Mechanisms of coronary microvascular tone regulation: Aging and sex differences

by

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Abstract

The coronary microcirculation is the principle site of blood flow control and myocardium oxygen delivery within the coronary artery tree. Coronary arteriole tone is determined by three major endothelium derived vasoactive substances: endothelin, nitric oxide (NO), and reactive oxygen species (ROS). The effects of these substances change with aging and differ between sexes. Endothelin-1 (ET-1), the primary endothelin isoform in the coronary circulation, acts on smooth muscle receptors endothelin-A (ET_A) and endothelin-B (ET_B) to induce vascular smooth muscle (VSM) contraction and vasoconstriction. Whereas ET-1 activation of the ET_B receptor on the endothelium initiates a cascade of events leading to NO production via endothelium derived NO synthase (eNOS) enzyme activation and VSM relaxation. Aged males maintain ET_A receptor expression and higher levels of vasoconstriction than do age-matched females. High levels of ET_A receptor activity are associated with hypertension, myocardial infarction, coronary artery spasm, atherosclerosis, and finally heart failure (HF). Additionally, NO can displace ET-1 from the VSM ET_A and ET_B receptors. Thus, with reduced eNOS activity and decreased NO production, there is a simultaneous loss of vasodilatory capacity and increase in vasoconstrictive capacity. In both rodent and human models aged males and females ROS production increases with age. ROS, such as superoxide, scavenge NO, decreasing its bioavailability and producing peroxynitrite. Peroxynitrite is a potent reactive nitrogen species that leads to endothelial cell apoptosis and eNOS enzyme dissociation, potentiating superoxide production and NO reduction. It has been shown that the reduction in NO bioavailability may be a primary mechanism of coronary artery disease. However, the ROS hydrogen peroxide, also increased with aging, produces a potent vasodilatory effect in the coronary microcirculation and seems to be one mechanism that buffers the loss of NO-induced vasodilation. In postmenopausal women

diminished estrogen levels further reduce eNOS production of NO. Males, however, tend to experience decrements in arteriole function a decade before women and estrogen may be one mechanism preserving vascular health into middle age that separates the chronology of coronary artery disease between sexes. Determining the mechanisms of disease onset that accompany the aging process will provide insight into potential therapies to preserve endothelium dependent dilation with aging such as exercise, dietary NO supplementation, and increased dietary anti-oxidant consumption.

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Dedication

To the curious, *nullius in verba.*

Chapter 1 - Mechanisms of coronary microvascular tone regulation: aging and sex differences

Introduction

Cardiovascular diseases (CVD) are the leading cause of death in both developed and developing countries (1). Specifically, coronary artery disease, due to arterial dysfunction, has the highest morbidity and mortality rate among cardiovascular diseases (1). The coronary arterial tree is tasked with the delivery of nutrients and removal of metabolic byproducts from the myocardium. The coronary microcirculation is the principal location in the coronary vascular tree responsible for matching oxygen (O_2) delivery (QO_2) to utilization (VO_2) for the network of parenchymal cells (i.e. myocardial cells), structural tissue, nerve and other cells types that dictate cardiac function and thus metabolic rate. The coronary microcirculation tightly manages arteriole tone to ensure that appropriate QO_2:VO_2 is met. Coronary vascular resistance is determined by vascular anatomy, extravascular cardiac compressive forces, and vascular smooth muscle contraction. Chilian et al. discovered that around 50% of total coronary resistance in the left ventricle resides in arterioles less than 150μm in diameter (2). Figure 37 in *Local regulation of microvascular perfusion* by Davis et al. (2008) illustrates the distribution of resistance along the coronary vascular tree, the predominant vasoactive influences within each segment of the coronary microcirculation and that the majority of coronary vascular resistance resides in the arterioles (3). Coronary arteriole tone is regulated by metabolic, myogenic, endothelial and neural influences (4, 5, 6). The relative impact of these mechanisms is dependent upon the size of the arteriole. This review will focus on three endothelial and myogenic regulators of coronary arteriole tone in healthy aging; a) endothelin (ET) b) endothelium-derived nitric oxide (NO) and c) reactive oxygen species (ROS).

The relationship between age and cardiovascular disease development is evident (7). It can be said that aging alone is a major risk factor for the development of CVD and, as stated by Seals et. al. that CVD are diseases of aging (7). Aging reduces cardiac function (8) as well as submaximal coronary blood flow in aged rats (9) and humans (10). Additionally, evidence of sex differences with aging in the onset of cardiovascular disease is mounting. Celermajer et al. demonstrated that endothelium dysfunction occurs 10 years earlier, on average, in men as compared to women (11). In coronary arterioles LeBlanc et al. demonstrated that endothelium-dependent dilation (EDD) loses effectiveness with age for male (12) and female rats (13); however, mechanisms of endothelium dysfunction can be sex specific (14, 15). Males demonstrate an increased or preserved responsiveness to vasoconstrictors (16) such as ET-1 (17), while post-menopausal females, having reduced estrogen levels, may lose the vasodilatory capacity buffer of estrogen allowing for an increased reactivity to vasoconstrictor mechanisms, such as ET-1, in coronary arterioles.

With these insights it is becoming ever clearer that coronary microvascular dysfunction in aging plays a critical role in the development of cardiovascular disease with the addition that sex may alter the mechanism of disease development. Given the increasing population of older adults, understanding the microcirculatory pathologies with healthy aging is essential. Gaining insights into the aging coronary microcirculation could deliver insights into therapeutic mechanisms for the delay or prevention of CVD as well as a greater understanding of the pathologies associated with coronary artery disease (CAD) and heart failure (HF) in the aged.

During the aging process, especially into the 6^{th} decade of life, the effectiveness of chemical vasoactives at the endothelial and smooth muscle level change. ET-1, endothelium derived NO (and/or NO bioavailability), and ROS become out of balance. The endothelin B

(ET_B) receptor, located on the endothelium, when activated by ET-1, leads to eNOS expression and NO production to dilate coronary arterioles, particularly <150μm (18). Functionality of ET_B on the endothelium increases in value as the endothelin A (ET_A) and endothelin B (ET_B) receptors, located on the vascular smooth muscle (VSM), maintain their potent vasoconstrictive capacity throughout the aging processes in male rats (13). Relatively high levels of ET_A expression lead to a chronic increase in vessel tone which is accompanied by a host of pathologies such as hypertension (19), myocardial infarction (20), coronary artery spasm (21), atherosclerosis (22), and finally HF (23). The compounding effect of high ET_A expression on the VSM with an increased buildup of arteriole plaques as humans age, is damaging the endothelium and abolishing EDD mechanisms.

Additionally, there tends to be an increase in the production of ROS such as hydrogen peroxide (H_2O_2) and superoxide (O_2^-) with aging (24). Superoxide, produced in parallel to mitochondrial production of ATP, will scavenge NO rendering it to peroxynitrite ($OONO^-$), a more potent oxidant that has no vasodilatory effects and induces cell death (25, 26, 27). On the other hand, H_2O_2, also produced in proportion to mitochondrial ATP production, has significant vasodilatory effects and functions to match coronary blood flow to myocardial oxygen demand (28). Superoxide can severely damage cell health but H_2O_2 is important for arteriole tone maintenance. They are produced simultaneously and thus the ramifications of both ROS must be considered when looking at therapies such as exercise or increasing NO bioavailability in the aged.

In order to understand the mechanisms of, and provide more effective treatments for CAD in the aged, we must first separately understand how the healthy aging process leads to coronary arteriole endothelial dysfunction because arterioles are the primary site of blood flow

regulation. This will provide clarity for what mechanisms of disease are solely due to aging and will help elucidate a stratified picture of CAD as diet, sedentary lifestyle, and genetic pathologies are layered atop this complex disease.

Endothelin

Endothelin is a 21-amino acid vasoactive peptide produced by coronary endothelial cells (28). While three isoforms of endothelin have been discovered (ET-1, ET-2, and ET-3) (28). ET-1 is the most abundant isoform in the coronary circulation, and plays a critical role in regulating basal coronary arteriole tone during periods of basal metabolism (29). However, its role in regulating the coronary circulation is then reduced with increases in metabolic demand (13, 30). ET-1 is primarily produced via cleavage of its non-active precursor preproendothelin and big ET (bET) (31, 32). bET is converted to ET-1 via endothelin-converting enzyme (33, 34) as demonstrated by Khimji et al. in *Endothelin – Biology and Disease* (2010) figure two (35).

ET-1 is released by endothelial cells with approximately 80% being released albuminally toward the vascular smooth muscle (32). The local release and utilization ET-1 leads to the belief that ET-1 functions primarily in a local paracrine rather than in a circulating endocrine, fashion (33). Some research suggests that ET-1 may act in an autocrine fashion, as well (33). ET-1 receptors type A (ET_A) and type B (ET_B) are expressed on vascular smooth muscle cells of coronary arterioles, but only ET_B receptors reside on the endothelium (34). ET-1 will bind to the G_q-protein-coupled ET_A and ET_B receptors on vascular smooth muscle cells resulting in vasoconstriction. Whereas, ET_B receptors activated by ET-1 on the endothelium lead to prostacyclin (PGI_2) and NO production which cross the vascular space attaching to guanylyl cyclase on the vascular smooth muscle. Guanylyl cyclase activates the cyclic guanosine monophosphate (cGMP) to protein kinase G (PKG) pathway which leads to smooth muscle

relaxation and vasodilation, as demonstrated in figure 4 of Endothelin-Biology and disease by Khimji et al. (2010) (35, 36, 37, 33, 38).

Recent evidence supports the role of ET-1 in mediating endothelial dysfunction with aging (39). Data from aged humans indicate that ET-1 vasoconstrictor activity is augmented with aging (40, 41) and contributes, at least in part, to diminished EDD in older adults (42). In human vascular endothelial cells Donato et al. demonstrated that ET-1 plasma concentrations and expression increase with age and are inversely associated with EDD (40). While Donato et al. obtained these results from the brachial artery, studies have suggested that brachial artery flow mediated dilation can serve as an index of coronary conduit artery endothelial function (43).

Responsiveness to ET-1 changes with age differently depending upon sex. In the human coronary circulation, reports indicate that age-related differences in ET-1 induced vasoconstriction occur principally as a result of decreased ET_A receptor protein levels. However, older men had a greater ET_A receptor-mediated vasoconstrictor tone than age-matched women (44, 45). Female rat responsiveness to ET-1 in the aorta declines with age (46). Contrarily, male rats have an increased responsiveness to ET-1 in large coronary arteries (47). These results are reversed in the coronary microcirculation. LeBlanc et al. discovered that aged male rats demonstrate a decreased responsiveness to ET-1 induced vasoconstriction in coronary resistance arterioles (46, 13). In aged male rats these age-related decrements in ET-1 induced vasoconstriction are accompanied by a decrease in ET_A receptor protein expression on the vascular smooth muscle, with a coinciding increase in ET_B receptor protein expression on the vascular arteriole endothelium (13). In addition, the ET_B receptor on the endothelium has been shown to modulate the vasoconstrictor effects of ET-1 bound to ET_A or ET_B receptors on the VSM primarily through NO production (48, 49). In age-matched female rats, LeBlanc et al

demonstrated an increased vasoconstrictive response to ET-1. However, in contrast to male rats, the coronary arterioles of aged female rats demonstrated an increased responsiveness to ET-1 despite unchanged ET_A or ET_B receptor protein expression levels (13), exemplifying that alterations in coronary EDD and constriction are heavily dictated by sex and age. Interestingly, this age related increased responsiveness to the vasoconstrictive effects of endothelin may provide some protection to the aging heart (12) as vasoconstriction can redirect coronary blood flow to the subendocardium, preventing excessive back flow from the coronary circulation during systole (45). This sex difference in vasoconstrictor tone to ET-1 via differences in ET_A expression may be a mechanism responsible for the sex differences in CAD between male and female older adults (44). However, endothelium-independent mechanisms, such as calcium (Ca^+) handling, may also be responsible for the vasoconstrictive differences, as exemplified by young and aged female rats' vasoconstrictive differences in arteries remaining even after blood vessel denudation and ET_B blockade (50). Finally, inhibition of ET-1 signaling with ET_A receptor antagonist improved EDD in old, but not young, mice (47). These results indicate that older mice either have an increased or preserved sensitivity to the vasoconstrictive effects of ET_A and/or that supportive mechanisms of vasodilation are not preserved in the aging process.

Endothelin and Nitric Oxide

In 1990, Boulanger and Luscher discovered that endothelium-derived NO production inhibited the production of endothelin through a cyclic GMP-dependent pathway (51), indicating that a reduced NO production or bioavailability, via eNOS inhibition, could lead to exacerbated production of endothelin and thus chronic vasoconstriction. NO, has also, been demonstrated to displace ET-1 from ET_A and ET_B receptors on VSM, inhibiting their vasoconstrictive effects (49). NO can also bind to thiol groups on the endothelin receptors producing s-nitrosothiols

which act as vasodilators (52, 53). Kang et al. have demonstrated impaired NO-mediated vasodilation in aged female rat coronary arterioles that were believed to be due to decreased circulating estrogen levels. (54). Contrarily, aged male rats exhibit an increase in eNOS mRNA (17), although, mRNA is not always indicative of protein expression. These sex-specific NO regulatory mechanisms could, in part, be responsible for the inverse ET-mediated constriction seen between aged male and female rat coronary arterioles (13).

Endothelium Derived Nitric Oxide

In vivo and *in vitro* studies have demonstrated that endothelium dependent release of NO is imperative for controlling coronary vascular resistance. NO protects the vascular system and is primarily produced by the enzyme eNOS on the endothelium. eNOS is activated by vascular shear stress induced by BF, chemical mediators, and/or calcium-calmodulin binding due to increased Ca^{+} signaling from VSM shear stress. Shear stress in the coronary circulation is principally regulated at the level of small arteries and arterioles <150μm in diameter, providing further evidence of eNOS activation primarily at these sites (55). eNOS produces NO via reduction of molecular oxygen by a two-step process. Firstly, eNOS, bound to co-factor (6R)-5,6,7,8-tetrahydro-L-biopterin (BH_4), hydroxylates L-arginine to N^{ω}- hydroxy-L-arginine which remains bound to eNOS. Secondly, eNOS oxidizes N^{ω}- hydroxy-L-arginine to L-citrulline and NO (56). NO dilates arterioles by stimulating soluble guanylyl cyclase to produce cGMP which activates PKG in smooth muscle cells to promote VSM relaxation. In addition, NO has been shown to inhibit platelet aggregation and adhesion, reducing VSM exposure to platelet-derived growth factors and thus fibrous plaque formation, leukocyte adhesion, DNA synthesis, mitogenesis, and proliferation of VSMC (57). These effects make NO one of the most important vasoprotective chemicals especially for healthy aging. However, several human and animal

studies correlate aging with endothelium dysfunction and secondarily with a decreased NO production and thus a depressed ability to regulate arteriole vessel tone (58, 59).

In porcine coronary resistance arterioles antagonists of eNOS produce vasoconstriction at rest, suggesting that a tonic release of NO is necessary for maintaining arteriole tone (60). Additionally, in isolated porcine coronary arterioles it has been demonstrated that clonidine (61), serotonin (61), and substance P (60) produce VSM relaxation through endothelium release of NO. In human and rat ventricular arterioles acetylcholine initiates EDD mediated by NO (62). However, in pigs, where acetylcholine acts as a vasoconstrictor, the release of endothelium derived NO is used to modulate the constrictive effects of acetylcholine (62). The vasoconstrictive effects of α_1- and α_2-adrenergic agonists in the coronary microcirculation are also attenuated by endothelium derived NO (63).

In one study, coronary arteriole flow-induced vasodilation was abolished by eNOS inhibition with L-NMMA, an L-arginine analog, obstructing the production of NO. These results were then reversed in the presence of excess L-arginine, demonstrating the necessity of the cofactor L-arginine in eNOS production of NO (60). Additionally, it is an insufficiency of L-arginine which can cause the eNOS enzyme to dissociate and produce superoxide instead of NO. This mechanism of superoxide production will be discussed further in the ROS section.

While some studies suggest that age enhances endothelial production of NO via increased eNOS protein expression, it is likely that the aged have a greater reliance on NO to mediate vessel tone due to other mechanisms of EDD not being preserved into old age. This is demonstrated well by Shipley et al. where young and old rats given NG-nitro-L-arginine methyl ester (L-NAME), a non-selective NOS inhibitor, both had significant increases in percent arteriole constriction. However, a statistically significant difference in percent constriction

remained between young and old. Additionally, with denudation of the arteriole no constrictive differences remained. This indicates that other EDD mechanisms buffer the loss of NO in the young, but these mechanisms are likely not preserved in healthy aged male rats (17).

Sex also seems to play a role in eNOS expression. Recent research suggests that decreased circulating estrogen levels in postmenopausal women lead to the downregulation of estrogen receptor alpha (ERα) and reduced EDD (64, 11). ERα, which modulates vascular function through estrogen (65), is shown to be reduced in postmenopausal women as compared to premenopausal women (66). This reduction in ERα activation impairs EDD in part due to a reduced eNOS expression. However, with estrogen administration EDD improves in some postmenopausal women (67) as a result of increased NO bioavailability (68). However, these results have been variable in the literature and more research is needed to confirm these results.

Reactive Oxygen Species and Nitric Oxide Bioavailability

Superoxide

The primary mechanism of endothelium damage and inhibition of the eNOS enzyme in aged humans and animals is production of ROS, in particular O_2^-, without anti-oxidant compensation (69-71). Superoxide is generated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (85), the mitochondrial respiratory chain (73,74), as well as eNOS uncoupling (75). Current research supports the hypothesis that aging exacerbates the production of superoxide (7). This supports the theory that the age-related reduction in NO is principally due to superoxide scavenging NO to produce the potent reactive nitrogen species (RNS) peroxynitrite, and/or uncouple eNOS, both of which produce superoxide anions (24). The superoxide anions reduce endothelium dependent production of NO and reduce NO bioavailability (76, 7). Reduction in NO bioavailability is likely a primary mechanism of reduced EDD with aging.

eNOS uncoupling, which leads to the production of a superoxide anion instead of NO, is the product of either reduced BH_4 bioavailability, reduced L-arginine bioavailability, and/ or ROS or RNS damage as demonstrated by figure three in *The Coronary Circulation in Health and Disease* by Muller-Delp (2013) (7, 24, 77, 78). BH_4 is easily oxidized by superoxide and peroxynitrite making it no longer functional as an eNOS cofactor (79). While the primary mechanism(s) causing reduced BH_4 levels with aging is up for debate, it is likely that the production of BH_4 remains the same in aged humans. This is supported by studies on aged rodents (80) where it has been demonstrated that increases in ROS are oxidizing BH_4 reducing its bioavailability. Studies have demonstrated that BH_4 administration causes an improvement in EDD in aged individuals that is not observed in young subjects. The vasodilatory effects of BH_4 administration are blocked NOS inhibition, confirming that BH_4 is a required eNOS cofactor for NO production (81, 82). Additionally, age-related differences in EDD when NOS was inhibited were abolished (82-85).

Hydrogen Peroxide

Hydrogen peroxide has been proposed to function as a feedforward mechanism coupling myocardial demand to coronary BF and O_2 delivery due to its direct relationship to O_2 utilization (24). In part, this is due to increased electron transport chain utilization resulting in greater production of ROS such as superoxide which is disumtated to H_2O_2. The vasodilatory effects of endothelium derived H_2O_2 were initially discovered in porcine and human coronary arterioles (86, 87, 25). Some work from Saitoh et al. indicates that a portion of H_2O_2 is produced in VSM mitochondria with increased metabolic demand. However, mitochondrial derived H_2O_2 is also produced in endothelial cells where, in a paracrine manner, it is taken up by the surrounding coronary arteriole VSM cells activating PKG opening Ca^+ activated potassium channels which

hyperpolarize the arteriole VSM and induce relaxation as demonstrated by figure three in *The Coronary Circulation in Health and Disease* by Muller-Delp (2013) (24, 88, 89).

More studies are needed to clearly identify what proportion of H_2O_2 comes from the VSM versus the endothelium during resting conditions, where metabolic demand is relatively low (25). For aged, sedentary individuals where minimal work could cause an increase in metabolic demand and thus mitochondrial superoxide production, H_2O_2 may be an important regulator of arteriole tone moment to moment as it may be used in a compensatory manner for NO. However, H_2O_2 is proinflammatory (90), and H_2O_2 production that supersedes the superoxide dismutase (SOD) buffering capacity of the endothelium or is dismutated in the presence of catalytic transition metals such as iron (Fe^{2+}) could impair endothelial function due to excess hydroxide (OH^-) production (24). Arteriole dilation is mediated by NO into the seventh decade of life (91). However, H_2O_2 may provide an ulterior mechanism for regulating coronary arteriole tone in the aged for both sexes. This production of H_2O_2 appears to be advantageous in producing a vasodilation in the coronary circulation versus the anticipated detrimental effects that it may have as a ROS (89).

Potential Therapies

Anti-oxidants

Askurza et al. demonstrated that anti-oxidants such as ascorbic acid and superoxide scavengers can restore EDD in the peripheral (brachial) conduit arteries of older sedentary human males but had no effect on both young and old endurance-exercise trained subjects (92, 10). Additional evidence in coronary resistance arteries of aged rats, where NO-mediated dilation is reduced, demonstrates significantly improved BF with treatment of scavengers of superoxide (i.e. SOD) (10). Future research will need to determine if it is the increased bioavailability of NO

or the increased production of H_2O_2 that mediates coronary BF improvements. Importantly, Donato et al. demonstrated that antioxidant enzyme expression in vascular endothelial cells was similar between young and old healthy adults (93). This indicates that the increase in ROS production during aging, without compensatory increase in anti-oxidant bioavailability, is likely a major contributing factor to hampered EDD and decreased NO bioavailability. Zhao et al. contend that animal cell apoptosis, programed cell death, can be prematurely induced by ROS and RNS such as O_2^- and peroxynitrite ($ONOO^-$). Peroxynitrite is formed through the combination of NO and O_2^- (26) and produces endothelial cell apoptosis which accelerates vascular endothelium dysfunction and thus impaired EDD (94). Accordingly, a therapy to preserve EDD in the coronary arterioles with aging would necessitate a dietary increase in anti-oxidant containing foods such as fruits and vegetables to reduce ROS and RNS which will preserve endothelial cell health and reduce NO scavenging.

Exercise

The literature has demonstrated a clear link between sedentary behaviors in aged individuals and a depressed coronary microvascular BF response to acute exercise (95). Studies consisting of both cross-sectional comparisons and interventional studies show that consistent aerobic exercise enhances EDD in older men likely through maintained basal NO production via eNOS, a reduction in NADPH oxidase activity, and an increase in SOD activity (95-98, 83).

Coronary microvascular resistance is determined by VSM contractility in coronary arterioles. Delp et al. have demonstrated that VSM contractile function is impaired in coronary arterioles from aged rats (95). In aged male rats, VSM exhibited a secretory phenotype and VSM proliferation in the arteriolar wall, decreased arteriole VSM myosin heavy chain 1, and increased expression of both phosphohistone H3 and synthetic ribosomal protein S6. Demonstrating that

the age-related contractile dysfunction of coronary arteriole VSM via these mechanisms may be in part responsible for the hampered BF responses to acute exercise. However, exercise training reversed all of these responses in age male rats restoring VSM contractile responsiveness (95). Taddei et al. and Eskura et al. demonstrated that regular aerobic exercise augments EDD through a reduction in ROS with a concurrent increase in NO bioavailability, in part due to the preservation of BH_4 in aged men (96, 97). Mechanisms of EDD preservation are likely shear stress driven as exercise increases vascular shear stress inducing eNOS expression, maintenance of NO bioavailability which acts as both a vasodilator and endothelium protector, and a retardation of plaque buildup (100, 101). This research demonstrates that exercise, even late in life, can reverse many of the deleterious effects of sedentary aging. Unfortunately, research has not demonstrated a similar effect of aerobic exercise on EDD preservation and recovery in aged, postmenopausal women in any arteries let alone coronary arterioles (102, 103). The mechanisms differentiating male and female EDD responses to exercise will be significant areas of further research.

Inorganic Nitric Oxide Supplementation

Numerous investigations demonstrate a decreased NO-mediated vasodilation with age due to a reduction in NO bioavailability (104, 85, 105). Even though eNOS protein expression has been demonstrated to increase with age (40), aging blunts NO production in response to increased shear stress (106) and aging is associated with an increase in ROS scavenging for NO. Thus, the increased eNOS protein expression is likely an attempt to compensate for the reduced NO bioavailability. Inorganic nitrate (NO_3^-) supplementation (e.g. beetroot juice) has become a therapy of extensive research due to its ability to be broken down into nitrite (NO_2^-) and NO via a step-wise, NOS independent mechanism (107). While much of the research has been conducted

in skeletal muscle, the systemic increase in plasma NO_3^- concentrations indicates that NO bioavailability is likely also increased in coronary arterioles. Increasing NO bioavailability through a NOS independent mechanism may provide an excellent therapeutic mechanism for improving coronary microvascular resistance in the aged.

Conclusion

In conclusion, the coronary artery is tasked with the delivery of O_2 and nutrients and removal of metabolic byproducts. The coronary microvasculature is the primary site of BF regulation which is determined by arteriole tone. Arteriole tone is regulated by VSM constriction and relaxation which is primary regulated by three strong vasoactive substances all working in tandem to control arteriole tone, namely ET-1, NO, and ROS. ET-1 works on ET_A and ET_B receptors on the VSM to induce vasoconstriction, whereas the ET_B receptor on the endothelium activates the eNOS pathway to produce NO and relax the VSM eliciting vasodilation. In the aged, ET_A receptors are typically preserved but ET_B receptors on the endothelium decrease in number typically due to endothelium damage via arteriole plaques and ROS and RNS damage. ROS, such as O_2^-, production is increased with age and uncouples eNOS and scavenges for NO, reducing its bioavailability, forcing the use of H_2O_2 as the primary vasodilator in coronary arterioles of the aged. Decreasing ROS via increased dietary intake of anti-oxidants, increased dietary inorganic nitrate consumption, and increased exercise in the aging can all preserve the health of the endothelium ensuring sustained vasodilatory capacity during the aging process. Unfortunately, female research is lagging far behind research in males and thus the mechanism(s) preserving coronary arteriole health in aging females is still to be revealed.

Future Research

While eNOS plays a major role in coronary arteriole BF regulation, neuronal NOS (nNOS) has also recently been demonstrated to play a role in controlling basal coronary BF and microvascular tone (108). Coronary nNOS is expressed on the VSM and coronary perivascular nerves in rats and humans (109, 110). Lamping et al. and Huang et al. have both demonstrated in the mouse coronary circulation the use of nNOS for EDD generated through NO in eNOS knockout mice (111, 112). No literature, to our knowledge, concerning how nNOS changes with age or sex in the coronary circulation currently exists. However, due to its newly discovered significance in the coronary circulation, it is another mechanism of regulation that deservers further exploration. Additionally, several studies indicate that inducible NOS (iNOS) expression and function in VSM increase with age (113, 59, 104, 114). However, results by Shipley et al. refute these findings demonstrating no significant alterations in vasoconstrictive responsiveness in coronary arterioles from old male rats (17). Research to clarify the functionality of each enzyme eNOS, nNOS, and iNOS within the coronary circulation as it relates to aging and sex is necessary to understand the EDD dysfunction that arises with age.

Finally, the sex specific response to ET-1 is comprised by a multifactorial response system. However, one major possible mechanism decreasing female vasoconstrictive responsiveness to ET-1 could lie within the VSM Ca^{2+} release and reuptake. ET-1 induced Ca^{2+} release has a long lasting vasoconstrictive effect. Therefore, it is reasonable to hypothesize that aged females have either a blunted Ca^{2+} release or a rapid Ca^{2+} reuptake that hampers the vasoconstrictive effects of ET-1. However, further research is needed to clearly identify and explain the mechanism of action.

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Chapter 2 - Reflection

As I review my time at Kansas State University it is humbling to imagine where I would be without the encouragement and mentorship of my professors and peers. Entering Kansas State as an undergraduate in Athletic Training I knew I had a passion for physiology. My first semester I enrolled in Basic Nutrition and discovered my interest in nutrition. Second semester freshman year I switched my major to Nutrition and Health and joined Drs. Timothy Musch and David Poole's Clarenburg Cardiopulmonary Research Laboratory. Through the combination of nutrition and chemistry heavy course work and my time researching the ramifications of heart failure on peripheral circulation I obtained an understanding of how the components of human health intersect to maintain proper physiological function. However, I struggled through my chemistry coursework throughout my time as an undergraduate. It is amazing now to look back at the chemistry I struggled with and see how I am applying it through my research today. As a sophomore taking organic chemistry, and struggling, I wanted to take a year off of college to spend time studying on my own. Through the encouragement of my mom I stuck with my studies and it is amazing to think I am now graduating with a Master of Science degree, a Certificate in Public Health, and continuing my research interests through a Fulbright to Semmelweis Medical School in Budapest, Hungary. Wanting to stop attending college for a year to pursue my own studies demonstrated to me one thing, I have a deep and pure passion for understanding physiology, unattached to academic success that might come with it. Sticking with my education taught me that persisting through the struggle leads to a stronger mindset on the other side and is the foundation for an iron-will to accomplish what I start.

At the end of the day I did get my time off. Taking a semester off half way through my master's program to travel around the globe, only to come back and finish, was one of the best

decisions I made. Against the will of my parents, I knew that time away was essential to my personal growth and health. It is only through my time abroad that I gained perspective on my educational process, I made the connection to establish my Fulbright in Hungary, and I strengthened my focus on a career in international health care.

College is a process of maturing mentally, emotionally, and spiritually. It is humbling to look back and imagine where I would be without the support of professors and peers around me. However, I've learned to trust myself. In many ways research has taught me how to question dogma in the literature and dogma in my own life. I am grateful for my time at Kansas State University in the Department of Nutrition, Dietetics and Sensory Sciences in the College of Human Ecology. I have found no better people than here and will miss them dearly.