

Mechanisms of action of Transient Potential Receptors (TRP) Channels: Relevance for Vascular Aging and Menopause

LIMA, F. O. (1); RIBEIRO, P. T (2);. (Franciely Oliveira De Lima; Thaís Porto Ribeiro)

Laboratório de Farmacologia Cardiovascular, Universidade Federal da Paraíba, João Pessoa, PB, Brazil (1)
francielylima-93@hotmail.com

*Laboratório de Farmacologia Cardiovascular, Universidade Federal da Paraíba; Faculdade Maurício de Nassau –
João Pessoa, UNINASSAU, João Pessoa, PB, Brazil (3) thaispribeiro@hotmail.com*

Summary:

The vascular effects of estrogen are affected by the stage of reproductive life, the time since menopause, and the extent of cardiovascular diseases (CVD) have been describe. The vascular longevity increase impairing the vascular function as well the hormone loss in menopause process age related, and both are non-modifiable risk factors, which lead to endothelial dysfunction for decrease of protective mechanisms of endothelial cells function. However, the mechanisms of vascular responsiveness to sex steroids during CVD development remain poorly understood in women and men. The aim of this review was to compare a search in the literature about the molecular mechanisms involved in endothelial dysfunction caused by aging-induced menopause and the TRPV4 role. For this, an exploratory and study carried out from searches in the databases: medline and pubmed, where we selected recent studies about this subject matter. Sexual hormones have mechanisms of action described as protectors of resistance mechanisms to stress and their reduction with menopause can aggravate the damages of the aging. Among the adverse mechanisms of endothelial cellular aging are the increase of oxidative stress, inflammation, reduction of NO bioavailability, autophagic flow reduction, mitochondrial dysfunction and alterations of cellular metabolism control pathways. In addition, Transient Potential Receptors (TRP) channels such as TRPV4 channels presents in the endothelial cells membranes may also have protective role during aging-related menopause by interacting with various intracellular proteins. The mechanisms by which the hormonal function and the TRPV4 channels can interfere with the resistance processes to cellular stress or how they may be altered during the process of menopausal aging are not still elucidated by the studies described in the literature. Therefore, the information available up to the present time so that the hypotheses raised on this subject later clarified from preclinical and clinical studies, thus being the basis for improved assistance in the dysfunctions caused by the menopause associated with aging. From this, we can conclude that to understand the mechanisms of this change is important for targeting new preventive and therapeutic strategies such as control endothelial dysfunction and others molecules alterations results of menopause aging process which can lead to more incidence of CVD in women.

Keywords: Menopause, Vascular Aging and TRPV4.

1. INTRODUCTION

The increase in life expectative of world population increases the vascular longevity and it is lead to more cardiovascular diseases. The aging is a process characterized by the gradual loss of the physiological processes that maintain the protection and

homeostasis of cellular and systemic functions, being in women, specifically, marked by alterations related to the decrease of the hormonal levels after the menopause and, both processes are risk factors for CVD marked by endothelial dysfunction [1].

The demographic transition caused by population aging has increased with collective and individual impacts for the entire world population since it requires systems and policies that govern health care according to particularities of both men and women. Although, only recently, because of the increase in the incidence of cardiovascular diseases (CVD) in women, as research has focused on the gender characteristics which can be involved in these pathophysiological process [1].

The menopausal aging process causes systemic, cellular and molecular changes due to the gradual loss of physiological functions and signaling mechanisms involved in the homeostatic processes of maintaining endothelial function during the aging process, which directly influenced by the reduction of hormonal function in the post menopause. Therefore recent studies propose an alert for the need for further studies on CVD in women [1-2].

The primary mechanisms for the development of aging-related endothelial dysfunction are increased oxidative stress, inflammation in response to increased intracellular oxidative enzymes, decreased nitric oxide (NO) bioavailability, increased activation of gene transcription pathways and release of pro-inflammatory cytokines. In

addition, the dilator response decrease in response to increased blood flow, shear stress and the application of vasodilators substances such as acetylcholine (ACh) in aging and postmenopausal experimental models [3-4].

The relevance of the present study is based on the need to develop studies that characterize the molecular mechanisms involved in vascular changes caused by the aging-related menopause process, including the effects of hormonal loss and TRPV4 channel function to guide future preclinical and clinical studies, from which prophylactic and therapeutic measures against CVD in women will be developed [2-5].

Based on these literature findings, the objectives of this study were to elucidate the molecular mechanisms involved in endothelial dysfunction caused by aging-induced menopause and the role of TRPV4 channels in this vascular dysfunction.

METHODS

This work developed through an exploratory research, developed from studies already described in the literature. Scientific articles searched and selected in the PUBMED and Google Scholar databases, from searches with the following descriptors for the articles: Menopause, Vascular Aging

and TRPV4, where we selected recent studies about this subject matter.

RESULTS AND DISCUSSION

Demographic studies show that the expectation for the 2050 year is that to 30% of the world population, including the Brazilian population, live more than 60 years of age due to the reduction of mortality of younger and older individuals in addition to the increase of life and about 1 billion of this population will

the functionality of the individual and increasing the risk factors for the affection by CVD [5].

AGING AND CARDIOVASCULAR DISEASES

Studies about the relationship between aging and its effects on the cardiovascular system was initiated since the 19th century when the Canadian physician, Dr. William Osler, proposed "longevity is a vascular issue", since certain habits and style of are

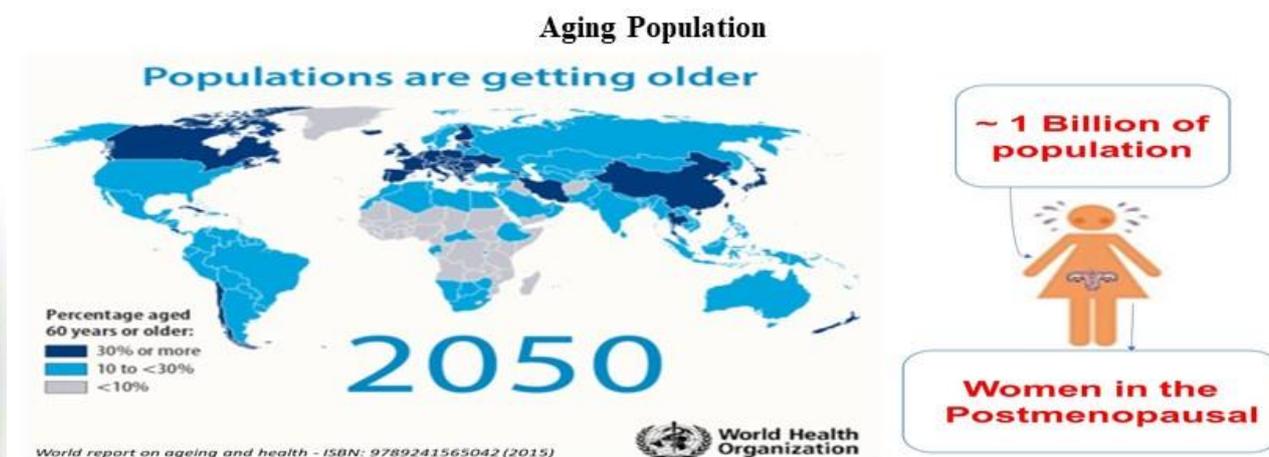


Figure 1. Population are getting older. The expectation for the year 2050 is that 10 to 30% of the world population, including the Brazilian population, live more than 60 years of age and about 1 billion of this population will be postmenopausal women (adapted from WHO, 2015).

be postmenopausal women [1-6].

The literature shown aging is the mere passage of time, senescence is the decrease of the functionality of the organism and senility is the appearance of pathological processes associated with age [7]. Thus, the aging is a physiological process of gradual decrease of molecular and systemic functions that maintain the cellular homeostasis decreasing

risk factors for CVD [1]. Since then, researches with different experimental models carried out and, currently, with a new perspective on the impact of the aging process on women's cardiovascular health because, in recent decades, the incidence of CVD in middle-aged and elderly women has increased [2].

One of the main causes of CVD is endothelial dysfunction. This is referred in the preclinical and clinical studies (cell culture, % of dilation to reactivity) during aging, that show impairment of vascular tone regulation, mediated by interactions between different receptors and intracellular molecules due to the downstream of physiological processes of stress resistance in the cell. This process are autophagy, which is the process by which cells eliminate damaged proteins and mitochondrial dysfunction, thus acting on the suppression of oxidative stress and inflammation, and energy sensor pathways and cellular metabolism such as adenosine monophosphate activated protein kinase (AMPK) and sirtuin-1 (SIRT-1), a NAD⁺-dependent deacetylase and ADP-ribosyltransferase protein [3].

Oxidative stress and inflammation resulting from excessive production of vasoactive factors and vascular growth also called "modifiable molecular mediators (MMM)," are involved in cell senescence and damage to vessels and organs, such as the heart and kidneys. Experimental evidences suggest modulating activity of life expectancy by participating in the damages in cell renewal and gene transcription that originate the pathophysiological processes of the main cardiovascular diseases associated with aging and menopause, such as systemic arterial hypertension, atherosclerosis, and heart failure [3-8].

MENOPAUSE AND CARDIOVASCULAR DISEASES AS A PROCESS RELATED TO AGE

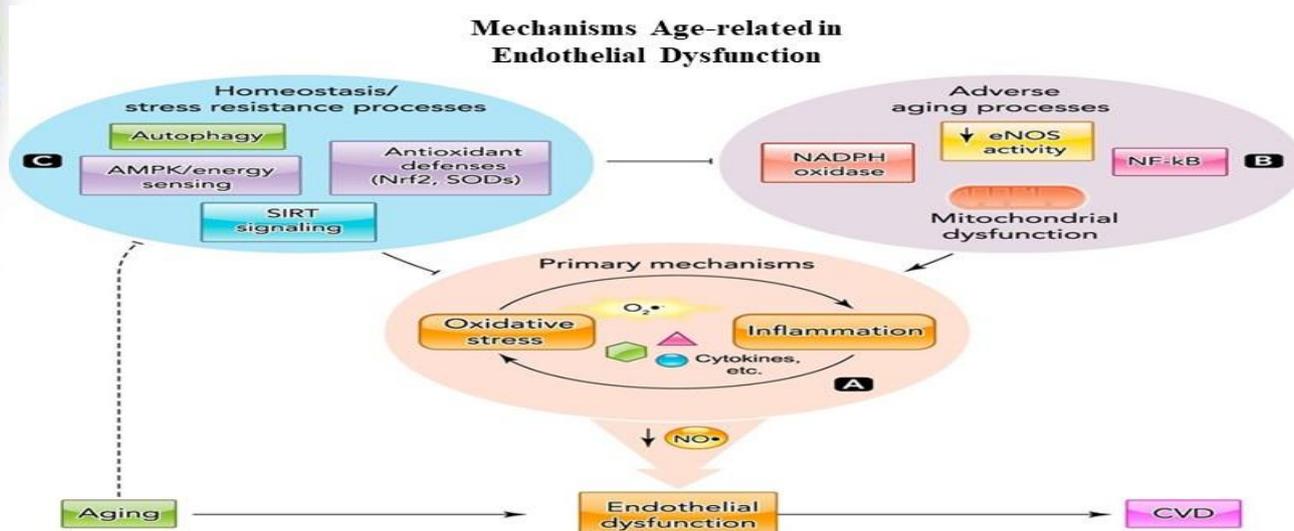


Figure 2. Mechanisms of age-related endothelial dysfunction. A: the primary mechanisms of endothelial dysfunction with aging are reduced NO bioavailability and increased oxidative stress/inflammation characterized by increased superoxide (O₂^{•-}) and inflammatory mediators. B: various adverse cellular aging processes contribute to the development of age-associated oxidative stress and inflammation. C: cellular homeostasis and stress resistance systems protect endothelial function with aging by preventing adverse events and directly reducing oxidative stress/inflammation. Age-related impairments in these protective processes contribute to oxidative stress-/inflammation-mediated NO insufficiency and endothelial dysfunction with aging (adapted from Seals, et. al., 2014).

promotes osteoclastogenesis or bone resorption and the production of the cytokine osteoprotegerin (OPG), secreted by osteoblasts to inhibit RANKL [4].

In the cardiovascular system an increase in the cardiovascular risk profile (growth regulation, inhibition of cardiomyocyte apoptosis and contractility, vasodilation, NO release, inhibition of endothelial cell proliferation and vascular smooth muscle cell and inhibition of cellular apoptosis (figure 4) [4].

rupture and damage of the endothelial cell barrier and dilatation of brachial artery in response to response to flow was also reduced [10].

The experimental model of menopause is described and used for different pathophysiological analyzes. However, there is still a need for studies that clearly characterize the impacts of this model associated with aging on endothelial cells and morphological, biochemical and functional alterations of the endothelium that are related to the increase of the CVD in postmenopausal

Vascular Alterations in the Menopause-age Process

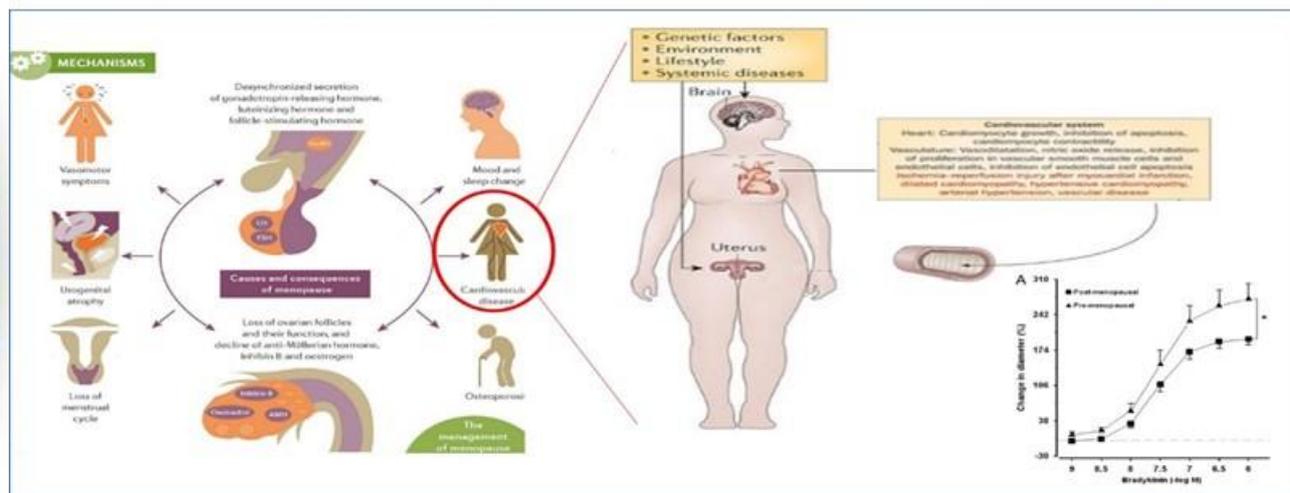


Figure 4. Menopause- age process alters vascular function. The menopause process lead to systemic alterations and the cardiovascular system is more impaired with endothelial dysfunction showed although decrease in dilatation perceptual in function of bradykinin in postmenopausal artery compared to premenopausal artery (adapted of DAVIS, et al., 2015; PROSSNITZ e BARTON, 2011).

Furthermore, the percentage of bradykinin-induced endothelium-dependent dilation has been shown to be reduced in small arteries of postmenopausal women compared to premenopausal women, functional abnormalities accompanied by

women, from middle to advanced age.

SO! IS AGING A DISEASE?

Sir William Osler suggested, "A man is just like the age of his arteries". Since then, studies described in the literature show

endothelial dysfunction as a process related to advancing age with experiments that show the Ach-induced response, which induces increased bioavailability of nitric oxide to cause vasorelaxation, is lower in elderly individuals compared to young individuals. It occur either in humans, in rats isolated from rats or in cultured endothelial cells to quantify NO by fluorescence microscopy [3-5].

In addition, the intracellular environment directly influences the DNA cell development and function, and aging is a series of signalling and cell turnover failures that occur over time in all cells that make up the organs and systems. This is due to changes in gene transcription, loss of density of some molecules and proteins, and consequent changes in oxidation, nitration and phosphorylation processes, which alter the intima and middle layers of the arteries to cause oxidative stress and inflammation, making the cardiovascular system the more associated with age-related failures [5-11].

Thus, is possible to affirm that the molecular alterations of the heart and arteries associated with accelerated aging it referred to as cardiovascular disease.

CELLULAR AND MOLECULAR
BIOLOGY OF ENDOTHELIAL CELL
AGING

Dr. Z. Lale Koldaş (2017) describe that at the cellular and mitochondrial level endothelial and vascular smooth muscle cells (VSMC) have key structural and functional alterations that promote cardiovascular disease (CVD) and is defined as cardiovascular aging.

The vascular endothelium is a cellular barrier that internally coats the arteries and releases biologically active molecules that act in autocrine and paracrine manner to induce balance between vasodilator (e.g., NO, prostacyclin and endothelium-derived hyperpolarizing factor) and vasoconstrictors (e.g., endothelin-1, Ang II and thromboxane A2) responses. It modulate arterial function in response to the stimuli to release these molecules [3-12].

Endothelial cells depend on signaling pathways between the molecules responsible for the maintenance of vascular basal tonus. In addition to mechanisms of resistance to intracellular stress, such as autophagy, energy sensor / AMPK pathway, antioxidant defense factors (Nrf2, SOD), which modulate adverse cellular aging processes, such as decreased eNOS activity, increased NADPH oxidase, NF-Kb and mitochondrial dysfunction, and are altered during the arterial aging process [3].

The suppression of these homeostatic mechanisms of aging allows the development of the primary mechanisms of endothelial dysfunction including oxidative stress and inflammation, being characterized mainly by the vascular insufficiency of NO [7]. This, according to evidence collected from human endothelial cells and arteries animals, results from changes in the activation state of eNOS, the availability of cofactor and the activation of NO, which impairs endothelium-derived dilation with aging [4].

The cellular molecules that interact with mechanisms to maintain the balance between vasodilator and vasoconstrictor responses, that may undergo changes with menopause and advancement of age, is the family of transient potential receptors channels (TRP) currently described as capable of interact with a large number of intracellular proteins and participate in arterial vasodilation. [11-12-13].

TRP CHANNELS

Transient potential receptors (TRPs) are a class of non-selective cation channels for intracellular calcium influx (Ca^{++}), separated according to the amino acid sequence in six subtypes with subtypes, distributed by different cell types, including endothelial cells and vascular smooth muscle

cells, which are involved in the control of vasoconstriction and vasodilation [6].

The subfamily of transient potential receptors of the vanilloid type (TRPV) has six subtypes (TRPV1-6). Currently, TRPV4 described as capable of interacting with a large number of intracellular proteins and participating in arterial vasodilation of rats when induced in the presence of the 4α -PDD agonist. Its effects on endothelial cells, its signaling mechanisms for protection against oxidative stress and its role in endothelium-dependent vasorelaxation are still unclear [11-12].

TRPV4 channels stand out due to the broad range of stimuli that lead to their activation, including physical (cell swelling, heat and mechanical) and chemical stimuli (endocannabinoids, arachidonic acid (AA), and 4α -phorbol esters) [14].

These TRPV4 channels are also involved in endothelial dilation by Ach-induced muscarinic (M) receptor stimulation through a signaling complex that also involves a protein kinase C (PKC) isomer and is associated with ancorine (AKP150) protein. This pathway locally increases intracellular calcium, which results in the activation of potassium channels sensitive to low calcium and intermediate conductance (S/IK

Calcium), NO production or 11,12-epoxyethanedioic acid (11,12-ETT) [8].

The studies shown that these channels are expressed in rat carotid artery endothelial cell junctions in the presence of the CD31 marker and were shown to participate in vasodilation in the presence of 4 α -PDD and eNOS and COX inhibitors [8].

This study shows that aging and menopause are risk factors to CVD because both lead to imbalance between vasodilators and vasoconstrictors substances involved on vascular tonus basal maintenance pathways. So, these alterations cause endothelial dysfunction.

Xu X., et al (2017) Showed that endothelial cellular senescence is another phenomenon that occurs in the aging endothelium and aggravates the endothelial dysfunction, which is a key antecedent of clinical vascular disease and may serve as a predictive and potential diagnostic marker of disease.

Starting from these findings, we observed that the main adverse process involved with the endothelial aging is initiated by damage of AMPK and SIRT pathways. On figure 5, we show that aging process blocks the AMPK and SIRT1 molecule activity that, consequently, reduces the inhibition of the NADPH oxidase or activation of FOXO3, leading to oxidative stress increase. Furthermore, these alterations

increase the cellular proliferation, reduce the pathway PI3-Akt-eNOS and decrease the autophagy process. All these altered pathways are involved with the endothelial dysfunction.

Knowing the damaged pathways involved in the vascular aging process, we observed too that the menopause aging process is involved with the reduction of these pathways because the hormonal protection affects reduction is related with these alterations because the estrogen receptors of membrane (GPER30) and nuclear (E α and E β) are both involved with the eNOS activity and gene transcription process. Therefore, these receptors can induce the vasodilation via endothelial and vascular smooth muscle cell although increase of nitric oxide (NO) bioavailability by non-genomic or genomic process [15-16].

In addition also shown on this review that is important to study the menopause-aging process because the dilator response decreases in response to increased blood flow, shear stress and the application of vasodilatory substances such as acetylcholine (ACh) in aging and postmenopausal experimental models [3-4].

This process can influence the TRPV4 channels involved in endothelial dilation by ACh-induced muscarinic (M) receptor stimulation through a signaling complex that

AMPK and SIRT1 Pathways Altered with Aging

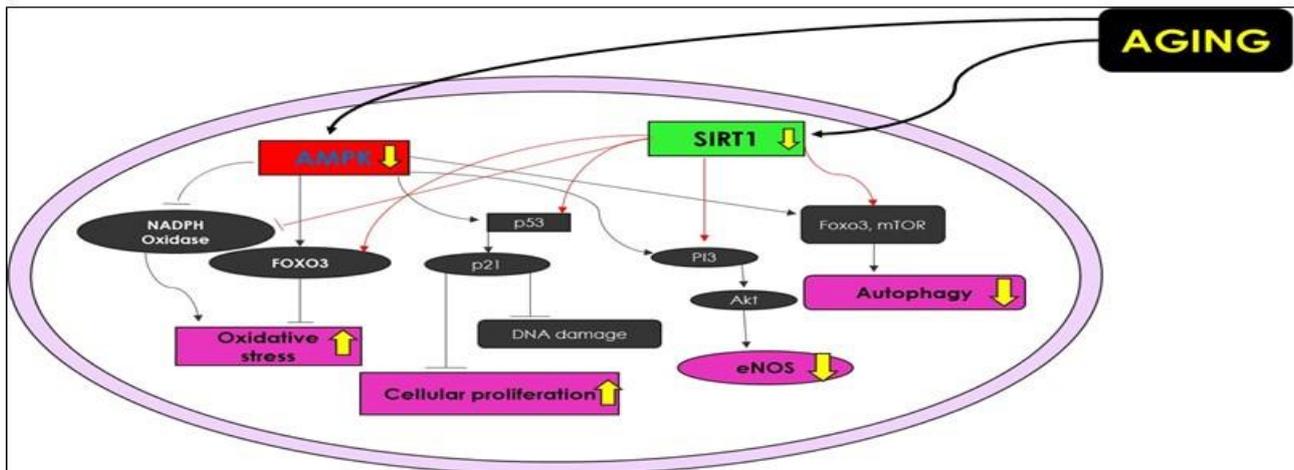


Figure 5. Vascular aging effects on adverse endothelial process pathways. In vascular endothelial cells, the aging process reduces the AMPK and SIRT1 activity increasing the pathways of oxidative stress or cellular proliferation, and decreasing the eNOS activity as such as autophagy process.

also involves a protein kinase C (PKC) isomer and is associated with ancorin (AKP150) protein [5].

An study of Juan Du, et. al. (2016), they concluded that impaired TRPV4-mediated Ca^{2+} signaling causes endothelial dysfunction and that TRPV4 is a potential target for clinical treatment of age-related vascular system diseases.

Thus, showing the relevance of to study the integrate role of this molecules of stress resistance, woman sexual hormones receptors and TRPV4 channels on endothelial cell for to understand the endothelial dysfunction of menopause aging process.

CONCLUSION

Although this study is possible conclude that to understand the mechanisms

of this change is important for targeting new preventive and therapeutic strategies such as control endothelial dysfunction and others molecules alterations results of menopause aging process which can lead to more incidence of CVD in womans.

REFERENCES

- [1] World report on ageing and health - ISBN: 9789241565042 (2015)
- [2] MAAS, Angela HEM et al. Red alert for women's heart: the urgent need for more research and knowledge on cardiovascular disease in women: proceedings of the workshop held in Brussels on gender differences in cardiovascular disease, 29 September 2010. **European Heart Journal**, v. 32, n. 11, p. 1362-1368, 2011.

- [3] SEALS, Douglas R. et al. You're Only as Old as Your Arteries: Translational Strategies for Preserving Vascular Endothelial Function with Aging. **Physiology**, v. 29, n. 4, p. 250-264, 2014.
- [4] DONATO, Anthony J. et al. Cellular and molecular biology of aging endothelial cells. **Journal of molecular and cellular cardiology**, v. 89, p. 122-135, 2015.
- [5] Davis SR, et al. Menopause. **Nature Reviews, Disease Primers**, v. 1, n. 1, p. 119, 2015.
- [6] Z. Lale Koldaş. What is aging and cardiovascular aging? **Turk Kardiyol Dern Ars.** 45(5): p.1-4, 2017.
- [7] MONACO, Thiago Oliveira; SILVEIRA, Paulo Sergio Panse. Aging is not senescence: a short computer demonstration and implications for medical practice. **Clinics**, v. 64, n. 5, p. 451-457, 2009.
- [8] LAKATTA, Edward G. So! What's aging? Is cardiovascular aging a disease?. **Journal of molecular and cellular cardiology**, v. 83, p. 1-13, 2015.
- [9] DU, Juan et al. Increasing TRPV4 expression restores flow-induced dilation impaired in mesenteric arteries with aging. **Scientific reports**, v. 6, p. 22780, 2016.
- [10] WHITE, John PM et al. TRPV4: molecular conductor of a diverse orchestra. **Physiological reviews**, v. 96, n. 3, p. 911-973, 2016.
- [11] LIU, DaoYan; XIONG, ShiQiang; ZHU, ZhiMing. Imbalance and dysfunction of transient receptor potential channels contribute to the pathogenesis of hypertension. **Science China. Life Sciences**, v. 57, n. 8, p. 818, 2014.
- [12] EARLEY, Scott et al. TRPV4-dependent dilation of peripheral resistance arteries influences arterial pressure. **American Journal of Physiology-Heart and Circulatory Physiology**, v. 297, n. 3, p. H1096-H1102, 2009.
- [13] XU, Xianglai et al. Age-related Impairment of Vascular Structure and Functions. **Aging and Disease**, v. 8, n. 5, p. 590-610, 2017.
- [14] FILOSA, Jessica A.; YAO, Xiaoqiang; RATH, Geraldine. TRPV4 and the regulation of vascular tone. **Journal of cardiovascular pharmacology**, v. 61, n. 2, p. 113, 2013.
- [15] KUBLICKIENE, Karolina et al. Small artery endothelial dysfunction in postmenopausal women: in vitro function, morphology, and modification by estrogen and selective estrogen receptor modulators. **The Journal of Clinical Endocrinology & Metabolism**, v. 90, n. 11, p. 6113-6122, 2005.

[16] PROSSNITZ, Eric R.; BARTON, Matthias. The G-protein-coupled estrogen receptor GPER in health and disease. **Nature Reviews Endocrinology**, v. 7, n. 12, p. 715-726, 2011.