA ($r^2 = 0.200$, $p = 0.005$) and significant negative correlation with telomere length ($r^2 = -0.146$, $p = 0.043$).

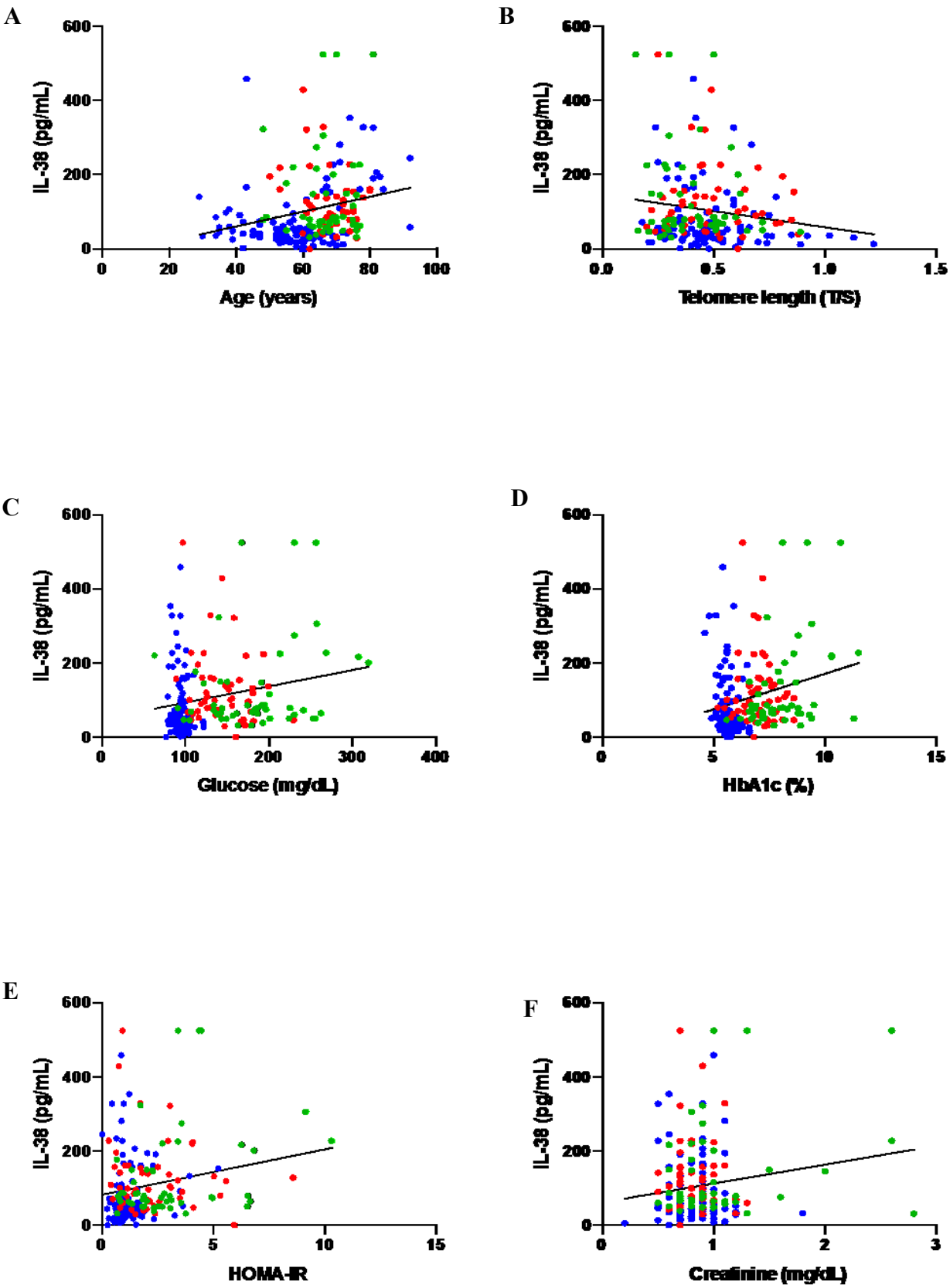


Figure 5. *Correlations between circulating IL-38 levels and selected biochemical variables in T2DM (NC and C) and CTR*. Scatter plots showing the relationship between IL-38 plasma levels and **A**. age, **B**. telomere length, **C.** glucose, **D**. HbA1c, **E**. HOMA-IR, **F**. creatinine in T2DM and CTR.

3.2. IL-38 plasma levels in T2DM patients

IL-38 concentration was investigate in plasma of T2DM patients and CTR subjects. A significant increase concentration of IL-38 was observed in T2DM patients (n=95) compared with CTR group (n=100) (T2DM-129,8 pg/mL versus CTR-81,27 pg/mL, P< 0.001) (Figure 6A). Moreover, was observed that plasma IL-38 concentrations in T2DM-C (n=50), such as nephropathy, chronic renal failure, retinopathy, lower limb arteriopathy and major adverse cardiovascular events (MACE) and T2DM-NC (n=50), were statistically significantly higher compared with CTR (n=95) (T2DM-NC-128,4 pg/mL versus CTR-81,27 pg/mL, p<0.05 and T2DM-C-131,1 pg/mL versus CTR-81,27 pg/mL, p<0.05) (Figure 6B).

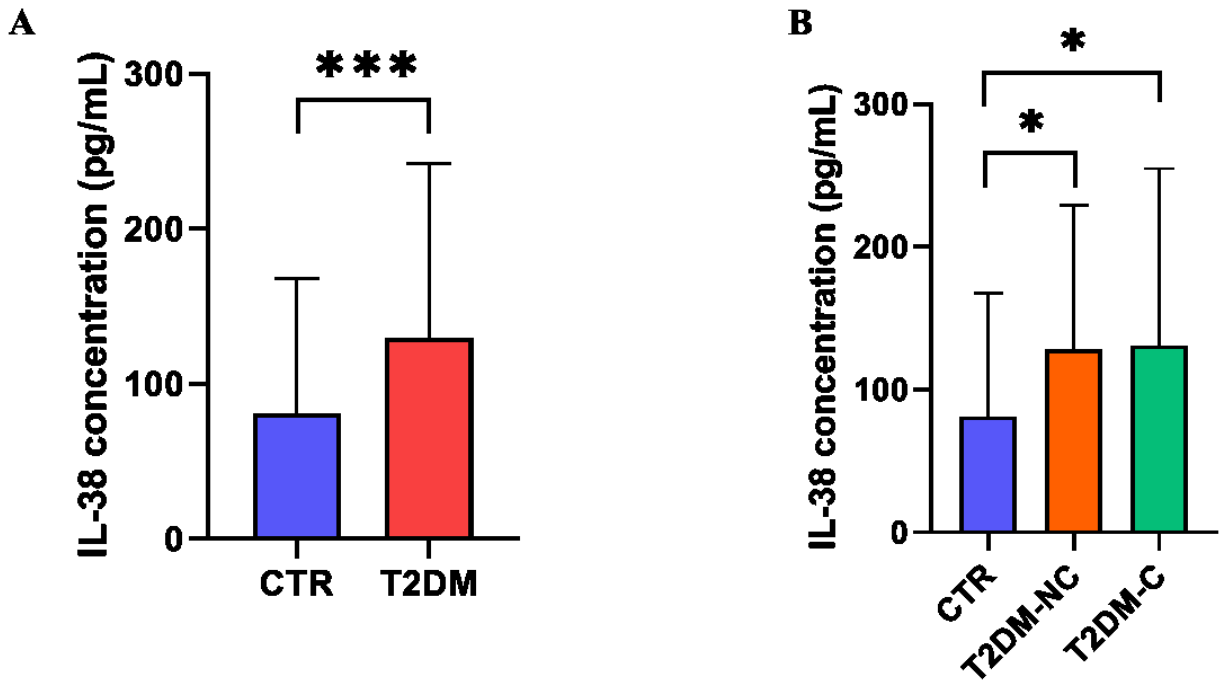


Figure 6. *IL-38 plasma concentration (pg/ml) in T2DM patients and CTR*. A. Plasma IL-38 levels in T2DM patients (NC and C) (n = 100) and healthy controls (CTR) (n = 95). B. IL-38 plasma concentration in T2DM patients with complications (T2DM-C) (n=50) and T2DM patients without complications (T2DM-NC) (n=50) compared with healthy controls (CTR) (n=100). Data were determined by IL-38 ELISA kit. ***p<0.01 and *p<0.05 following analysis of co-variance (ANOVA) with Bonferroni correction.

The concentration levels of IL-38 were significantly higher in patients with diabetic nephropathy (n=15) compared to the T2DM-NC (n=50) (Diabetic nephropathy-187,3 pg/mL versus T2DM-NC-118,4 pg/mL, $p<0.05$). This data was normalized to age and gender (Figure 7).

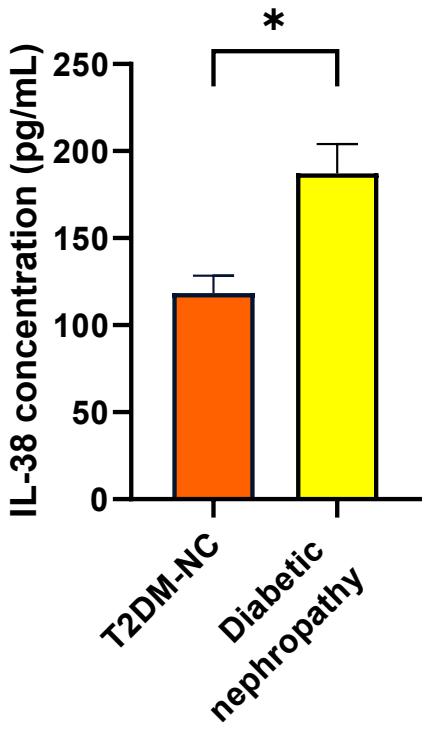


Figure 7. *IL-38 plasma concentration (pg/ml) in patients with diabetic nephropathy.* *p<0.05 following analysis of co-variance (ANCOVA) with Bonferroni correction.

To evaluate the potential of IL-38 as a disease marker for T2DM, plasma IL-38 levels were used for ROC curve analysis (Figure 8). Compared with CTR, the T2DM patients (NC and C) have an AUC: 0.7 ($p<0.01$), a sensitivity of 76.0%, and a specificity of 65.3% with a optimal cutoff value of 61.08 pg/mL.

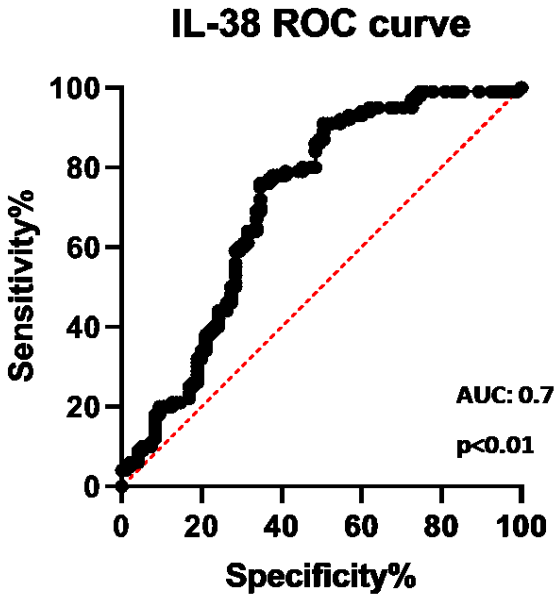


Figure 8. *Receiver-operating characteristic (ROC) curve analysis of plasma IL-38 for the diagnosis of T2DM*. The AUC for IL-38 is provided with its associated 70% confidence intervals. Mann-Whitney U test and associated p values are indicated. The ROC curves specificity % on the x-axis versus the sensitivity% on the y-axis.

Discussion

Is well established the correlation between inflammation and diabetes mellitus. Insulin resistance has been associated with highly produced pro-inflammatory cytokines, e.g. IL-1β, IL-6, C reactive protein and TNF, and decreased production of anti-inflammatory mediators such as IL-4 and IL-10 (Xiao et al. 2014). Nevertheless, IL-38 expression associated with insulin resistance has not been sufficiently investigated.

In our current study there was significantly increased IL-38 levels in T2DM patient, as well as with complication and without complication, compared to healthy subjects. Such findings suggest that anti-inflammatory role of elevated IL-38 may not be effective in reducing inflammation, due to uncontrolled diabetes in susceptible patients. Moreover, in patients with diabetic nephropathy IL-38 concentration was significantly higher compared with T2DM patients without complication. Our study also showed a significant positive correlation between IL-38 levels in T2DM patients and health subjects with age, glucose, HbA1c, creatinine, HOMA. The negative correlations were identified only with telomere length.

Such finding is supported by Zhiyan Yu et al., who demonstrated significantly increased IL-38 in the arteries and veins in the GDM umbilical cord, as well as in the GDM chorionic villi, compared to that of the Non-GDM subjects. Also, this study showed that serum IL-38 was positively correlated with fasting blood glucose (Yu et al. 2017).

In summary, the present study showed that IL-38 may be involve in the development of T2DM.

4. Synergistic anti-inflammatory activity of natural compounds on endothelial and monocytic cells

4.1. Curcuma, Resveratrol and β-Caryophyllene effects on HUVECs viability

Curcuma (CUR), resveratrol (RSV) and β-Caryophyllene (BCP) cytotoxic effects were evaluated on young HUVECs after 3 h and 24 h of treatments by MTT assay.

Further experiments were performed considering approximately 80% viability of treated cells (2 µg/ml CUR and RSV, 20 µg/ml BCP and for their mix 1.6 µg/ml CUR, 1.6 µg/ml BCP, 0.32 µg/ml RSV (ratio 1:1:0.2) (Figure 9).

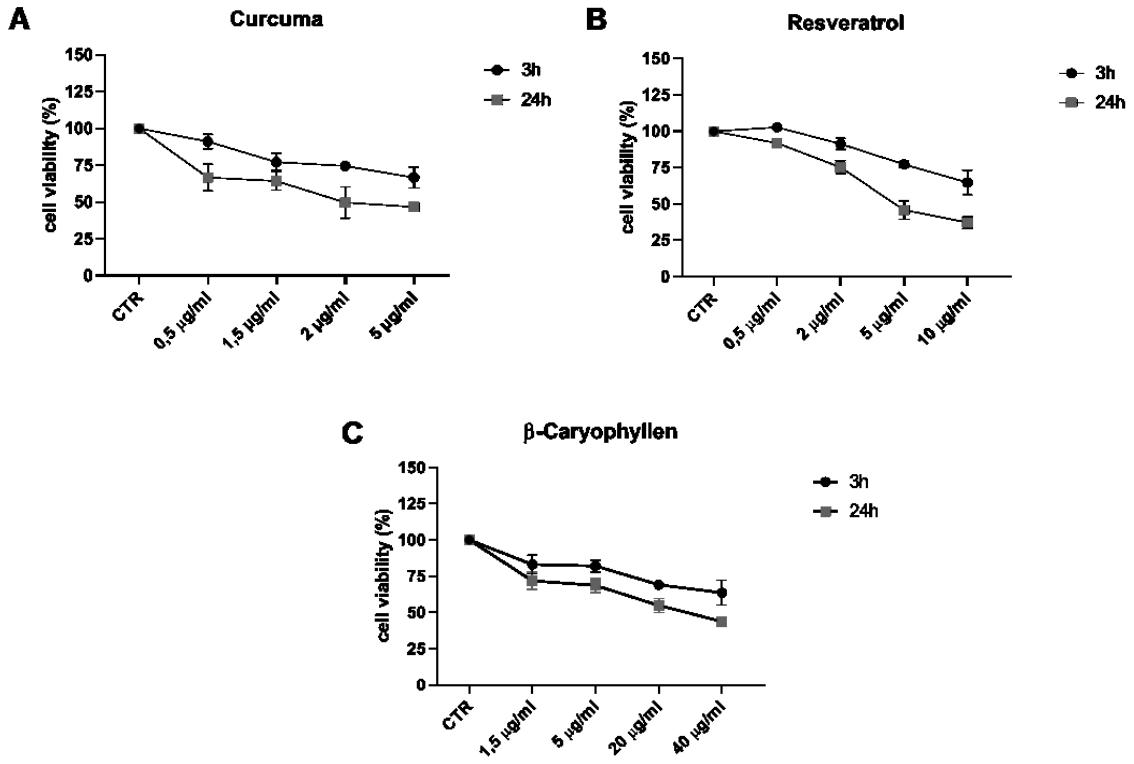


Figure 9. Young HUVEC cell viability after 3h and 24h treatment with different concentrations of **A.** CUR, **B.** RSV and **C.** BCP.

4.2. Replicative and doxorubicin-induced senescence in HUVECs

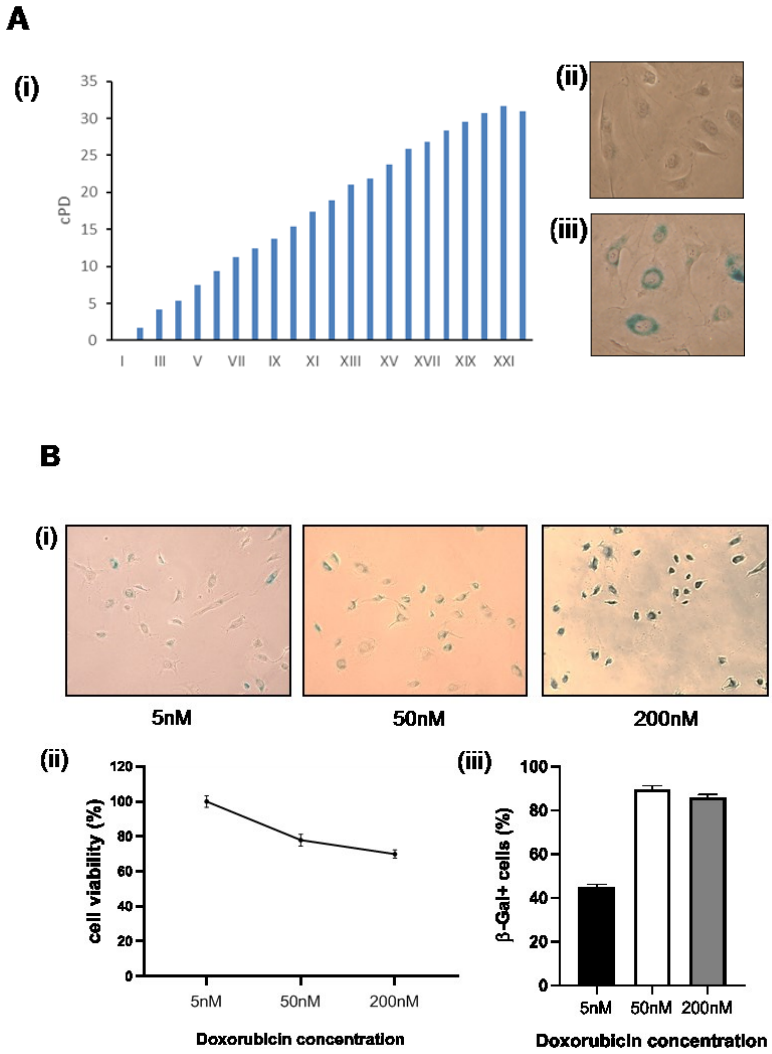
The effects of natural compounds were analysed in young (yHUVEC) and replicative or doxorubicin-induced senescent (RS-HUVEC or IS-HUVEC) HUVEC cells.

Replicative senescence (RS) corresponded to cPD > 30, whereas yHUVECs corresponded to cPD < 15 (Figure 10A).

Doxorubicin cytotoxicity was tested by MTT assay and the concentration with the 80% of cell viability was selected.

Doxorubicin-induced senescence (IS) was achieved with 50 nM treatment for 24h (SA- β-Gal positive cells > 60%) (Figure 10B).

P16 (Ink4a) was analysed in both type of senescent cells, showing a significant increase of both mRNA (Figure 10C) and protein (Figure 10D) compared to younger cells.



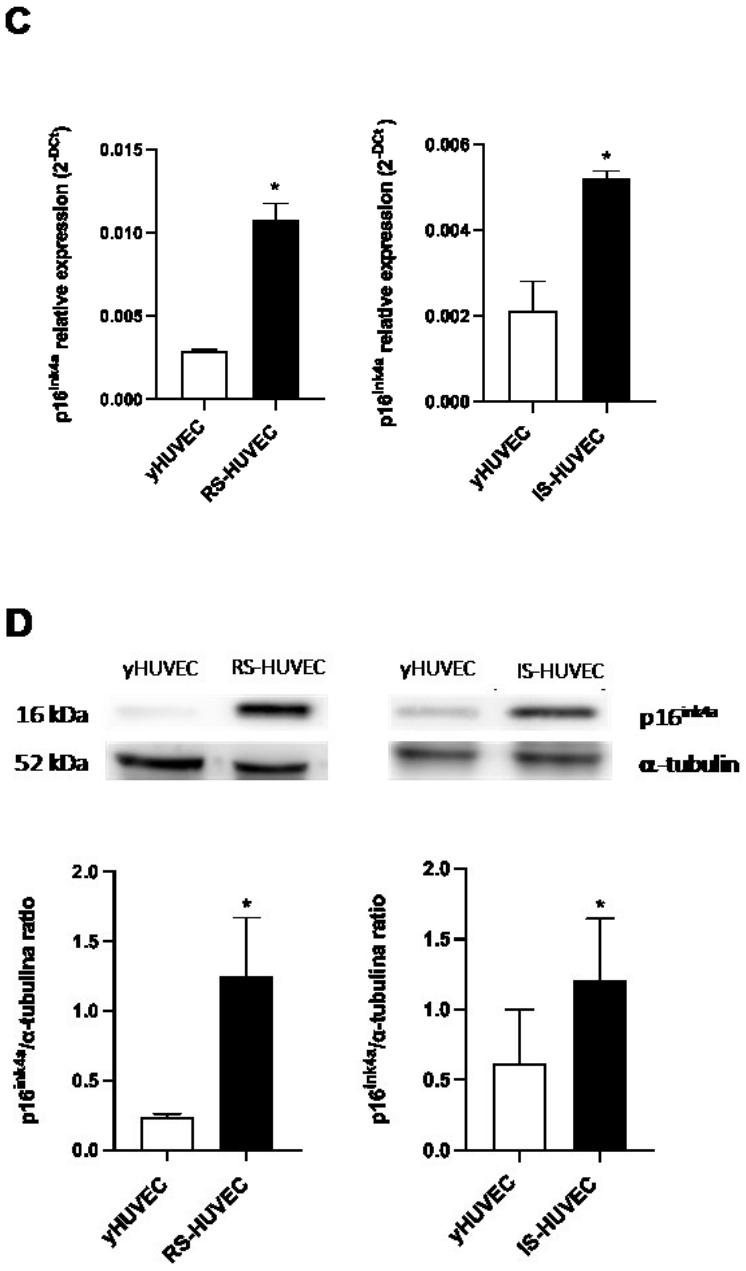
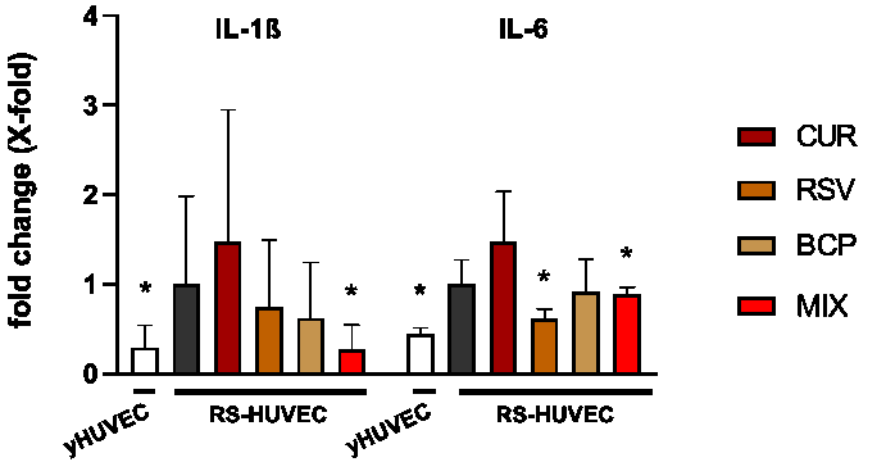


Figure 10. *Characterization of HUVEC in replicative (RS) and doxorubicin-induced senescence (IS).***A**. Cumulative number of population doublings (cPD) in RS-HUVEC. X-axis: cell passages from P1 to P21 **(i)**; SA-β-Gal staining of young (**ii**) and senescent (**iii**) HUVEC. **B**. SA-β-Gal staining (**i**), cell viability (**ii**) and percentage of SA- β-Gal positive cells (**iii**) of IS-HUVEC after treatment 5nM, 50nM and 200 nM of doxorubicin. **C**. Relative expression of $p16^{ink4a}$ mRNA in yHUVEC (p4), RS-HUVEC (p21) and IS-HUVEC. **D**. Western Blot of $p16^{ink4a}$ protein and relative densitometric analysis for RS- (**i**) and IS-HUVEC (**ii**). *Student's t test, $p < 0.05$.

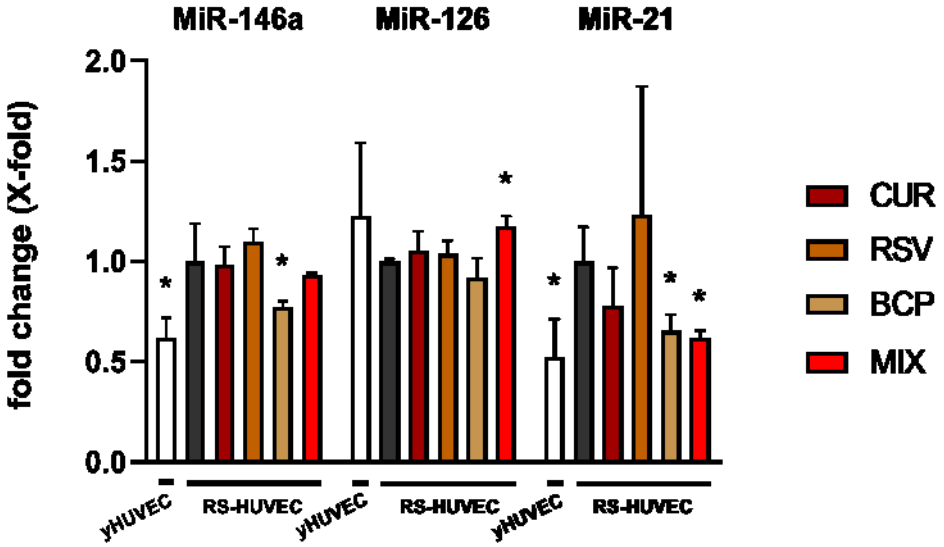
4.3. Synergistic anti-SASP activity of natural compounds in RS and IS HUVECs

Replicative and doxorubicin-induced senescent HUVECs were analyzed to assay the expression of genes related to SASP after the treatment with single natural compounds (CUR-2μg/ml, RSV-2μg/ml and BCP-20μg/ml) or the mix of these substances (1.6μg/ml-CUR: 1.6μg/ml-BCP: 0.32μg/ml-RSV in ratio 1:1:0.2). The expression of IL-1β, IL-6, miR-21, miR-146a and miR-126 and the secretion of the IL-6 were significantly increased in RS- and IS-HUVECs compared to yHUVECs. The MIX was able to reduce more efficiently and significantly IL-1β and IL-6 expression levels in both type of senescence (Figure 11A and Figure 11D) compared to the single compounds. Notably, the treatment with mix induced a significant down-regulation of miR-21 in RS-HUVECs (Figure 11B) and of miR-146a expression in IS-HUVECs (Figure 11E), as well as a significant up-regulation of miR-126 level in both senescent HUVEC models (Figure 11B and Figure 11E). A significant reduction of IL-6 release was observed after treatment with mix in RS-HUVECs (Figure 11C).

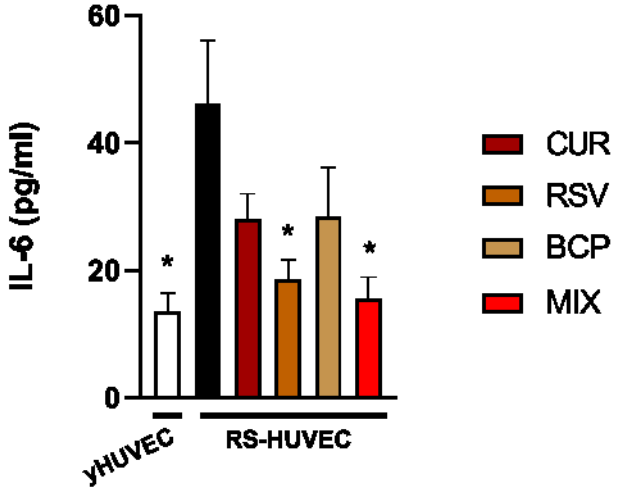
A


IL-1ß
IL-6
fold change (X-fold)
4
3
2
1
0
*
yHUVEC
RS-HUVEC
CUR
RSV
BCP
MIX

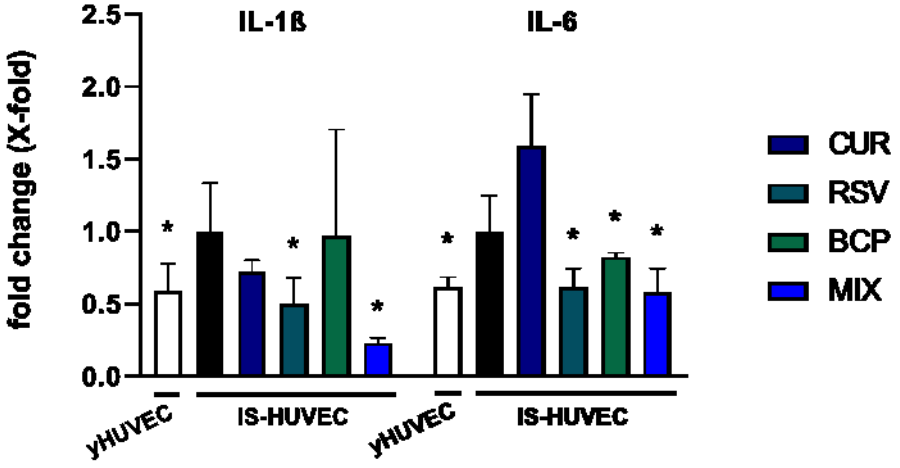
B


MiR-146a
MiR-126
MiR-21
fold change (X-fold)
2.0
1.5
1.0
0.5
0.0
*
yHUVEC
RS-HUVEC
CUR
RSV
BCP
MIX

C


60
40
20
0
IL-6 (pg/ml)
*
*
*
CUR
RSV
BCP
MIX
yHUVEC
RS-HUVEC

D


2.5
2.0
1.5
1.0
0.5
0.0
fold change (X-fold)
IL-1ß
IL-6
*
*
*
*
*
*
*
*
CUR
RSV
BCP
MIX
yHUVEC
IS-HUVEC
yHUVEC
IS-HUVEC

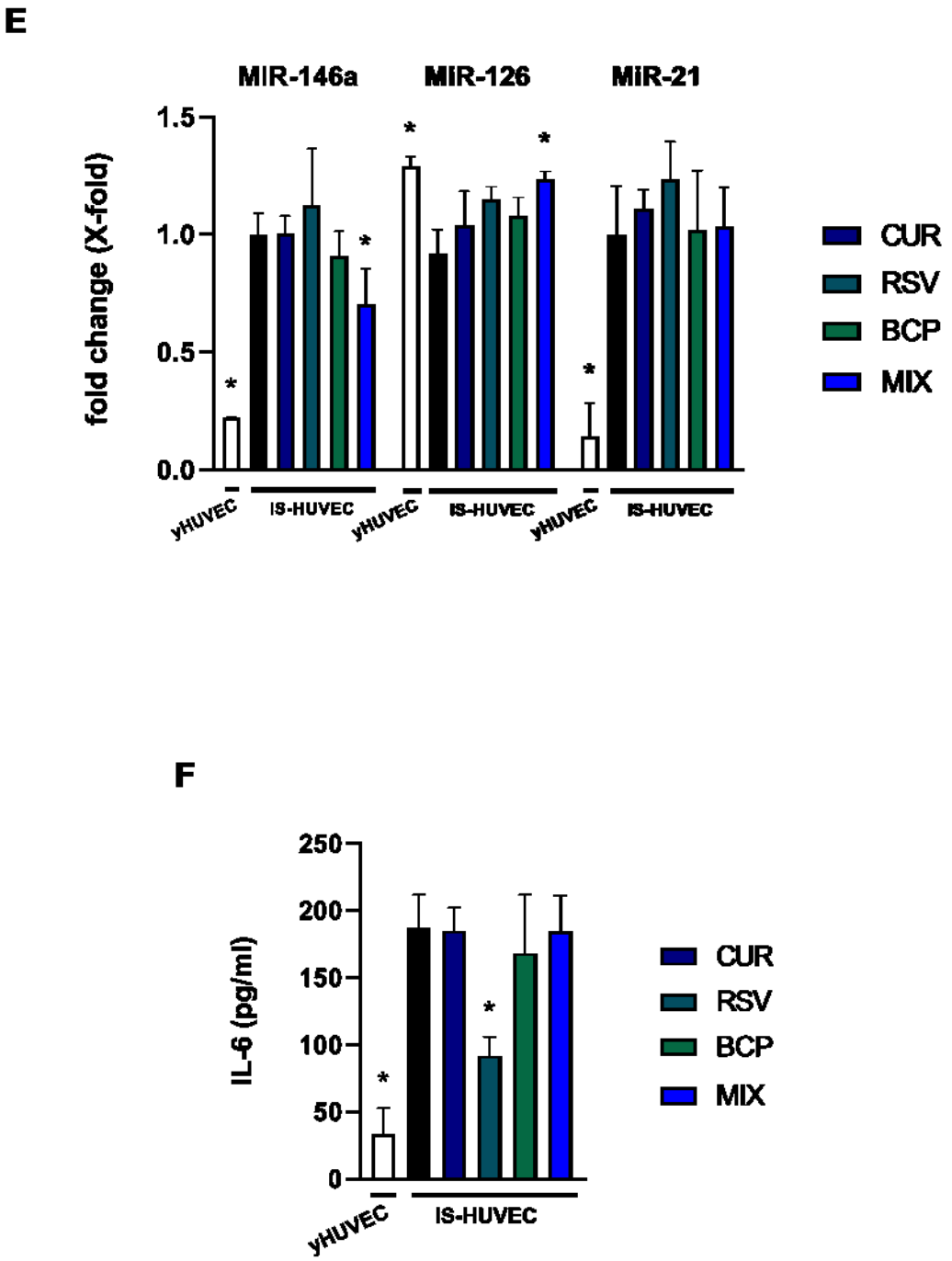


Figure 11. *Analysis of mRNA and miRNA expression and IL-6 secretion after 3 hours of treatment with CUR, RSV and BCP as single compound and in combination in RS and IS HUVEC.* **A**. and **D.** mRNA expression of IL-1β and IL-6 in RS- and IS-HUVEC, respectively. **B** and **E.** miRNA expression of miR-146a, miR-21 and miR-126 in RS- and IS-HUVEC. Data were reported as fold change *vs* untreated RS-and IS-HUVEC, respectively. **C.** and **F.** Concentration (pg/ml) of IL-6 in culture medium of RS- and IS-HUVEC. Histograms represent the mean of three different experiments ± SD. *Student's t test, $p < 0.05$.

4.4. SIRT1, Caspase-1 and p16^{ink4a} protein levels in RS and IS HUVECs treated with Curcuma, Resveratrol and β-Caryophyllene alone or as mix

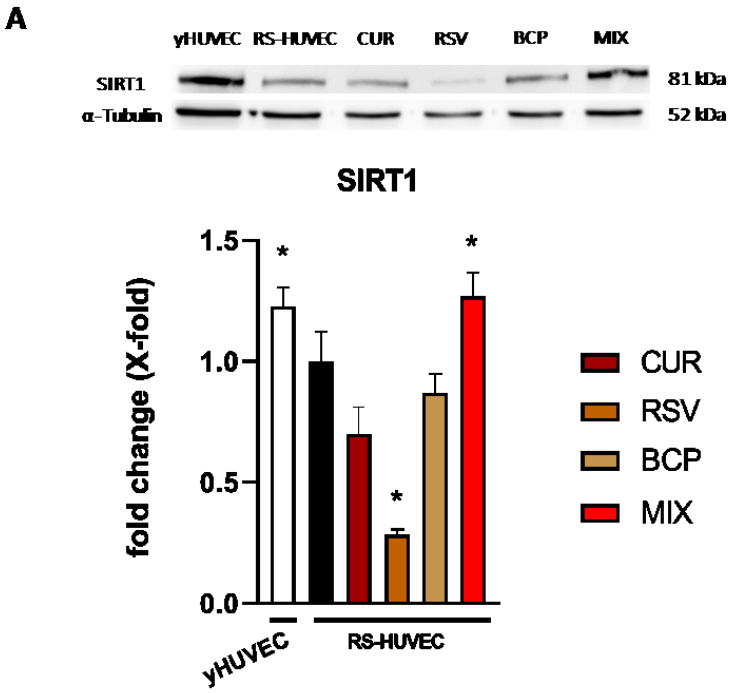
Some proteins are involved in the acquisition of SASP, such as SIRT1, Caspase-1 and p16^{ink4a} were analysed in HUVECs.

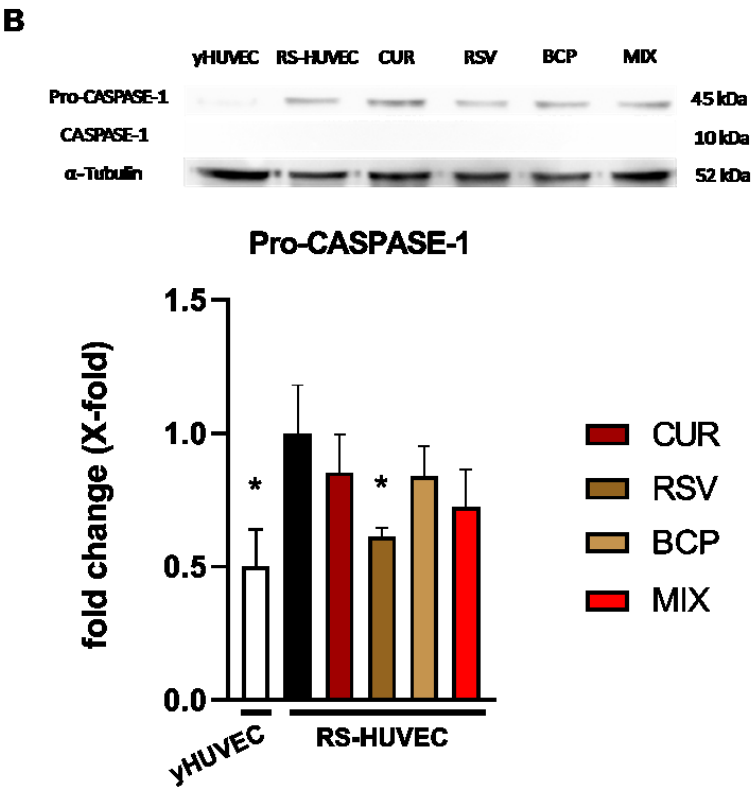
SIRT1 is the most studied sirtuin in mammal, with many roles in several tissues and organs, including the ability to restrain SASP at the transcriptional level (Hayakawa et al. 2015); it also regulates the cellular response to stressors and delays vascular senescence by activating eNOS and suppressing the p53 and NF-kB pathways (Kida and Goligorsky 2016).

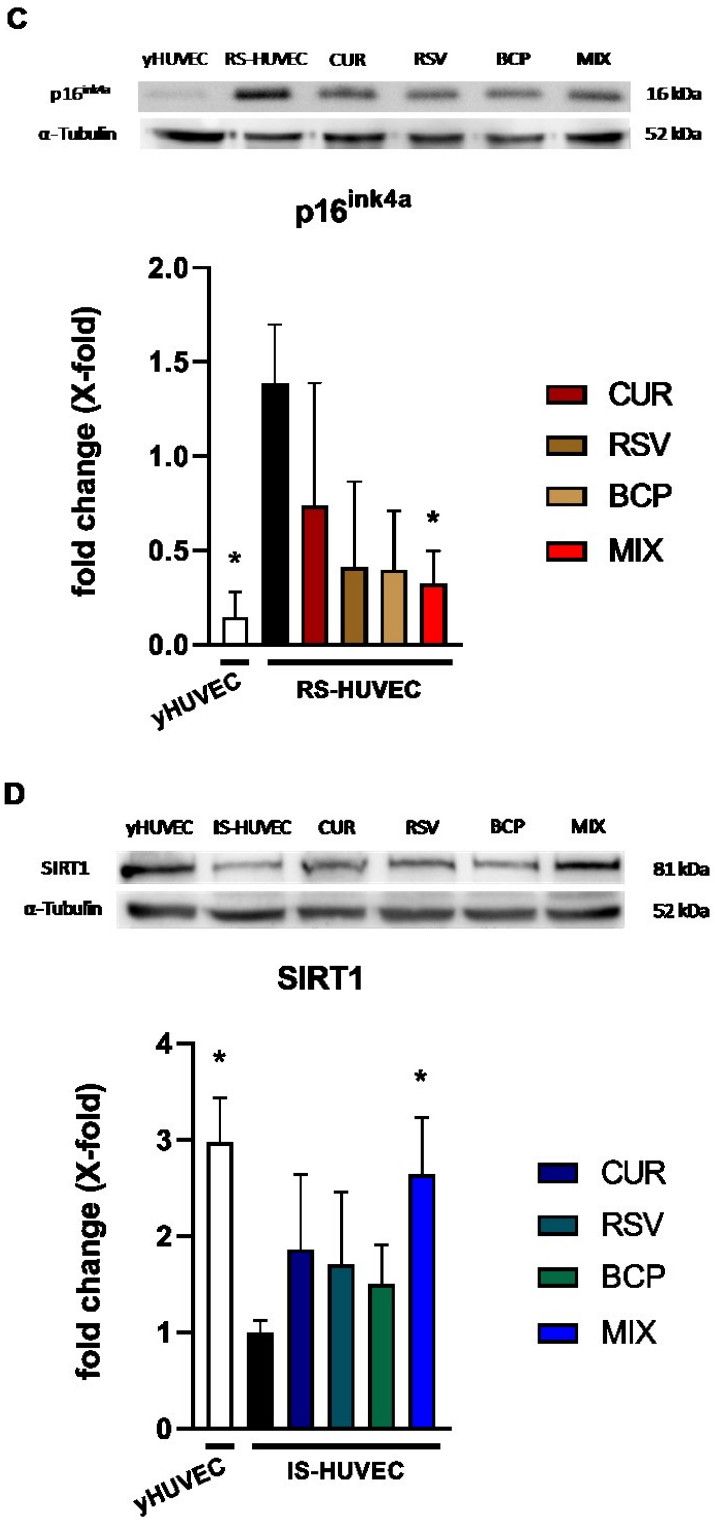
RS- and IS-HUVECs, were treated with single natural compounds (CUR-2µg/ml, RSV-2µg/ml and BCP 20 µg/ml) or the mix of these substances (1.6 µg/ml CUR: 1.6 µg/ml BCP: 0.32 µg/ml RSV in ratio 1:1:0.2) for 3h.

Both RS- and –IS-HUVECs showed a significant reduced level of SIRT1 and a significant increase of pro-Caspase-1 and p16^{ink4a} levels compared with yHUVECs.

The MIX significantly restore the expression of SIRT1 and p16^{Ink4a} in both RS- and IS-HUVECs (Figure 12A,C,D,F).


A
yHUVEC
RS-HUVEC
CUR
RSV
BCP
MIX
SIRT1
α-Tubulin
81 kDa
52 kDa
SIRT1
fold change (X-fold)
1.5
1.0
0.5
0.0
*
*
*
CUR
RSV
BCP
MIX
yHUVEC
RS-HUVEC


B
yHUVEC
RS-HUVEC
CUR
RSV
BCP
MIX
Pro-CASPASE-1
CASPASE-1
α-Tubulin
45 kDa
10 kDa
52 kDa
Pro-CASPASE-1
fold change (X-fold)
1.5
1.0
0.5
0.0
*
*
CUR
RSV
BCP
MIX
yHUVEC
RS-HUVEC


C
yHUVEC
RS-HUVEC
CUR
RSV
BCP
MIX
p16ink4a
16 kDa
α-Tubulin
52 kDa
p16ink4a
fold change (X-fold)
2.0
1.5
1.0
0.5
0.0
*
*
yHUVEC
RS-HUVEC
CUR
RSV
BCP
MIX
D
yHUVEC
IS-HUVEC
CUR
RSV
BCP
MIX
SIRT1
81 kDa
α-Tubulin
52 kDa
SIRT1
fold change (X-fold)
4
3
2
1
0
*
*
yHUVEC
IS-HUVEC
CUR
RSV
BCP
MIX

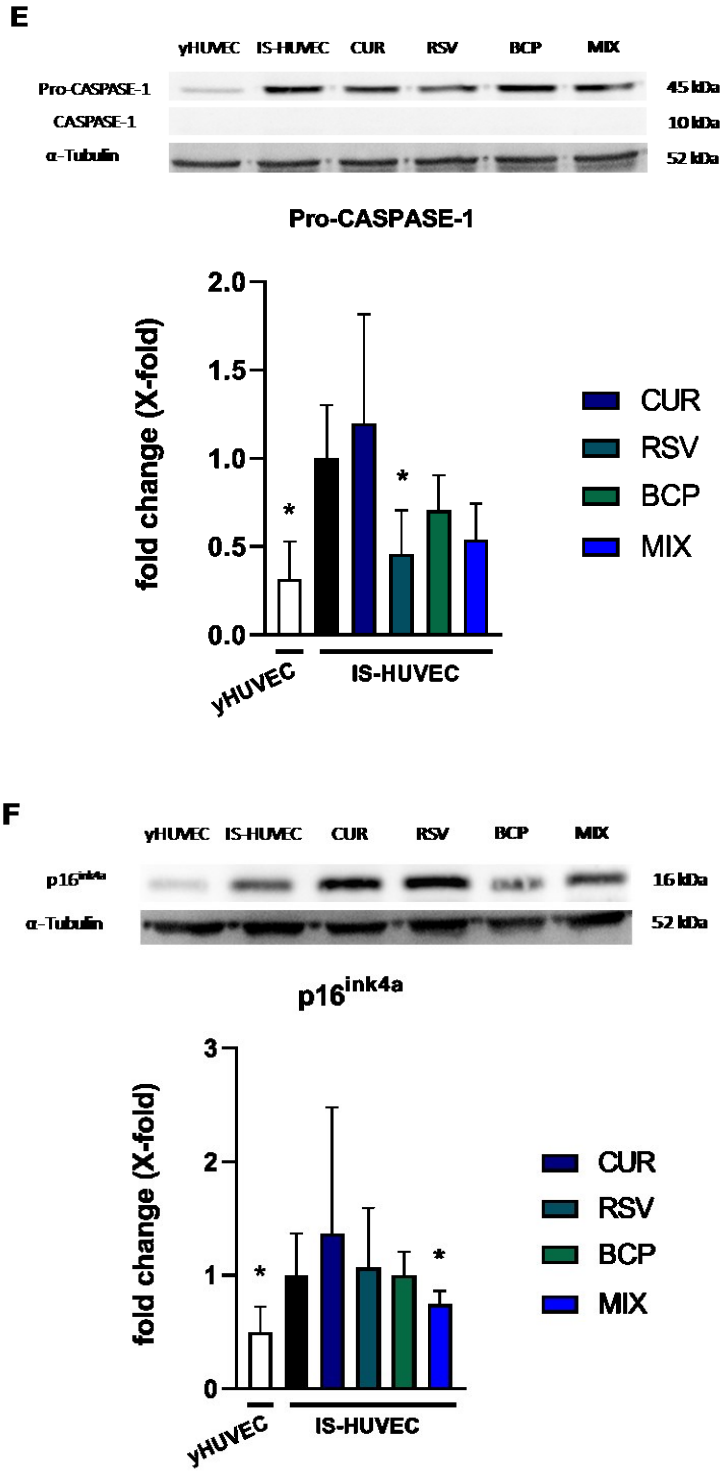


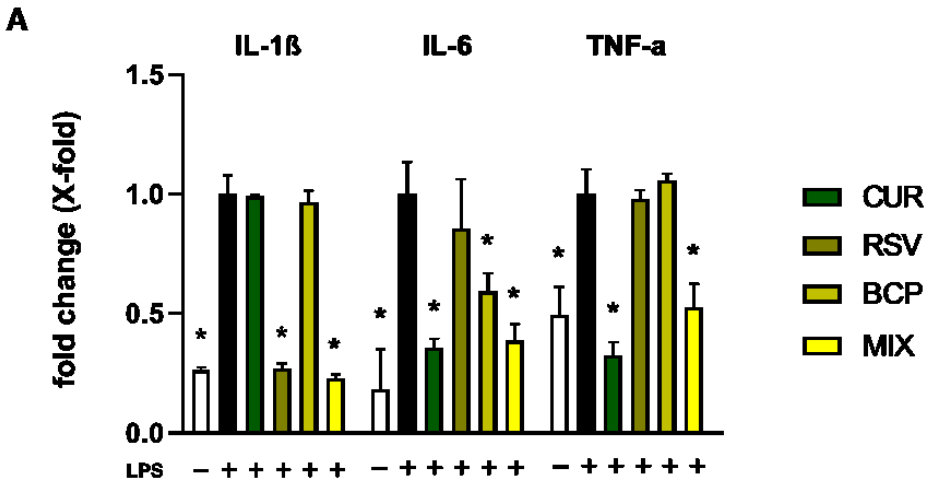
Figure 12. Analysis of *SIRT1, CASPASE-1 and p16^{ink4a} proteins levels after treatment with CUR, RSV and BCP and their mix in RS- and IS-HUVEC.* **A.** and **D**. SIRT-1 protein levels and densitometric analysis RS- and IS-HUVEC. **B.** and **E**. Pro-caspase-1 protein level and densitometric analysis RS- and IS-HUVEC. **C.** and **F**. p16^{ink4a} protein level and densitometric analysis RS- and IS-HUVEC. Data were reported as fold change *vs* RS- and IS-HUVEC. All data were normalized with α-tubulin. Bands were quantified by ImageJ; histograms represent the mean of three different experiments ± SD. *Student's t test, $p < 0.05$.

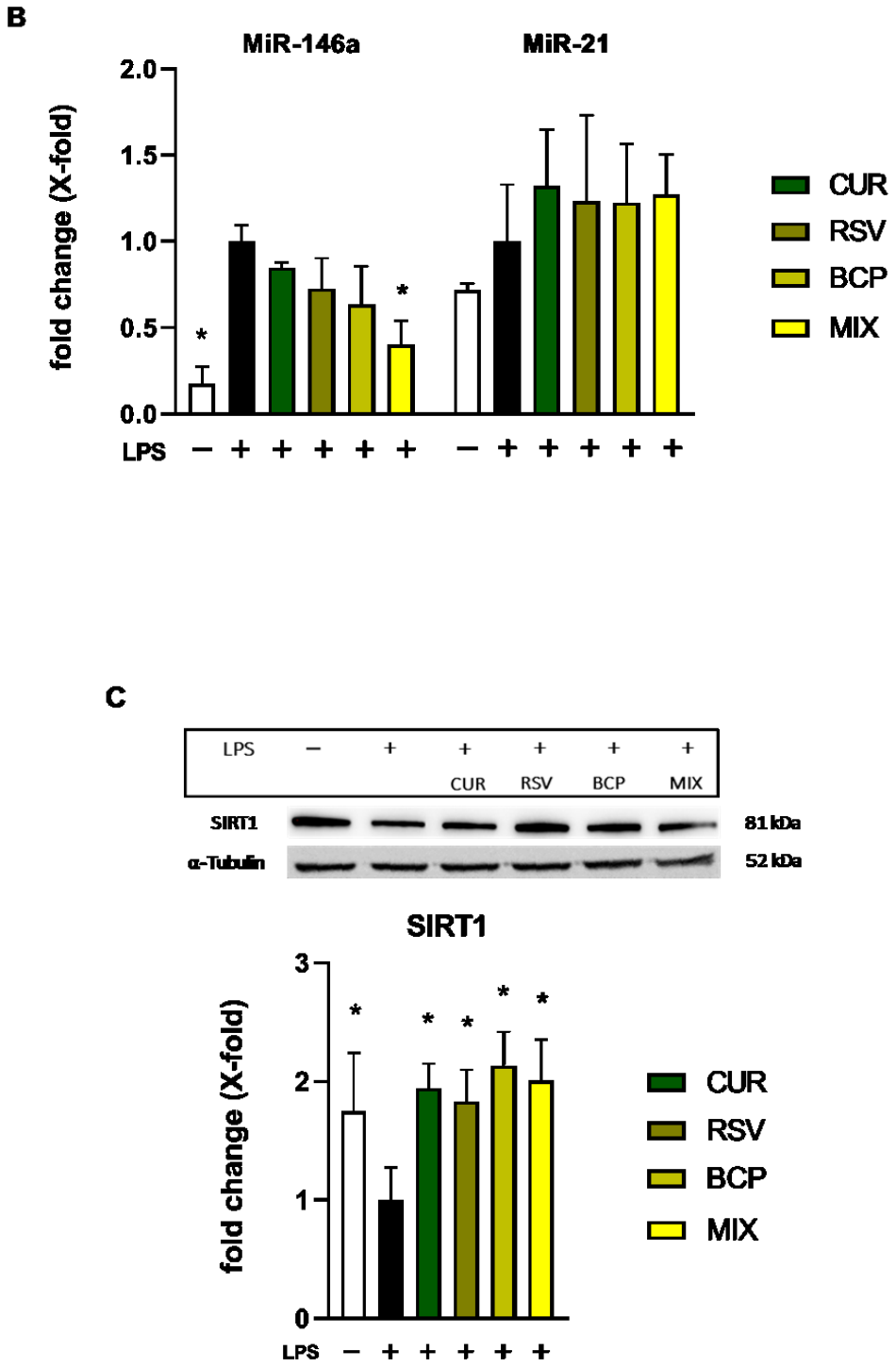
4.5. Synergistic anti-inflammatory effect of natural compound in human monocytic cell line

The potential anti-inflammatory activity of the natural compounds alone or in combination was evaluated analyzing the expression of IL-1β, IL-6, TNF-α, miR-21 and miR-146a in LPS-stimulated THP-1 cells.

The mix significantly decreased the expression of all tested cytokines (IL-1β, IL-6, TNF-α) (Figure 13A) and of miR-146a (Figure 13B) in LPS-stimulated THP-1 more efficiently than the single compounds. MiR-21 was not significantly modulated (Figure 13B).

In addition, we investigated the modulatory effect of natural compounds on SIRT1 levels and Pro-caspase 1 activation. There was a significant down-regulation of Pro-caspase-1 activation only after treatment with the MIX of natural compounds (Figure 13D). All the natural extracts, including the mix, significantly increased SIRT1 protein level (Figure 13C).




B
MiR-146a
MiR-21
fold change (X-fold)
2.0
1.5
1.0
0.5
0.0
LPS
CUR
RSV
BCP
MIX
C
LPS
CUR
RSV
BCP
MIX
SIRT1
α-Tubulin
81 kDa
52 kDa
SIRT1
fold change (X-fold)
3
2
1
0
LPS
CUR
RSV
BCP
MIX

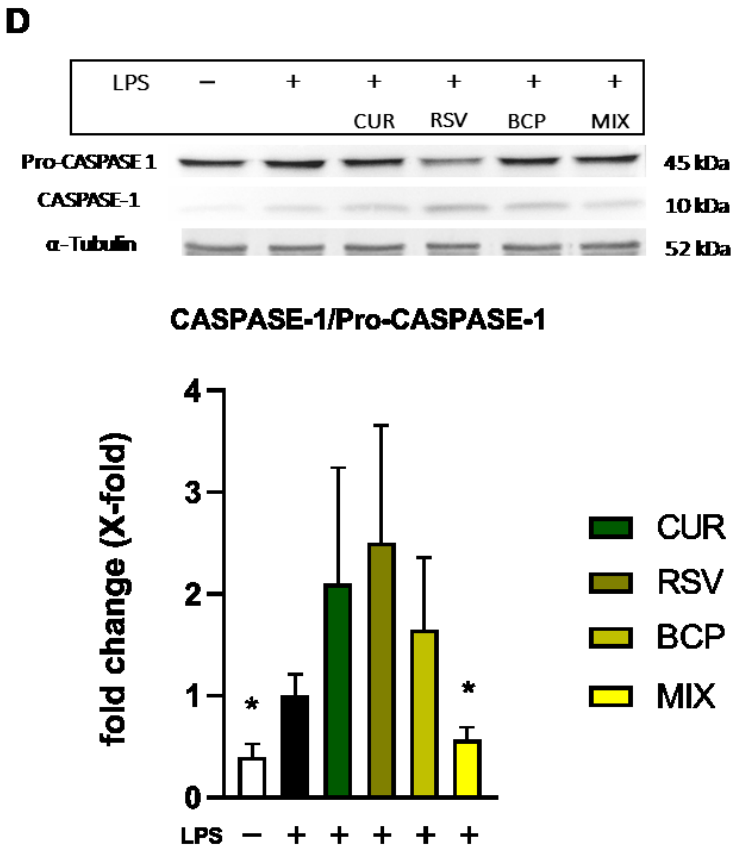


Figure 13. *Analysis of the anti-inflammatory activity of CUR, RSV and BCP as single compound and in combination in LPS-stimulated THP-1 cells.* **A**. IL-1β, IL-6 and TNFα mRNA expression **B**. miR-146a and miR-21 expression. Data were reported as fold change *vs* inflamed THP-1 cells. **C**. and **D**. SIRT1 and Caspase-1/pro-Caspase-1 protein level and densitometry. Data were normalized with α-tubulin and reported as fold change vs inflamed THP-1 cells. Bands were quantified by ImageJ; histograms represent the mean of three different experiments ± SD. *Student's t test, $p < 0.05$.

Discussion

Cellular senescence is one of the main trigger of aging process. A number of natural compound have been investigated for their anti-senescence and anti-aging potential effects through the modulation of senescence-associated secretoma (SASP). The pro-inflammatory cytokines IL-1β and IL-6 are the most investigated cytokines associated with SASP (Orjalo et al., 2009). Also some miRNAs, named inflammamiRs are related to SASP, as well as epigenetic-related enzymes, i.e. SIRT1. We investigated the anti-SASP activity of CUR, RSV and BCP, alone or combined (mix), on HUVEC and THP-1.

Our main results suggest a significant synergistic anti-inflammatory and anti-SASP effects of a mix, compared to the single compounds, both on HUVECs and THP1 cells.

In various study with *in vitro* and *in vivo* models of inflammation and pre-clinical tests and clinical trials in humans, curcumin, colchicine, resveratrol, capsaicin, epigallocatechin-3-gallate, quercetin have reduced cytokines levels and inhibited COX-2, 5-LOX and NF-κB activity (Furst and Zundorf, 2014).

CUR and RSV have been deeply described for their anti-inflammatory property both in monocytic and endothelial cells (Minakshi Rana, 2016; Yueliu Sun, 2015; Joseph Schwager, 2017), whereas BCP was recently considered to attenuate inflammation (Masayoshi Yamaguchi, 2019).

However, to our knowledge, our data are the first evidence of a synergistic activity of CUR, RSV and BCP combined together.

Moreover, to evaluate the anti-SASP activity of the mix, we took advantage of two models of senescence, such as replicative and doxorubicin-induced senescence. This cellular model was never investigated to evaluate the anti-SASP activity of CUR, RSV and BCP.

Furthermore, our study provided evidences that natural compounds exert their biological effects also modulating microRNAs expression (Lin et al., 2017). It has been previously reported that

RSV down-regulates some microRNAs involved in the development of cancers (miR-21, miR-30a-5p, miR-19) (Wang et al., 2015; (McCubrey et al., 2017).

We observed that the mix was able to reduce the expression of some *inflamma-miRs* (Olivieri, Rippo et al. 2013), such as miR-21 and miR-146a and to increase miR-126 in senescent HUVECs, while in THP1 cell the mix was able to down-regulate miR-146a level compared to single compound. Notably, miR-146a and miR-21 targets some proteins belonging to NF-kB pathway. MiR-146a targets both tumor necrosis factor receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK-1) (Taganov et al., 2006), whereas miR-21 down-regulates the expression of IRAK and MyD88 (Chen et al., 2013), as well as of programmed cell death protein 4 (PDCD4) (Sheddy et al., 2010).

MiR-126 plays a pivotal role in modulating vascular development and homeostasis, targeting specific mRNAs such as CXCL12, VCAM-1, SPRED-1 and PIK3R2 (Olivieri et al, 2014).

These data demonstrated that the mix of substances has a synergistic activity in modulating inflammation and vascular cells homeostasis.

Furthermore, SIRT1, Caspase 1 and inflammasome play important roles in inflammation, aging and ADRs (Cordero et al., 2018). It was reported that SIRT1 blocks the monocyte transmigration into the arterial wall, showing anti-inflammatory effects in endothelial cells (Kida and Goligorsky, 2016). There are suggestions that dietary intake of resveratrol, catechins, EGCG, propolis extracts, creosol, and luteoloside may promote health and extend the lifespan via multiple mechanisms, including the reduction of oxidative stress, induction of autophagy, and suppression of NLRP3 activation (Chuang et al., 2014). The activation of SIRT1 may inhibit the NLRP3 inflammasome activation and subsequent caspase-1 cleavage as well as interleukin IL-1β secretion. On the contrary SIRT1 knockdown enhances the activation of NLRP3 inflammasome as demonstrated in endothelial and monocyte cell (Li et al., 2017; Zhao et al., 2019). Our data

demonstrated that the treatment with the mix of substances increased SIRT-1 expression in both cellular models, and reduced the Caspase 1 expression in inflamed THP1.

Overall, our results demonstrate that the mix of CUR, RSV and BCP exerts a synergistic anti-inflammatory/anti-SASP activity in monocytic and endothelial cells. Since endothelial dysfunction is a common risk factors for the ARDs, our results suggest that some specific combinations of nutraceutics can contribute to achieve an healthier aging.

Conclusion

ARD development has been associated to deregulation of a number of pathways including mitochondrial function, oxidative stress balance, and systemic inflammation. SC are characterized by alteration of the same pathways, suggesting that their accumulation during aging could be key major mechanism underlying organismal aging. Experimental data have indicated that acquisition of the senescence-associated secretory phenotype (SASP) by SC could contribute to the tissue dysfunction—including a reduced proliferation of stem and progenitor cell pools—induced by SC accumulation. *In vivo* studies have described an increased level of factors associated with the SASP in all the aged organisms analysed. Since the SASP is a DNA damage response (DDR), it promotes NF-kB and inflammasome activation, which is associated to release of potent proinflammatory factors such as interleukins (IL-1, IL-6, IL-8, and TNF-α), chemokines, growth factors, matrix-degrading enzymes, and reactive oxygen species (ROS). Besides, SASP-related factors are able to influence nearby younger cells, spreading the SASP at the systemic level by fuelling inflammaging, and ultimately promoting ARD development. Increased levels of SASP-related compounds have been described in a number of human ARD such as diabetes.

Several miRNAs are modulated during aging and inflammatory processes in different tissues and organisms. Changes in miRNAs expression have been proposed as potential biomarkers in chronic inflammatory diseases, such as T2DM. In this context, circulating miR-146a could be used as a novel age-related biomarker of healthy/unhealthy aging trajectories.

Cytokine modulation is believed to play a key role in the immune system at older age, with evidence pointing to an inability to fine-control systemic inflammation, which seems to be a marker of aging. IL-38 anti-inflammatory cytokines expression was increases in T2DM patients.

This data may discuss that an enhanced anti-inflammatory phenotype could be beneficial as a contributor to longevity by effectively controlling the pro-inflammatory background.

The findings from the present study corroborate the idea that circulating biomarkers of ARDs such as cytokine and miRNAs, may be useful to early detect deviations from a physiological aging trajectory, allowing to adopt therapeutic or lifestyle interventions to delay the onset of T2DM.

In a whole, the results collected in this work underline that a nutraceutical approach targeting inflammatory condition could be effective in counteracting the detrimental effects of senescence and especially of SASP.

Materials and methods

HUVEC and THP-1 culture

HUVECs, primary human umbilical endothelial cells, obtained from a pool of donors, were purchased from Clonetics (Lonza, Switzerland) and cultured in endothelial basal medium (EBM-2, CC-3156, Lonza) supplemented with SingleQuot Bullet Kit (CC-4176, Lonza) containing 0.1% human recombinant epidermal growth factor (rh-EGF), 0.04% hydrocortisone, 0.1% vascular endothelial growth factor (VEGF), 0.4% human recombinant fibroblast growth factor (rh-FGF-B), 0.1% insulin-like growth factor-1 with the substitution of arginine for glutamic acid at position 3 (R3- IGF-1), 0.1% ascorbic acid, 0.1% heparin, 0.1% gentamicin and amphotericin-B (GA-1000), and 2% fetal bovine serum (FBS). The cells were seeded at a density of 5000/cm^2 in T 75 flasks (Corning Costar, Sigma Aldrich, St. Louis MO, USA). Once that 80-90% confluence was reached, cells were harvested by trypsinization, counted using hemocytometer and replated at lower density.

Induction and characterization of senescent cells

Replicative senescence (RS) was achieved after a number of replicative passages (measured as population doubling-PD). Population doublings (PDs) were calculated by the formula: (log10(F) – log10(I)) / log10(2), where F is the number of cells at the end of the passage and I is the number of seeded cells. Cumulative population doublings (cPD) were calculated as the sum of PD changes.

Drug-induced senescence (IS) was obtained by treating HUVECs with doxorubicin hydrochloride (50 nM) (Sigma Aldrich, Italy) for 24 hours. Treated cells were harvested following a 96-hour recovery period with fresh medium.

HUVECs were classified as young or senescent based on cPD and on senescence-associated (SA)-β-galactosidase activity and p16 expression. Young cells were characterized by cPD < 15 and SA-β-Gal positive cells < 5%. Senescent cells were characterized by cPD >30 and SA-β-Gal positive cells > 60%.

SA-β-Gal activity was detected by using Senescence Detection Kit (BioVision Inc., Milpitas, CA, USA). Briefly, non-confluent, 12-wells plates cultured HUVECs were fixed for 15 minutes at room temperature, and then washed twice in phosphate-buffered saline (PBS) and incubated overnight at 37°C with Staining Solution Mix (containing X-gal). The percentage of β-gal-positive cells was determined by counting at least 200 cells per well using light microscopy. $p16^{INK4a}$.-expression was evaluated by RT-qPCR and western blot analysis.

Human monocytic THP-1 cells were purchased from ATCC (Rockville, MD, USA) and maintained in RPMI-1640 medium supplemented with 10 % heat-inactivated fetal bovine serum, 1 % penicillin/streptomycin, 1 % L-glutamine and 0.1 % β-mercaptoethanol (50mM) (all from Euroclone, Milano, Italy)

The cells were seeded at a density of 500000 cells /ml in T 75 flasks in suspension.

THP-1 cells were treated with 500 ng/ml of LPS (lipopolysaccharide) for 3h to induce an inflammatory response.

Cell viability assay

The (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to test cell viability. Cells were grown in 48-well plates at a density of 5000 cells/well. After 24 hours were treated with differential doses of natural compound for 3h and 24h. The 50 µl MTT (1 mg/ml) was added and incubated for 4 h; the formazan salt that formed was solubilized by adding

200 µl dimethyl sulfoxide and its amount was determined by measuring optical density at 540 nm using a microplate reader (MPT Reader, Invitrogen, Milano, Italy).

HUVEC and THP-1 treatments

The substances Curcuma (CUR), Resveratrol (RSV) and β-caryophyllene (BCP) were purchased in purified form Mivell, Italy (an innovative start-up). Based on the results of viability assay, cells were treated with Curcuma - 2 µg/ml, Resveratrol - 2 µg/ml and Beta-caryophyllene 20 µg/ml. To evaluate the synergistic effect of the three natural compounds, the mix was composed of the same amount of polyphenols as in the single compound and with the ratio 1:1:0.2 (Curcuma 1.6 µg/ml, β-caryophyllene 1.6 µg/ml and Resveratrol 0.32 µg/ml).

All substances were dissolved in DMSO and the final concertation of DMSO was 0.1% in all of solution. The treatments with single compounds and mixed was carried out for 3h.

THP-1 cells were stimulated with 500 µg/ml of LPS (lipopolysaccharide) for 3h together with natural compound.

RNA isolation

Total RNA from HUVECs was isolated using the Norgen Biotek Kit (#37500, Thorold, ON, Canada), according to the manufacturer's instructions. RNA was stored at −80 °C until use.

RT-PCR and qRT-PCR of mRNA

RNA amount was determined by spectrophotometric quantification with Nanodrop ONE (NanoDrop Technologies, Wilmington, DE, USA). Total RNA was reverse-transcribed using TAKARA Kit (PrimeScript™ RT reagent Kit with gDNA Eraser, Cat: RR047A) according to the

manufacturer's instructions. qRT-PCR was performed in a Rotor-Gene Q (Qiagen) using TB Green™ Premix Ex Taq™(Cat:RR420A) in a 10 µl reaction volume. Cycling conditions were: 95 °C for 2 m, and 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 30 s (40 cycles). Samples were run in duplicate. mRNA quantification was assessed using the 2−DDCt method.Gapdh and Beta-actin were used as an endogenous control.

RT-PCR and qRT-PCR of mature miRNAs

MiRNAs expression was quantified by quantitative real-time PCR (RT-qPCR) using TaqMan miRNA assay (Catalog #4427012 - TermoFisher Scientific), according to the manufacturer's protocol. Briefly, miRNA was reverse transcribed with the TaqMan MicroRNA reverse transcription kit (4366596 – ThermoFisher Scientific), using miR- specific stem-loop primer. 10 µl of RT mix contained 2 µl of each miR-specific stem-loop primer, 3.34 µl of input RNA, 1 µl of 10 mM dNTPs, 0.67 µl of reverse transcriptase, 1 µl of 10× buffer, 1.26 µl of RNase inhibitor diluted 1:10, and 0.73 µl of H2O. The mixture was incubated at 16 °C for 30 min, at 42 °C for 30 min, and at 85 °C for 5 min. The 10 µl qRT- PCR reaction mix included 0.5 µl 20x TaqMan MicroRNA Assay, which contained the PCR primers and probes (5'-FAM), 5 µl 2x TaqMan Universal Master mix no UNG (4440040 – ThermoFisher Scientific), and 2.66 µl RT product. The reaction presented an initial step at 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. Data were analyzed with Rotor Gene Q (Qiagen, Hilden, Germany) with the automatic comparative threshold (Ct) setting for adapting baseline. qRT-PCR data were standardized to RNU44. The 2−ΔCT method was used to determine miRNA expression.

Western Blot analyses

Cell lysates were collected using RIPA buffer (150 mMNaCl, 10 mM Tris, pH 7.2, 0.1 % SDS, 1.0 % Triton X-100, 5 mM EDTA, pH 8.0) with protease inhibitor Cocktail (Roche Applied Science, Indianapolis, IN, USA). After centrifugation to clarify the lysates, the amount of protein content was evaluated with Bradford assay. Aliquots (25-40 μg) of the lysates were then subjected to either 10% and 15% SDS-PAGE and transferred to PVDF membranes (Bio-Rad, Hercules, CA, USA). 5% skim milk was used to block the membrane that was then incubated overnight with the primary antibody.

The primary antibodies used are: mouse anti-SIRT1 (abcam), rabbit anti-caspase-1 p10 (Santa Cruz Biotechnology), anti-p16 (Santa Cruz) and rabbit anti-α-tubulin (Cell Signaling), subsequently they were incubated with a secondary antibody conjugated to horseradish peroxidase for 1 h at room temperature. Immunoreactive proteins were visualized using Clarity ECL chemiluminescence substrate (Bio-Rad). Bands were quantified using ImageJ software. Each measure was normalized with α-tubulin as a control.

ELISA assay

Culture supernatants were collected at the end of each incubation, centrifuged, and stored at −20°C until use in the assays. Human IL1F10 ELISA Kit (Wuhan Fine Biotech Co.) and IL-6 ELISA Kit (Cayman chemical) were used to measure the concentrations according with the manufacturer's instructions.

Patients IL-38 study

A total of 100 T2DM patients from central Italy and 95 healthy control subjects (CTRs) gave their informed consent to be enrolled in the study. The study protocol was approved by the Ethics Committee of INRCA-IRCCS (Ancona, Italy). T2DM was diagnosed according to American Diabetes Association Criteria. The information collected included information on vital signs, anthropometric data, medical history and behaviours, and exercise.

The presence/absence of diabetic complications was established as follows:

 retinopathy was defined as dilated pupils detected on fundoscopy and/or fluorescence angiography;

 incipient nephropathy was a urinary albumin excretion rate > 30 mg ⁄ 24 h and normal creatinine clearance;

 chronic renal failure was defined as an estimated glomerular filtration rate < 60 mL/min per 1.73 m2;

 neuropathy was established by electromyography;

 ischemic heart disease was diagnosed by clinical history and/or ischaemic electrocardiographic alterations; these patients had had ST- or non-ST elevation myocardial infarction, which was defined as a major acute cardiac event (MACE). Mean time from the MACE was 9±8 years;

 peripheral vascular disease, including arteriosclerosis obliterans and cerebrovascular disease, was diagnosed based on history, physical examination, and Doppler imaging.

Laboratory assays

Total white blood cells, monocyte and platelet counts were performed by standard automated procedures. Serum concentrations of HbA1c, fasting insulin, fibrinogen, apolipoprotein A–I and

B, total and HDL cholesterol, triglycerides, creatinine, fasting glucose and highly sensitive C-reactive protein were measured by standard procedures in all subjects.

Statistical analysis

Continuous data were tested for normality by Shapiro Wilk's test and are reported as mean ± standard deviation (SD). Partial correlation was used to test for correlations between IL-38 and other biochemical variables in CTR subjects and in T2DM patients. Analysis of covariance (ANCOVA) followed by post-hoc tests for multiple comparisons was used to compare the mean differences in biochemical, clinical, and anthropometric variables after adjustment for age and sex. Data analysis was performed using IBM SPSS Statistics for MacOS, version 25.0 (IBM Corp, Armonk, NY, USA). Statistical significance was defined as two-tailed p-value <0.05.

References

Abe, K., Y. Hashimoto, S. Yatsushiro, S. Yamamura, M. Bando, Y. Hiroshima, J. Kido, M. Tanaka, Y. Shinohara, T. Ooie, Y. Baba, and M. Kataoka. 2013. 'Simultaneous immunoassay analysis of plasma IL-6 and TNF-alpha on a microchip', *PLoS One*, 8: e53620.

Arai, Y., C. M. Martin-Ruiz, M. Takayama, Y. Abe, T. Takebayashi, S. Koyasu, M. Suematsu, N. Hirose, and T. von Zglinicki. 2015. 'Inflammation, But Not Telomere Length, Predicts Successful Ageing at Extreme Old Age: A Longitudinal Study of Semi-supercentenarians', *EBioMedicine*, 2: 1549-58.

Arca, M., G. Pigna, and C. Favoccia. 2012. 'Mechanisms of diabetic dyslipidemia: relevance for atherogenesis', *Curr Vasc Pharmacol*, 10: 684-6.

Argyropoulou, A., N. Aligiannis, I. P. Trougakos, and A. L. Skaltsounis. 2013. 'Natural compounds with anti-ageing activity', *Nat Prod Rep*, 30: 1412-37.

Bahadoran, Z., P. Mirmiran, and F. Azizi. 2013. 'Dietary polyphenols as potential nutraceuticals in management of diabetes: a review', *J Diabetes Metab Disord*, 12: 43.

Baker, D. J., B. G. Childs, M. Durik, M. E. Wijers, C. J. Sieben, J. Zhong, R. A. Saltness, K. B. Jeganathan, G. C. Verzosa, A. Pezeshki, K. Khazaie, J. D. Miller, and J. M. van Deursen. 2016. 'Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan', *Nature*, 530: 184-9.

Baldissera, M. D., C. F. Souza, T. H. Grando, P. H. Doleski, A. A. Boligon, L. M. Stefani, and S. G. Monteiro. 2017. 'Hypolipidemic effect of beta-caryophyllene to treat hyperlipidemic rats', *Naunyn Schmiedebergs Arch Pharmacol*, 390: 215-23.

Barnes, P. J. 2015. 'Mechanisms of development of multimorbidity in the elderly', *Eur Respir J*, 45: 790-806.

Basha, R. H., and C. Sankaranarayanan. 2016. 'beta-Caryophyllene, a natural sesquiterpene lactone attenuates hyperglycemia mediated oxidative and inflammatory stress in experimental diabetic rats', *Chem Biol Interact*, 245: 50-8.

Belwal, T., S. F. Nabavi, S. M. Nabavi, and S. Habtemariam. 2017. 'Dietary Anthocyanins and Insulin Resistance: When Food Becomes a Medicine', *Nutrients*, 9.

Bhaumik, D., G. K. Scott, S. Schokrpur, C. K. Patil, A. V. Orjalo, F. Rodier, G. J. Lithgow, and J. Campisi. 2009. 'MicroRNAs miR-146a/b negatively modulate the senescence-associated inflammatory mediators IL-6 and IL-8', *Aging (Albany NY)*, 1: 402-11.

Biesalski, H. K., L. O. Dragsted, I. Elmadfa, R. Grossklaus, M. Muller, D. Schrenk, P. Walter, and P. Weber. 2009. 'Bioactive compounds: definition and assessment of activity', *Nutrition*, 25: 1202-5.

Bilen, J., N. Liu, and N. M. Bonini. 2006. 'A new role for microRNA pathways: modulation of degeneration induced by pathogenic human disease proteins', *Cell Cycle*, 5: 2835-8.

Biomarkers Definitions Working, Group. 2001. 'Biomarkers and surrogate endpoints: preferred definitions and conceptual framework', *Clin Pharmacol Ther*, 69: 89-95.

Boutet, M. A., G. Bart, M. Penhoat, J. Amiaud, B. Brulin, C. Charrier, F. Morel, J. C. Lecron, M. Rolli-Derkinderen, A. Bourreille, S. Vigne, C. Gabay, G. Palmer, B. Le Goff, and F. Blanchard. 2016. 'Distinct expression of interleukin (IL)-36alpha, beta and gamma, their antagonist IL-36Ra and IL-38 in psoriasis, rheumatoid arthritis and Crohn's disease', *Clin Exp Immunol*, 184: 159-73.

Boutet, M. A., A. Najm, G. Bart, R. Brion, S. Touchais, V. Trichet, P. Layrolle, C. Gabay, G. Palmer, F. Blanchard, and B. Le Goff. 2017. 'IL-38 overexpression induces anti-inflammatory effects in mice arthritis models and in human macrophages in vitro', *Ann Rheum Dis*, 76: 1304-12.

Busse, P. J., and S. K. Mathur. 2010. 'Age-related changes in immune function: effect on airway inflammation', *J Allergy Clin Immunol*, 126: 690-9; quiz 700-1.

Cai, E. P., and J. K. Lin. 2009. 'Epigallocatechin gallate (EGCG) and rutin suppress the glucotoxicity through activating IRS2 and AMPK signaling in rat pancreatic beta cells', *J Agric Food Chem*, 57: 9817-27.

Campisi, J., and F. d'Adda di Fagagna. 2007. 'Cellular senescence: when bad things happen to good cells', *Nat Rev Mol Cell Biol*, 8: 729-40.

Caro, M., A. Sansone, J. Amezaga, V. Navarro, C. Ferreri, and I. Tueros. 2017. 'Wine lees modulate lipid metabolism and induce fatty acid remodelling in zebrafish', *Food & Function*, 8: 1652-59.

Catapano, A. L., I. Graham, G. De Backer, O. Wiklund, M. J. Chapman, H. Drexel, A. W. Hoes, C. S. Jennings, U. Landmesser, T. R. Pedersen, Z. Reiner, G. Riccardi, M. R. Taskinen, L. Tokgozoglu, W. M. M. Verschuren, C. Vlachopoulos, D. A. Wood, J. L. Zamorano, M. T. Cooney, and E. S. C. Scientific Document Group. 2016. '2016 ESC/EAS Guidelines for the Management of Dyslipidaemias', *Eur Heart J*, 37: 2999-3058.

Chang, J. J., D. J. Chung, Y. J. Lee, B. H. Wen, H. Y. Jao, and C. J. Wang. 2017. 'Solanum nigrum Polyphenol Extracts Inhibit Hepatic Inflammation, Oxidative Stress, and Lipogenesis in High-Fat-Diet-Treated Mice', *J Agric Food Chem*, 65: 9255-65.

Chang, T. C., M. F. Hsu, and K. K. Wu. 2015. 'High glucose induces bone marrow-derived mesenchymal stem cell senescence by upregulating autophagy', *PLoS One*, 10: e0126537.

Chatterjee, S., K. Khunti, and M. J. Davies. 2017. 'Type 2 diabetes', *Lancet*, 389: 2239-51.

Chen, G., H. Wang, X. Zhang, and S. T. Yang. 2014. 'Nutraceuticals and functional foods in the management of hyperlipidemia', *Crit Rev Food Sci Nutr*, 54: 1180-201.

Cheng, D. M., P. Kuhn, A. Poulev, L. E. Rojo, M. A. Lila, and I. Raskin. 2012. 'In vivo and in vitro antidiabetic effects of aqueous cinnamon extract and cinnamon polyphenol-enhanced food matrix', *Food Chem*, 135: 2994-3002.

Cheng, Y., Z. Dong, and S. Liu. 2014. 'beta-Caryophyllene ameliorates the Alzheimer-like phenotype in APP/PS1 Mice through CB2 receptor activation and the PPARgamma pathway', *Pharmacology*, 94: 1-12.

Childs, B. G., M. Durik, D. J. Baker, and J. M. van Deursen. 2015. 'Cellular senescence in aging and age-related disease: from mechanisms to therapy', *Nat Med*, 21: 1424-35.

Chu, M., I. M. Chu, E. C. Yung, C. W. Lam, T. F. Leung, G. W. Wong, and C. K. Wong. 2016. 'Aberrant Expression of Novel Cytokine IL-38 and Regulatory T Lymphocytes in Childhood Asthma', *Molecules*, 21.

Chu, M., L. S. Tam, J. Zhu, D. Jiao, H. Liu, Z. Cai, J. Dong, C. W. Kai Lam, and C. K. Wong. 2017. 'In vivo anti-inflammatory activities of novel cytokine IL-38 in Murphy Roths Large (MRL)/lpr mice', *Immunobiology*, 222: 483-93.

Chuang, S. Y., C. H. Lin, and J. Y. Fang. 2014. 'Natural compounds and aging: between autophagy and inflammasome', *Biomed Res Int*, 2014: 297293.

Collado, M., M. A. Blasco, and M. Serrano. 2007. 'Cellular senescence in cancer and aging', *Cell*, 130: 223-33.

Coppe, J. P., P. Y. Desprez, A. Krtolica, and J. Campisi. 2010. 'The senescence-associated secretory phenotype: the dark side of tumor suppression', *Annu Rev Pathol*, 5: 99-118.

Coppe, J. P., C. K. Patil, F. Rodier, Y. Sun, D. P. Munoz, J. Goldstein, P. S. Nelson, P. Y. Desprez, and J. Campisi. 2008. 'Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor', *PLoS Biol*, 6: 2853-68.

Cordero, M. D., M. R. Williams, and B. Ryffel. 2018. 'AMP-Activated Protein Kinase Regulation of the NLRP3 Inflammasome during Aging', *Trends Endocrinol Metab*, 29: 8-17.

Cortez, M. A., and G. A. Calin. 2009. 'MicroRNA identification in plasma and serum: a new tool to diagnose and monitor diseases', *Expert Opin Biol Ther*, 9: 703-11.

Crimmins, E., S. Vasunilashorn, J. K. Kim, and D. Alley. 2008. 'Biomarkers related to aging in human populations', *Adv Clin Chem*, 46: 161-216.

Cuccioloni, M., M. Mozzicafreddo, M. Spina, C. N. Tran, M. Falconi, A. M. Eleuteri, and M. Angeletti. 2011. 'Epigallocatechin-3-gallate potently inhibits the in vitro activity of hydroxy-3-methyl-glutaryl-CoA reductase', *J Lipid Res*, 52: 897-907.

Czech, M. P. 2017. 'Insulin action and resistance in obesity and type 2 diabetes', *Nat Med*, 23: 804-14.

Davalli, P., T. Mitic, A. Caporali, A. Lauriola, and D. D'Arca. 2016. 'ROS, Cell Senescence, and Novel Molecular Mechanisms in Aging and Age-Related Diseases', *Oxid Med Cell Longev*, 2016: 3565127.

Deepa, P., K. Sowndhararajan, S. Kim, and S. J. Park. 2018. 'A role of Ficus species in the management of diabetes mellitus: A review', *J Ethnopharmacol*, 215: 210-32.

Dimmeler, S., and P. Nicotera. 2013. 'MicroRNAs in age-related diseases', *EMBO Mol Med*, 5: 180-90.

Dimri, G. P., X. Lee, G. Basile, M. Acosta, G. Scott, C. Roskelley, E. E. Medrano, M. Linskens, I. Rubelj, O. Pereira-Smith, and et al. 1995. 'A biomarker that identifies senescent human cells in culture and in aging skin in vivo', *Proc Natl Acad Sci U S A*, 92: 9363-7.

Dodig, S., I. Cepelak, and I. Pavic. 2019. 'Hallmarks of senescence and aging', *Biochem Med (Zagreb)*, 29: 030501.

Dominguez Avila, J. A., J. Rodrigo Garcia, G. A. Gonzalez Aguilar, and L. A. de la Rosa. 2017. 'The Antidiabetic Mechanisms of Polyphenols Related to Increased Glucagon-Like Peptide-1 (GLP1) and Insulin Signaling', *Molecules*, 22.

Duenas, M., I. Munoz-Gonzalez, C. Cueva, A. Jimenez-Giron, F. Sanchez-Patan, C. Santos-Buelga, M. V. Moreno-Arribas, and B. Bartolome. 2015. 'A survey of modulation of gut microbiota by dietary polyphenols', *Biomed Res Int*, 2015: 850902.

Durazzo, A., M. Lucarini, E. B. Souto, C. Cicala, E. Caiazzo, A. A. Izzo, E. Novellino, and A. Santini. 2019. 'Polyphenols: A concise overview on the chemistry, occurrence, and human health', *Phytother Res*.

Edwards, C. A., J. Havlik, W. Cong, W. Mullen, T. Preston, D. J. Morrison, and E. Combet. 2017. 'Polyphenols and health: Interactions between fibre, plant polyphenols and the gut microbiota', *Nutr Bull*, 42: 356-60.

Estruch, R., E. Ros, J. Salas-Salvado, M. I. Covas, D. Corella, F. Aros, E. Gomez-Gracia, V. Ruiz-Gutierrez, M. Fiol, J. Lapetra, R. M. Lamuela-Raventos, L. Serra-Majem, X. Pinto, J. Basora, M. A. Munoz, J. V. Sorli, J. A. Martinez, M. Fito, A. Gea, M. A. Hernan, M. A. Martinez-Gonzalez, and Predimed Study Investigators. 2018. 'Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts', *N Engl J Med*, 378: e34.

Feng, D., L. Ohlsson, and R. D. Duan. 2010. 'Curcumin inhibits cholesterol uptake in Caco-2 cells by down-regulation of NPC1L1 expression', *Lipids Health Dis*, 9: 40.

Feng, J., W. Xing, and L. Xie. 2016. 'Regulatory Roles of MicroRNAs in Diabetes', *Int J Mol Sci*, 17.

Fernandez-Castillejo, S., R. M. Valls, O. Castaner, L. Rubio, U. Catalan, A. Pedret, A. Macia, M. L. Sampson, M. I. Covas, M. Fito, M. J. Motilva, A. T. Remaley, and R. Sola. 2016. 'Polyphenol rich olive oils improve lipoprotein particle atherogenic ratios and subclasses profile: A randomized, crossover, controlled trial', *Mol Nutr Food Res*, 60: 1544-54.

Ferrare, K., L. P. R. Bidel, A. Awwad, P. Poucheret, G. Cazals, F. Lazennec, J. Azay-Milhau, M. Tournier, A. D. Lajoix, and D. Tousch. 2018. 'Increase in insulin sensitivity by the association of chicoric acid and chlorogenic acid contained in a natural chicoric acid extract (NCRAE) of chicory (Cichorium intybus L.) for an antidiabetic effect', *J Ethnopharmacol*, 215: 241-48.

Ferrieres, J. 2004. 'The French paradox: lessons for other countries', *Heart*, 90: 107-11.

Fidyt, K., A. Fiedorowicz, L. Strzadala, and A. Szumny. 2016. 'beta-caryophyllene and beta-caryophyllene oxide-natural compounds of anticancer and analgesic properties', *Cancer Med*, 5: 3007-17.

Franceschi, C. 2007. 'Inflammaging as a major characteristic of old people: can it be prevented or cured?', *Nutr Rev*, 65: S173-6.

———. 2017. 'Healthy ageing in 2016: Obesity in geroscience - is cellular senescence the culprit?', *Nat Rev Endocrinol*, 13: 76-78.

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

Franceschi, C., M. Capri, D. Monti, S. Giunta, F. Olivieri, F. Sevini, M. P. Panourgia, L. Invidia, L. Celani, M. Scurti, E. Cevenini, G. C. Castellani, and S. Salvioli. 2007. 'Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans', *Mech Ageing Dev*, 128: 92-105.

Freund, A., A. V. Orjalo, P. Y. Desprez, and J. Campisi. 2010. 'Inflammatory networks during cellular senescence: causes and consequences', *Trends Mol Med*, 16: 238-46.

Freund, A., C. K. Patil, and J. Campisi. 2011. 'p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype', *EMBO J*, 30: 1536-48.

Fujii, Y., N. Osaki, T. Hase, and A. Shimotoyodome. 2015. 'Ingestion of coffee polyphenols increases postprandial release of the active glucagon-like peptide-1 (GLP-1(7-36)) amide in C57BL/6J mice', *J Nutr Sci*, 4: e9.

Furst, R., and I. Zundorf. 2014. 'Plant-derived anti-inflammatory compounds: hopes and disappointments regarding the translation of preclinical knowledge into clinical progress', *Mediators Inflamm*, 2014: 146832.

Galbiati, A., C. Beausejour, and F. d'Adda di Fagagna. 2017. 'A novel single-cell method provides direct evidence of persistent DNA damage in senescent cells and aged mammalian tissues', *Aging Cell*, 16: 422-27.

Gillery, P., and S. Jaisson. 2013. 'Usefulness of non-enzymatic post-translational modification derived products (PTMDPs) as biomarkers of chronic diseases', *J Proteomics*, 92: 228-38.

Gobbens, R. J., M. A. van Assen, and M. J. Schalk. 2014. 'The prediction of disability by self-reported physical frailty components of the Tilburg Frailty Indicator (TFI)', *Arch Gerontol Geriatr*, 59: 280-7.

Gonzalez-Abuin, N., N. Martinez-Micaelo, M. Margalef, M. Blay, A. Arola-Arnal, B. Muguerza, A. Ardevol, and M. Pinent. 2014. 'A grape seed extract increases active glucagon-like peptide-1 levels after an oral glucose load in rats', *Food & Function*, 5: 2357-64.

Gonzalez-Covarrubias, V. 2013. 'Lipidomics in longevity and healthy aging', *Biogerontology*, 14: 663-72.

Gorinstein, S., H. Leontowicz, M. Leontowicz, R. Krzeminski, M. Gralak, E. Delgado-Licon, A. L. Martinez Ayala, E. Katrich, and S. Trakhtenberg. 2005. 'Changes in plasma lipid and antioxidant activity in rats as a result of naringin and red grapefruit supplementation', *J Agric Food Chem*, 53: 3223-8.

Gorisse, L., C. Pietrement, V. Vuiblet, C. E. Schmelzer, M. Kohler, L. Duca, L. Debelle, P. Fornes, S. Jaisson, and P. Gillery. 2016. 'Protein carbamylation is a hallmark of aging', *Proc Natl Acad Sci U S A*, 113: 1191-6.

Gurau, F., S. Baldoni, F. Prattichizzo, E. Espinosa, F. Amenta, A. D. Procopio, M. C. Albertini, M. Bonafe, and F. Olivieri. 2018. 'Anti-senescence compounds: A potential nutraceutical approach to healthy aging', *Ageing Res Rev*, 46: 14-31.

Han, D., E. Williams, and E. Cadenas. 2001. 'Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space', *Biochem J*, 353: 411-6.

Hanhineva, K., R. Torronen, I. Bondia-Pons, J. Pekkinen, M. Kolehmainen, H. Mykkanen, and K. Poutanen. 2010. 'Impact of dietary polyphenols on carbohydrate metabolism', *Int J Mol Sci*, 11: 1365-402.

Harris, T. A., M. Yamakuchi, M. Ferlito, J. T. Mendell, and C. J. Lowenstein. 2008. 'MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1', *Proc Natl Acad Sci U S A*, 105: 1516-21.

Hastings, M. L., and A. R. Krainer. 2001. 'Pre-mRNA splicing in the new millennium', *Curr Opin Cell Biol*, 13: 302-9.

Hayakawa, T., M. Iwai, S. Aoki, K. Takimoto, M. Maruyama, W. Maruyama, and N. Motoyama. 2015. 'SIRT1 suppresses the senescence-associated secretory phenotype through epigenetic gene regulation', *PLoS One*, 10: e0116480.

Hobbs, F. D. 2003. 'Cardiovascular disease and lipids. Issues and evidence for the management of dyslipidaemia in primary care', *Eur J Gen Pract*, 9: 16-24.

Hohn, A., D. Weber, T. Jung, C. Ott, M. Hugo, B. Kochlik, R. Kehm, J. Konig, T. Grune, and J. P. Castro. 2017. 'Happily (n)ever after: Aging in the context of oxidative stress, proteostasis loss and cellular senescence', *Redox Biol*, 11: 482-501.

Houtkooper, R. H., R. W. Williams, and J. Auwerx. 2010. 'Metabolic networks of longevity', *Cell*, 142: 9-14.

Howitz, K. T., K. J. Bitterman, H. Y. Cohen, D. W. Lamming, S. Lavu, J. G. Wood, R. E. Zipkin, P. Chung, A. Kisielewski, L. L. Zhang, B. Scherer, and D. A. Sinclair. 2003. 'Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan', *Nature*, 425: 191-6.

Huan, T., G. Chen, C. Liu, A. Bhattacharya, J. Rong, B. H. Chen, S. Seshadri, K. Tanriverdi, J. E. Freedman, M. G. Larson, J. M. Murabito, and D. Levy. 2018. 'Age-associated microRNA expression in human peripheral blood is associated with all-cause mortality and age-related traits', *Aging Cell*, 17.

Huntzinger, E., and E. Izaurralde. 2011. 'Gene silencing by microRNAs: contributions of translational repression and mRNA decay', *Nat Rev Genet*, 12: 99-110.

Jagger, A., Y. Shimojima, J. J. Goronzy, and C. M. Weyand. 2014. 'Regulatory T cells and the immune aging process: a mini-review', *Gerontology*, 60: 130-7.

Jaul, E., and J. Barron. 2017. 'Age-Related Diseases and Clinical and Public Health Implications for the 85 Years Old and Over Population', *Front Public Health*, 5: 335.

Johnson, S. C., M. Sangesland, M. Kaeberlein, and P. S. Rabinovitch. 2015. 'Modulating mTOR in aging and health', *Interdiscip Top Gerontol*, 40: 107-27.

Jun, J. I., and L. F. Lau. 2010. 'Cellular senescence controls fibrosis in wound healing', *Aging (Albany NY)*, 2: 627-31.

Jung, E. H., S. R. Kim, I. K. Hwang, and T. Y. Ha. 2007. 'Hypoglycemic effects of a phenolic acid fraction of rice bran and ferulic acid in C57BL/KsJ-db/db mice', *J Agric Food Chem*, 55: 9800-4.

Jung, U. J., M. K. Lee, Y. B. Park, M. A. Kang, and M. S. Choi. 2006. 'Effect of citrus flavonoids on lipid metabolism and glucose-regulating enzyme mRNA levels in type-2 diabetic mice', *Int J Biochem Cell Biol*, 38: 1134-45.

Keane, K. N., V. F. Cruzat, R. Carlessi, P. I. de Bittencourt, Jr., and P. Newsholme. 2015. 'Molecular Events Linking Oxidative Stress and Inflammation to Insulin Resistance and beta-Cell Dysfunction', *Oxid Med Cell Longev*, 2015: 181643.

Kida, Y., and M. S. Goligorsky. 2016. 'Sirtuins, Cell Senescence, and Vascular Aging', *Can J Cardiol*, 32: 634-41.

Kim, B., Y. Park, C. J. Wegner, B. W. Bolling, and J. Lee. 2013. 'Polyphenol-rich black chokeberry (Aronia melanocarpa) extract regulates the expression of genes critical for intestinal cholesterol flux in Caco-2 cells', *J Nutr Biochem*, 24: 1564-70.

Kim, M. K., H. S. Jung, C. S. Yoon, J. H. Ko, H. J. Chun, T. K. Kim, M. J. Kwon, S. H. Lee, K. S. Koh, B. D. Rhee, and J. H. Park. 2010. 'EGCG and quercetin protected INS-1 cells in oxidative stress via different mechanisms', *Front Biosci (Elite Ed)*, 2: 810-7.

Kim, Y., J. B. Keogh, and P. M. Clifton. 2016. 'Polyphenols and Glycemic Control', *Nutrients*, 8.

Kirkland, J. L., T. Tchkonia, Y. Zhu, L. J. Niedernhofer, and P. D. Robbins. 2017. 'The Clinical Potential of Senolytic Drugs', *J Am Geriatr Soc*, 65: 2297-301.

Kobayashi, M., T. Kawano, Y. Ukawa, Y. M. Sagesaka, and I. Fukuhara. 2016. 'Green tea beverages enriched with catechins with a galloyl moiety reduce body fat in moderately obese adults: a randomized double-blind placebo-controlled trial', *Food & Function*, 7: 498-507.

Kobayashi, M., M. Nishizawa, N. Inoue, T. Hosoya, M. Yoshida, Y. Ukawa, Y. M. Sagesaka, T. Doi, T. Nakayama, S. Kumazawa, and I. Ikeda. 2014. 'Epigallocatechin gallate decreases the micellar solubility of cholesterol via specific interaction with phosphatidylcholine', *J Agric Food Chem*, 62: 2881-90.

Krecman, V., N. Skottova, D. Walterova, J. Ulrichova, and V. Simanek. 1998. 'Silymarin inhibits the development of diet-induced hypercholesterolemia in rats', *Planta Med*, 64: 138-42.

Krishnamurthy, J., C. Torrice, M. R. Ramsey, G. I. Kovalev, K. Al-Regaiey, L. Su, and N. E. Sharpless. 2004. 'Ink4a/Arf expression is a biomarker of aging', *J Clin Invest*, 114: 1299-307.

Kumar, S., and P. H. Reddy. 2016. 'Are circulating microRNAs peripheral biomarkers for Alzheimer's disease?', *Biochim Biophys Acta*, 1862: 1617-27.

Kuo, L. J., and L. X. Yang. 2008. 'Gamma-H2AX - a novel biomarker for DNA double-strand breaks', *In Vivo*, 22: 305-9.

Lee, J. Y., K. R. Yu, B. C. Lee, I. Kang, J. J. Kim, E. J. Jung, H. S. Kim, Y. Seo, S. W. Choi, and K. S. Kang. 2018. 'GATA4-dependent regulation of the secretory phenotype via MCP-1 underlies lamin A-mediated human mesenchymal stem cell aging', *Exp Mol Med*, 50: 63.

Ley, S. H., O. Hamdy, V. Mohan, and F. B. Hu. 2014. 'Prevention and management of type 2 diabetes: dietary components and nutritional strategies', *Lancet*, 383: 1999-2007.

Li, A. N., S. Li, Y. J. Zhang, X. R. Xu, Y. M. Chen, and H. B. Li. 2014. 'Resources and biological activities of natural polyphenols', *Nutrients*, 6: 6020-47.

Li, J., L. Liu, W. Rui, X. Li, D. Xuan, S. Zheng, Y. Yu, J. Zhang, N. Kong, X. Zhu, H. Zou, W. Wan, and Y. Xue. 2017. 'New Interleukins in Psoriasis and Psoriatic Arthritis Patients: The Possible Roles of Interleukin-33 to Interleukin-38 in Disease Activities and Bone Erosions', *Dermatology*, 233: 37-46.

Li, Y., X. Yang, Y. He, W. Wang, J. Zhang, W. Zhang, T. Jing, B. Wang, and R. Lin. 2017. 'Negative regulation of NLRP3 inflammasome by SIRT1 in vascular endothelial cells', *Immunobiology*, 222: 552-61.

Lim, H., H. Park, and H. P. Kim. 2015. 'Effects of flavonoids on senescence-associated secretory phenotype formation from bleomycin-induced senescence in BJ fibroblasts', *Biochem Pharmacol*, 96: 337-48.

Lin, H., A. S. Ho, D. Haley-Vicente, J. Zhang, J. Bernal-Fussell, A. M. Pace, D. Hansen, K. Schweighofer, N. K. Mize, and J. E. Ford. 2001. 'Cloning and characterization of IL-1HY2, a novel interleukin-1 family member', *J Biol Chem*, 276: 20597-602.

Lin, Q., L. Ma, Z. Liu, Z. Yang, J. Wang, J. Liu, and G. Jiang. 2017. 'Targeting microRNAs: a new action mechanism of natural compounds', *Oncotarget*, 8: 15961-70.

Lin, S. H., K. J. Huang, C. F. Weng, and D. Shiuan. 2015. 'Exploration of natural product ingredients as inhibitors of human HMG-CoA reductase through structure-based virtual screening', *Drug Des Devel Ther*, 9: 3313-24.

Lindsey, L. P., C. M. Daphney, A. Oppong-Damoah, P. N. Uchakin, S. E. Abney, O. N. Uchakina, R. D. Khusial, A. Akil, and K. S. Murnane. 2019. 'The cannabinoid receptor 2 agonist, beta-caryophyllene, improves working memory and reduces circulating levels of specific proinflammatory cytokines in aged male mice', *Behav Brain Res*, 372: 112012.

Liu, D., W. Zhen, Z. Yang, J. D. Carter, H. Si, and K. A. Reynolds. 2006. 'Genistein acutely stimulates insulin secretion in pancreatic beta-cells through a cAMP-dependent protein kinase pathway', *Diabetes*, 55: 1043-50.

Lo Piparo, E., H. Scheib, N. Frei, G. Williamson, M. Grigorov, and C. J. Chou. 2008. 'Flavonoids for controlling starch digestion: structural requirements for inhibiting human alpha-amylase', *J Med Chem*, 51: 3555-61.

Low Wang, C. C., C. N. Hess, W. R. Hiatt, and A. B. Goldfine. 2016. 'Clinical Update: Cardiovascular Disease in Diabetes Mellitus: Atherosclerotic Cardiovascular Disease and Heart Failure in Type 2 Diabetes Mellitus - Mechanisms, Management, and Clinical Considerations', *Circulation*, 133: 2459-502.

Lu, W., Y. Zhang, D. Liu, Z. Songyang, and M. Wan. 2013. 'Telomeres-structure, function, and regulation', *Exp Cell Res*, 319: 133-41.

Lujambio, A. 2016. 'To clear, or not to clear (senescent cells)? That is the question', *Bioessays*, 38 Suppl 1: S56-64.

Malavolta, M., L. Costarelli, R. Giacconi, F. Piacenza, A. Basso, E. Pierpaoli, F. Marchegiani, M. Cardelli, M. Provinciali, and E. Mocchegiani. 2014. 'Modulators of cellular senescence: mechanisms, promises, and challenges from in vitro studies with dietary bioactive compounds', *Nutr Res*, 34: 1017-35.

Manzano, S., and G. Williamson. 2010. 'Polyphenols and phenolic acids from strawberry and apple decrease glucose uptake and transport by human intestinal Caco-2 cells', *Mol Nutr Food Res*, 54: 1773-80.

Martinez-Lopez, S., B. Sarria, J. L. Sierra-Cinos, L. Goya, R. Mateos, and L. Bravo. 2014. 'Realistic intake of a flavanol-rich soluble cocoa product increases HDL-cholesterol without inducing anthropometric changes in healthy and moderately hypercholesterolemic subjects', *Food & Function*, 5: 364-74.

Massudi, H., R. Grant, N. Braidy, J. Guest, B. Farnsworth, and G. J. Guillemin. 2012. 'Age-associated changes in oxidative stress and NAD+ metabolism in human tissue', *PLoS One*, 7: e42357.

McCubrey, J. A., K. Lertpiriyapong, L. S. Steelman, S. L. Abrams, L. V. Yang, R. M. Murata, P. L. Rosalen, A. Scalisi, L. M. Neri, L. Cocco, S. Ratti, A. M. Martelli, P. Laidler, J. Dulinska-Litewka, D. Rakus, A. Gizak, P. Lombardi, F. Nicoletti, S. Candido, M. Libra, G. Montalto, and M. Cervello. 2017. 'Effects of resveratrol, curcumin, berberine and other nutraceuticals on aging, cancer development, cancer stem cells and microRNAs', *Aging (Albany NY)*, 9: 1477-536.

McHugh, D., and J. Gil. 2018. 'Senescence and aging: Causes, consequences, and therapeutic avenues', *J Cell Biol*, 217: 65-77.

Meydani, M., and S. T. Hasan. 2010. 'Dietary polyphenols and obesity', *Nutrients*, 2: 737-51.

Millar, C. L., Q. Duclos, and C. N. Blesso. 2017. 'Effects of Dietary Flavonoids on Reverse Cholesterol Transport, HDL Metabolism, and HDL Function', *Adv Nutr*, 8: 226-39.

Mollace, V., M. Scicchitano, S. Paone, F. Casale, C. Calandruccio, M. Gliozzi, V. Musolino, C. Carresi, J. Maiuolo, S. Nucera, A. Riva, P. Allegrini, M. Ronchi, G. Petrangolini, and E. Bombardelli. 2019. 'Hypoglycemic and Hypolipemic Effects of a New Lecithin Formulation of Bergamot Polyphenolic Fraction: A Double Blind, Randomized, Placebo- Controlled Study', *Endocr Metab Immune Disord Drug Targets*, 19: 136-43.

Montecino-Rodriguez, E., B. Berent-Maoz, and K. Dorshkind. 2013. 'Causes, consequences, and reversal of immune system aging', *J Clin Invest*, 123: 958-65.

Montoliu, I., M. Scherer, F. Beguelin, L. DaSilva, D. Mari, S. Salvioli, F. P. Martin, M. Capri, L. Bucci, R. Ostan, P. Garagnani, D. Monti, E. Biagi, P. Brigidi, M. Kussmann, S. Rezzi, C. Franceschi, and S. Collino. 2014. 'Serum profiling of healthy aging identifies phospho- and sphingolipid species as markers of human longevity', *Aging (Albany NY)*, 6: 9-25.

Morey, C., and P. Avner. 2004. 'Employment opportunities for non-coding RNAs', *FEBS Lett*, 567: 27-34.

Mulvihill, E. E., A. C. Burke, and M. W. Huff. 2016. 'Citrus Flavonoids as Regulators of Lipoprotein Metabolism and Atherosclerosis', *Annu Rev Nutr*, 36: 275-99.

Munir, K. M., S. Chandrasekaran, F. Gao, and M. J. Quon. 2013. 'Mechanisms for food polyphenols to ameliorate insulin resistance and endothelial dysfunction: therapeutic implications for diabetes and its cardiovascular complications', *Am J Physiol Endocrinol Metab*, 305: E679-86.

Murota, K., Y. Nakamura, and M. Uehara. 2018. 'Flavonoid metabolism: the interaction of metabolites and gut microbiota', *Biosci Biotechnol Biochem*, 82: 600-10.

Nagamine, R., S. Ueno, M. Tsubata, K. Yamaguchi, K. Takagaki, T. Hira, H. Hara, and T. Tsuda. 2014. 'Dietary sweet potato (Ipomoea batatas L.) leaf extract attenuates hyperglycaemia by enhancing the secretion of glucagon-like peptide-1 (GLP-1)', *Food & Function*, 5: 2309-16.

Nekohashi, M., M. Ogawa, T. Ogihara, K. Nakazawa, H. Kato, T. Misaka, K. Abe, and S. Kobayashi. 2014. 'Luteolin and quercetin affect the cholesterol absorption mediated by epithelial cholesterol transporter niemann-pick c1-like 1 in caco-2 cells and rats', *PLoS One*, 9: e97901.

Nelson, R. H. 2013. 'Hyperlipidemia as a risk factor for cardiovascular disease', *Prim Care*, 40: 195-211.

O'Connell, R. M., D. S. Rao, A. A. Chaudhuri, M. P. Boldin, K. D. Taganov, J. Nicoll, R. L. Paquette, and D. Baltimore. 2008. 'Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder', *J Exp Med*, 205: 585-94.

Ogawa, K., S. Hirose, S. Nagaoka, and E. Yanase. 2016. 'Interaction between Tea Polyphenols and Bile Acid Inhibits Micellar Cholesterol Solubility', *J Agric Food Chem*, 64: 204-9.

Olivieri, F., M. Capri, M. Bonafe, C. Morsiani, H. J. Jung, L. Spazzafumo, J. Vina, and Y. Suh. 2017. 'Circulating miRNAs and miRNA shuttles as biomarkers: Perspective trajectories of healthy and unhealthy aging', *Mech Ageing Dev*, 165: 162-70.

Olivieri, F., M. R. Rippo, A. D. Procopio, and F. Fazioli. 2013. 'Circulating inflamma-miRs in aging and age-related diseases', *Front Genet*, 4: 121.

Olivieri, F., L. Spazzafumo, G. Santini, R. Lazzarini, M. C. Albertini, M. R. Rippo, R. Galeazzi, A. M. Abbatecola, F. Marcheselli, D. Monti, R. Ostan, E. Cevenini, R. Antonicelli, C. Franceschi, and A. D. Procopio. 2012. 'Age-related differences in the expression of circulating microRNAs: miR-21 as a new circulating marker of inflammaging', *Mech Ageing Dev*, 133: 675-85.

Orjalo, A. V., D. Bhaumik, B. K. Gengler, G. K. Scott, and J. Campisi. 2009. 'Cell surface-bound IL-1alpha is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network', *Proc Natl Acad Sci U S A*, 106: 17031-6.

Ozcan, S., N. Alessio, M. B. Acar, E. Mert, F. Omerli, G. Peluso, and U. Galderisi. 2016. 'Unbiased analysis of senescence associated secretory phenotype (SASP) to identify common components following different genotoxic stresses', *Aging (Albany NY)*, 8: 1316-29.

Palacios, O. M., M. Kramer, and K. C. Maki. 2019. 'Diet and prevention of type 2 diabetes mellitus: beyond weight loss and exercise', *Expert Rev Endocrinol Metab*, 14: 1-12.

Pant, A., V. Mishra, S. K. Saikia, V. Shukla, J. Asthana, B. A. Akhoon, and R. Pandey. 2014. 'Beta-caryophyllene modulates expression of stress response genes and mediates longevity in Caenorhabditis elegans', *Exp Gerontol*, 57: 81-95.

Passos, J. F., G. Nelson, C. Wang, T. Richter, C. Simillion, C. J. Proctor, S. Miwa, S. Olijslagers, J. Hallinan, A. Wipat, G. Saretzki, K. L. Rudolph, T. B. Kirkwood, and T. von Zglinicki. 2010. 'Feedback between p21 and reactive oxygen production is necessary for cell senescence', *Mol Syst Biol*, 6: 347.

Pazoki-Toroudi, H., H. Amani, M. Ajami, S. F. Nabavi, N. Braidy, P. D. Kasi, and S. M. Nabavi. 2016. 'Targeting mTOR signaling by polyphenols: A new therapeutic target for ageing', *Ageing Res Rev*, 31: 55-66.

Peltz, L., J. Gomez, M. Marquez, F. Alencastro, N. Atashpanjeh, T. Quang, T. Bach, and Y. Zhao. 2012. 'Resveratrol exerts dosage and duration dependent effect on human mesenchymal stem cell development', *PLoS One*, 7: e37162.

Pereira, D. F., L. H. Cazarolli, C. Lavado, V. Mengatto, M. S. Figueiredo, A. Guedes, M. G. Pizzolatti, and F. R. Silva. 2011. 'Effects of flavonoids on alpha-glucosidase activity: potential targets for glucose homeostasis', *Nutrition*, 27: 1161-7.

Piepoli, M. F., A. W. Hoes, S. Agewall, C. Albus, C. Brotons, A. L. Catapano, M. T. Cooney, U. Corra, B. Cosyns, C. Deaton, I. Graham, M. S. Hall, F. D. R. Hobbs, M. L. Lochen, H. Lollgen, P. Marques-Vidal, J. Perk, E. Prescott, J. Redon, D. J. Richter, N. Sattar, Y. Smulders, M. Tiberi, H. B. van der Worp, I. van Dis, W. M. M. Verschuren, S. Binno, and E. S. C. Scientific Document Group. 2016. '2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR)', *Eur Heart J*, 37: 2315-81.

Poland, G. A., I. G. Ovsyannikova, R. B. Kennedy, N. D. Lambert, and J. L. Kirkland. 2014. 'A systems biology approach to the effect of aging, immunosenescence and vaccine response', *Curr Opin Immunol*, 29: 62-8.

Prattichizzo, F., A. Giuliani, E. Mensa, J. Sabbatinelli, V. De Nigris, M. R. Rippo, L. La Sala, A. D. Procopio, F. Olivieri, and A. Ceriello. 2018. 'Pleiotropic effects of metformin: Shaping the microbiome to manage type 2 diabetes and postpone ageing', *Ageing Res Rev*, 48: 87-98.

Raederstorff, D. G., M. F. Schlachter, V. Elste, and P. Weber. 2003. 'Effect of EGCG on lipid absorption and plasma lipid levels in rats', *J Nutr Biochem*, 14: 326-32.

Rea, I. M., D. S. Gibson, V. McGilligan, S. E. McNerlan, H. D. Alexander, and O. A. Ross. 2018. 'Age and Age-Related Diseases: Role of Inflammation Triggers and Cytokines', *Front Immunol*, 9: 586.

Ressler, S., J. Bartkova, H. Niederegger, J. Bartek, K. Scharffetter-Kochanek, P. Jansen-Durr, and M. Wlaschek. 2006. 'p16INK4A is a robust in vivo biomarker of cellular aging in human skin', *Aging Cell*, 5: 379-89.

Richard, J. L., F. Cambien, and P. Ducimetiere. 1981. '[Epidemiologic characteristics of coronary disease in France]', *Nouv Presse Med*, 10: 1111-4.

Rodier, F., and J. Campisi. 2011. 'Four faces of cellular senescence', *J Cell Biol*, 192: 547-56.

Rouse, M., A. Younes, and J. M. Egan. 2014. 'Resveratrol and curcumin enhance pancreatic beta-cell function by inhibiting phosphodiesterase activity', *J Endocrinol*, 223: 107-17.

Rudloff, I., J. Godsell, C. A. Nold-Petry, J. Harris, A. Hoi, E. F. Morand, and M. F. Nold. 2015. 'Brief Report: Interleukin-38 Exerts Antiinflammatory Functions and Is Associated With Disease Activity in Systemic Lupus Erythematosus', *Arthritis Rheumatol*, 67: 3219-25.

Salminen, A., A. Kauppinen, and K. Kaarniranta. 2012. 'Emerging role of NF-kappaB signaling in the induction of senescence-associated secretory phenotype (SASP)', *Cell Signal*, 24: 835-45.

Sanders, J. L., and A. B. Newman. 2013. 'Telomere length in epidemiology: a biomarker of aging, age-related disease, both, or neither?', *Epidemiol Rev*, 35: 112-31.

Sayej, W. N., P. R. Knight Iii, W. A. Guo, B. Mullan, P. J. Ohtake, B. A. Davidson, A. Khan, R. D. Baker, and S. S. Baker. 2016. 'Advanced Glycation End Products Induce Obesity and Hepatosteatosis in CD-1 Wild-Type Mice', *Biomed Res Int*, 2016: 7867852.

Scavello, F., F. Zeni, C. C. Tedesco, E. Mensa, F. Veglia, A. D. Procopio, A. R. Bonfigli, F. Olivieri, and A. Raucci. 2019. 'Modulation of soluble receptor for advanced glycation end-products (RAGE) isoforms and their ligands in healthy aging', *Aging (Albany NY)*, 11: 1648-63.

Schilder, Y. D., E. H. Heiss, D. Schachner, J. Ziegler, G. Reznicek, D. Sorescu, and V. M. Dirsch. 2009. 'NADPH oxidases 1 and 4 mediate cellular senescence induced by resveratrol in human endothelial cells', *Free Radic Biol Med*, 46: 1598-606.

Schofield, J. D., Y. Liu, P. Rao-Balakrishna, R. A. Malik, and H. Soran. 2016. 'Diabetes Dyslipidemia', *Diabetes Ther*, 7: 203-19.

Schumacher, B., I. van der Pluijm, M. J. Moorhouse, T. Kosteas, A. R. Robinson, Y. Suh, T. M. Breit, H. van Steeg, L. J. Niedernhofer, W. van Ijcken, A. Bartke, S. R. Spindler, J. H. Hoeijmakers, G. T. van der Horst, and G. A. Garinis. 2008. 'Delayed and accelerated aging share common longevity assurance mechanisms', *PLoS Genet*, 4: e1000161.

Sen, P., P. P. Shah, R. Nativio, and S. L. Berger. 2016. 'Epigenetic Mechanisms of Longevity and Aging', *Cell*, 166: 822-39.

Shin, S. K., T. Y. Ha, R. A. McGregor, and M. S. Choi. 2011. 'Long-term curcumin administration protects against atherosclerosis via hepatic regulation of lipoprotein cholesterol metabolism', *Mol Nutr Food Res*, 55: 1829-40.

Siderakis, M., and M. Tarsounas. 2007. 'Telomere regulation and function during meiosis', *Chromosome Res*, 15: 667-79.

Sirtori, C. R., C. Galli, J. W. Anderson, and A. Arnoldi. 2009. 'Nutritional and nutraceutical approaches to dyslipidemia and atherosclerosis prevention: Focus on dietary proteins', *Atherosclerosis*, 203: 8-17.

So, A. Y., J. W. Jung, S. Lee, H. S. Kim, and K. S. Kang. 2011. 'DNA methyltransferase controls stem cell aging by regulating BMI1 and EZH2 through microRNAs', *PLoS One*, 6: e19503.

Sobolova, L., N. Skottova, R. Vecera, and K. Urbanek. 2006. 'Effect of silymarin and its polyphenolic fraction on cholesterol absorption in rats', *Pharmacol Res*, 53: 104-12.

Song, Z., G. von Figura, Y. Liu, J. M. Kraus, C. Torrice, P. Dillon, M. Rudolph-Watabe, Z. Ju, H. A. Kestler, H. Sanoff, and K. Lenhard Rudolph. 2010. 'Lifestyle impacts on the aging-associated expression of biomarkers of DNA damage and telomere dysfunction in human blood', *Aging Cell*, 9: 607-15.

Soto-Gamez, A., and M. Demaria. 2017. 'Therapeutic interventions for aging: the case of cellular senescence', *Drug Discov Today*, 22: 786-95.

St Sauver, J. L., C. M. Boyd, B. R. Grossardt, W. V. Bobo, L. J. Finney Rutten, V. L. Roger, J. O. Ebbert, T. M. Therneau, B. P. Yawn, and W. A. Rocca. 2015. 'Risk of developing multimorbidity across all ages in an historical cohort study: differences by sex and ethnicity', *BMJ Open*, 5: e006413.

Stein, S., N. Schafer, A. Breitenstein, C. Besler, S. Winnik, C. Lohmann, K. Heinrich, C. E. Brokopp, C. Handschin, U. Landmesser, F. C. Tanner, T. F. Luscher, and C. M. Matter. 2010. 'SIRT1 reduces endothelial activation without affecting vascular function in ApoE-/- mice', *Aging (Albany NY)*, 2: 353-60.

Stoccoro, A., H. L. Karlsson, F. Coppede, and L. Migliore. 2013. 'Epigenetic effects of nano-sized materials', *Toxicology*, 313: 3-14.

Szarc vel Szic, K., K. Declerck, M. Vidakovic, and W. Vanden Berghe. 2015. 'From inflammaging to healthy aging by dietary lifestyle choices: is epigenetics the key to personalized nutrition?', *Clin Epigenetics*, 7: 33.

Tafesse, T. B., A. Hymete, Y. Mekonnen, and M. Tadesse. 2017. 'Antidiabetic activity and phytochemical screening of extracts of the leaves of Ajuga remota Benth on alloxan-induced diabetic mice', *BMC Complement Altern Med*, 17: 243.

Taganov, K. D., M. P. Boldin, K. J. Chang, and D. Baltimore. 2006. 'NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses', *Proc Natl Acad Sci U S A*, 103: 12481-6.

Takenaka, S. I., S. Kaieda, T. Kawayama, M. Matsuoka, Y. Kaku, T. Kinoshita, Y. Sakazaki, M. Okamoto, M. Tominaga, K. Kanesaki, A. Chiba, S. Miyake, H. Ida, and T. Hoshino. 2015. 'IL-38: A new factor in rheumatoid arthritis', *Biochem Biophys Rep*, 4: 386-91.

Taslimi, P., and I. Gulcin. 2017. 'Antidiabetic potential: in vitro inhibition effects of some natural phenolic compounds on alpha-glycosidase and alpha-amylase enzymes', *J Biochem Mol Toxicol*, 31.

Tchkonia, T., Y. Zhu, J. van Deursen, J. Campisi, and J. L. Kirkland. 2013. 'Cellular senescence and the senescent secretory phenotype: therapeutic opportunities', *J Clin Invest*, 123: 966-72.

Tili, E., J. J. Michaille, A. Cimino, S. Costinean, C. D. Dumitru, B. Adair, M. Fabbri, H. Alder, C. G. Liu, G. A. Calin, and C. M. Croce. 2007. 'Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock', *J Immunol*, 179: 5082-9.

Vaiserman, A. M., O. V. Lushchak, and A. K. Koliada. 2016. 'Anti-aging pharmacology: Promises and pitfalls', *Ageing Res Rev*, 31: 9-35.

van de Veerdonk, F. L., A. K. Stoeckman, G. Wu, A. N. Boeckermann, T. Azam, M. G. Netea, L. A. Joosten, J. W. van der Meer, R. Hao, V. Kalabokis, and C. A. Dinarello. 2012. 'IL-38 binds to the IL-36 receptor and has biological effects on immune cells similar to IL-36 receptor antagonist', *Proc Natl Acad Sci U S A*, 109: 3001-5.

van Deursen, J. M. 2014. 'The role of senescent cells in ageing', *Nature*, 509: 439-46.

van Stee, M. F., A. A. de Graaf, and A. K. Groen. 2018. 'Actions of metformin and statins on lipid and glucose metabolism and possible benefit of combination therapy', *Cardiovasc Diabetol*, 17: 94.

Ventura, M. T., M. Casciaro, S. Gangemi, and R. Buquicchio. 2017. 'Immunosenescence in aging: between immune cells depletion and cytokines up-regulation', *Clin Mol Allergy*, 15: 21.

Vermeulen, J., J. C. Neyens, E. van Rossum, M. D. Spreeuwenberg, and L. P. de Witte. 2011. 'Predicting ADL disability in community-dwelling elderly people using physical frailty indicators: a systematic review', *BMC Geriatr*, 11: 33.

Villa-Rodriguez, J. A., A. Kerimi, L. Abranko, S. Tumova, L. Ford, R. S. Blackburn, C. Rayner, and G. Williamson. 2018. 'Acute metabolic actions of the major polyphenols in chamomile: an in vitro mechanistic study on their potential to attenuate postprandial hyperglycaemia', *Sci Rep*, 8: 5471.

Wagner, K. H., D. Cameron-Smith, B. Wessner, and B. Franzke. 2016. 'Biomarkers of Aging: From Function to Molecular Biology', *Nutrients*, 8.

Wang, G., F. Dai, K. Yu, Z. Jia, A. Zhang, Q. Huang, C. Kang, H. Jiang, and P. Pu. 2015. 'Resveratrol inhibits glioma cell growth via targeting oncogenic microRNAs and multiple signaling pathways', *Int J Oncol*, 46: 1739-47.

Wang, Y., T. Chen, H. Yan, H. Qi, C. Deng, T. Ye, S. Zhou, and F. R. Li. 2013. 'Role of histone deacetylase inhibitors in the aging of human umbilical cord mesenchymal stem cells', *J Cell Biochem*, 114: 2231-9.

Watanabe, S., S. Kawamoto, N. Ohtani, and E. Hara. 2017. 'Impact of senescence-associated secretory phenotype and its potential as a therapeutic target for senescence-associated diseases', *Cancer Sci*, 108: 563-69.

Wessner, B., L. Gryadunov-Masutti, H. Tschan, N. Bachl, and E. Roth. 2010. 'Is there a role for microRNAs in exercise immunology? A synopsis of current literature and future developments', *Exerc Immunol Rev*, 16: 22-39.

Williamson, G. 2013. 'Possible effects of dietary polyphenols on sugar absorption and digestion', *Mol Nutr Food Res*, 57: 48-57.

Wolfram, S., D. Raederstorff, M. Preller, Y. Wang, S. R. Teixeira, C. Riegger, and P. Weber. 2006. 'Epigallocatechin gallate supplementation alleviates diabetes in rodents', *J Nutr*, 136: 2512-8.

Xia, X., W. Chen, J. McDermott, and J. J. Han. 2017. 'Molecular and phenotypic biomarkers of aging', *F1000Res*, 6: 860.

Xiao, J., J. Li, L. Cai, S. Chakrabarti, and X. Li. 2014. 'Cytokines and diabetes research', *J Diabetes Res*, 2014: 920613.

Xu, W. D., and A. F. Huang. 2018. 'Role of Interleukin-38 in Chronic Inflammatory Diseases: A Comprehensive Review', *Front Immunol*, 9: 1462.

Yamashita, S., K. Ogawa, T. Ikei, M. Udono, T. Fujiki, and Y. Katakura. 2012. 'SIRT1 prevents replicative senescence of normal human umbilical cord fibroblast through potentiating the transcription of human telomerase reverse transcriptase gene', *Biochem Biophys Res Commun*, 417: 630-4.

Yan, S., T. Wang, S. Huang, Y. Di, Y. Huang, X. Liu, Z. Luo, W. Han, and B. An. 2016. 'Differential expression of microRNAs in plasma of patients with prediabetes and newly diagnosed type 2 diabetes', *Acta Diabetol*, 53: 693-702.

Yousefzadeh, M. J., Y. Zhu, S. J. McGowan, L. Angelini, H. Fuhrmann-Stroissnigg, M. Xu, Y. Y. Ling, K. I. Melos, T. Pirtskhalava, C. L. Inman, C. McGuckian, E. A. Wade, J. I. Kato, D. Grassi, M. Wentworth, C. E. Burd, E. A. Arriaga, W. L. Ladiges, T. Tchkonia, J. L. Kirkland, P. D. Robbins, and L. J. Niedernhofer. 2018. 'Fisetin is a senotherapeutic that extends health and lifespan', *EBioMedicine*, 36: 18-28.

Youssef, D. A., H. M. El-Fayoumi, and M. F. Mahmoud. 2019. 'Beta-caryophyllene alleviates diet-induced neurobehavioral changes in rats: The role of CB2 and PPAR-gamma receptors', *Biomed Pharmacother*, 110: 145-54.

Yu, Z., J. Liu, R. Zhang, X. Huang, T. Sun, Y. Wu, B. D. Hambly, and S. Bao. 2017. 'IL-37 and 38 signalling in gestational diabetes', *J Reprod Immunol*, 124: 8-14.

Yuan, X., X. Peng, Y. Li, and M. Li. 2015. 'Role of IL-38 and its related cytokines in inflammation', *Mediators Inflamm*, 2015: 807976.

Zampetaki, A., P. Willeit, L. Tilling, I. Drozdov, M. Prokopi, J. M. Renard, A. Mayr, S. Weger, G. Schett, A. Shah, C. M. Boulanger, J. Willeit, P. J. Chowienczyk, S. Kiechl, and M. Mayr. 2012. 'Prospective study on circulating MicroRNAs and risk of myocardial infarction', *J Am Coll Cardiol*, 60: 290-9.

Zhang, B., M. Kang, Q. Xie, B. Xu, C. Sun, K. Chen, and Y. Wu. 2011. 'Anthocyanins from Chinese bayberry extract protect beta cells from oxidative stress-mediated injury via HO-1 upregulation', *J Agric Food Chem*, 59: 537-45.

Zhang, J., R. Zhao, J. Chen, J. Jin, Y. Yu, Y. Tian, W. Li, W. Wang, H. Zhou, and S. Bo Su. 2017. 'The Effect of Interleukin 38 on Angiogenesis in a Model of Oxygen-induced Retinopathy', *Sci Rep*, 7: 2756.

Zhang, T., C. Lv, L. Li, S. Chen, S. Liu, C. Wang, and B. Su. 2013. 'Plasma miR-126 is a potential biomarker for early prediction of type 2 diabetes mellitus in susceptible individuals', *Biomed Res Int*, 2013: 761617.

Zhao, D. R., Y. S. Jiang, J. Y. Sun, H. H. Li, X. L. Luo, and M. M. Zhao. 2019. 'Anti-inflammatory Mechanism Involved in 4-Ethylguaiacol-Mediated Inhibition of LPS-Induced Inflammation in THP-1 Cells', *J Agric Food Chem*, 67: 1230-43.

Zhong, Y., K. Yu, X. Wang, X. Wang, Q. Ji, and Q. Zeng. 2015. 'Elevated Plasma IL-38 Concentrations in Patients with Acute ST-Segment Elevation Myocardial Infarction and Their Dynamics after Reperfusion Treatment', *Mediators Inflamm*, 2015: 490120.

Zhu, Y., T. Tchkonia, T. Pirtskhalava, A. C. Gower, H. Ding, N. Giorgadze, A. K. Palmer, Y. Ikeno, G. B. Hubbard, M. Lenburg, S. P. O'Hara, N. F. LaRusso, J. D. Miller, C. M. Roos, G. C. Verzosa, N. K. LeBrasseur, J. D. Wren, J. N. Farr, S. Khosla, M. B. Stout, S. J. McGowan, H. Fuhrmann-Stroissnigg, A. U. Gurkar, J. Zhao, D. Colangelo, A. Dorronsoro, Y. Y. Ling, A. S. Barghouthy, D. C. Navarro, T. Sano, P. D. Robbins, L. J. Niedernhofer, and J. L. Kirkland. 2015. 'The Achilles' heel of senescent cells: from transcriptome to senolytic drugs', *Aging Cell*, 14: 644-58.