DOI:10.14753/SE.2020.2305

HEMODYNAMIC AND ENDOCRINE FACTORS IN THE BIOMECHANICAL REMODELING OF THE VASCULAR WALL

PhD thesis

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Budapest
2019

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I. Abbreviations

5-HT_2	5-hydroxytryptamine
α	Alpha
AA	Arachidonic acid
Ach	Acetylcholine
ADP	Adenosine diphosphate
AngII	Angiotensin II
ASK1	Apoptosis signal-regulating kinase 1
ATP	Adenosine triphosphate
AT1R	Angiotensin II receptor type 1
β	Beta
BK	Bradykinin
bw	Body weight
C	Capacitance
CV	Vascular Compliance
Ca^2	Calcium
CaCl	Calcium chloride
cAMP	Cyclic adenosine monophosphate
CD68	Cluster of Differentiation 68 protein
cGMP	Cyclic guanosine monophosphate
CD18	Integrin beta-2
COX	Cyclooxygenase
CSE	Cystathionine γ-lyase
c-SRC	Proto-oncogene tyrosine-protein kinase
D	Distensibility

d	Diameter
DAG	Diacylglycerol
E	Elastic modulus
E	Estrogen
ECM	Extracellular matrix
EDRF	Endothelium derived relaxing factor
EDHF	Endothelium derived hyperpolarizing factor
EDTA	Ethylenediaminetetraacetic acid
g	Gram
h	Wall thickness
H	Histamine
I	Current
i.p.	Intra peritoneal
IP_3	1,4,5-trisphosphate
IM	Intramuscular
JNK	Jun N-terminal kinases
k	Proportionality constant
K^+	Potassium
KCl	Potassium chloride
kg	Kilogram
kPa	Kilopascal
l	Length
m	Meter
M	Muscarinic
$MgSO_4$	Magnesium sulfate
MBP	Mean Blood Pressure

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MAP kinase	Mitogen-activated protein kinase
min	Minute
mm	Millimeter
mmHg	Millimeters of mercury
μm	Micrometers
MMP	Matrix metalloproteinase
MPa	Megapascal
ng	nanogram
Na^+	Sodium
NA	Norepinephrine
NaCl	Sodium chloride
NAD(P)H	Nicotinamide adenine dinucleotide phosphate
$NaHCO_3$	Sodim bicarbonate
NaH_2PO_4	Sodium phosphate
nKR	Normal Krebs-Ringer solution
NO	Nitric oxide
NOS	Nitric oxide synthase
O_2	Oxygen
OV	Ovariectomy
OVX	Ovariectomy
P	Pressure
PG	Prostaglandin
PGH_2	Prostaglandin H2
PGI_2	Prostaglandin I2
Q	Flow
r	Radius

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R	Resistance
RF	Resorchin-fuchsin
SD	Standard deviation
SHR	Spontaneously hypertensive rat
SEM	Standard error of mean
SMA	Smooth muscle actin
SVR	Systemic vascular resistance
TRX	Thioredoxin
TxA2	Thromboxane A2
TXNIP	Thioredoxin-Binding Protein
U	Voltage
U46619	9,11-Dideoxy-9alpha,11alpha-methanoepoxy prostaglandin
V	Volume
VCAM1	Vascular cell adhesion protein 1

DOI:10.14753/SE.2020.2305

II. Introduction

The vascular system plays a diverse role in the human body. It acts as a conduit, transferring material, energy and information (functions of "conduction"). It is capable of mechanical adaptation, plays a role in the formation, conduction and dissipation of hemodynamic waves. It has receptor functions neurotransmitters are released in them and are important components of synthesis of different tissues. It plays a role in the biochemical conversion of substances in blood plasma in hemostasis and transmural transport of different substances.

Hypertension is a well-recognized risk factor for cardiovascular and renal diseases and may lead to increased risk of stroke (Bergan (Bergan, Pascarella, & Schmid-Schönbein, 2008). A study of men and women aged 45 to 83 years showed the age-standardized prevalence of hypertension at baseline was 74.3% for men and 70.2% for women (Lacruz ., 2015) . Small resistance arteries play a key role in the control of blood pressure. These segments are responsible for the most significant decrease of hydrostatic pressure along the circulatory system. The rise in blood pressure affects the structure and morphology of the vessel wall (Hayashi & Naiki, 2009). Angiotensin II is greatly implicated in vascular remodeling of small-resistance arteries (Intengan & Schiffrin, 2000; Neves et al., 2004; Neves, Virdis, & Schiffrin, 2003). Sex differences in hypertension have been described, and are at least in part thought to be due to the effects of estradiol and testosterone (Barton, Prossnitz, & Meyer, 2012), but little is known about the mechanisms through which sex hormones affect the remodeling of coronary resistance arteries.

A cross-sectional study of 2,211 men and women revealed that varicose veins affect 28% of the adult female population compared with in 15% of the adult males (Raffetto et al., 2010).The primary cause of the development of lower limb varicosity has not been established, but pressure loading, vein valve dysfunction, diabetes, obesity, smoking and age, pregnancy, standing for long periods of time have been implicated as major contributing factors to this to the progression of this disease. (Raffetto & Khalil, 2008; Raffetto et al., 2010). The pathomechanisms underlying the development of varicose vein is still inconclusive, and little is known about the effects of flow disturbances on venous pathology.

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Histology and physiology of the vessel wall

Histology of blood vessels (Figure 1.).

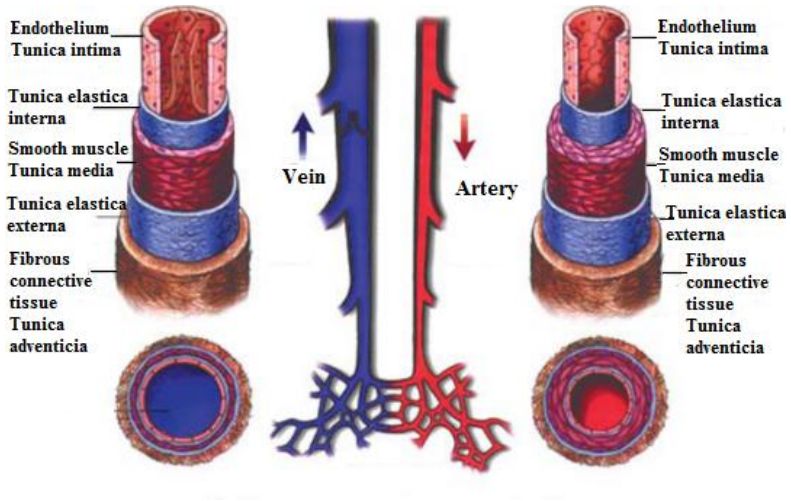

Figure 1.

Histology of veins and arteries.

(Figure from - (Torres-Vázquez, Kamei, & Weinstein, 2003)) Veins and arteries and veins are both composed of similar tissue layers, an inner endothelium (tunica intima) surrounded by internal elastic tissue, a layer of smooth muscle cells (tunica media), external elastic tissue, and fibrous connective tissue (tunica adventitia).

Arteries and veins have are composed of the same three layers, the tunica intima, the tunica media and the tunica adventitia. The tunica intima lines the lumen of the blood vessels. It consists of simple squamous epithelium and a thin basal membrane. The simple squamous epithelium provides a smooth surface for unperturbed blood flow. The tunica intima is collectively referred to as the endothelium. The tunica media is made of smooth muscle and elastic fibers. It is responsible for vasodialation and vasoconstriction of the blood vessels. The tunica adventitia is the most superficial layer. It is made of collagen fibers running in all directions for strength in many different directions. In arterioles the tunica intima consists of a single layer of squamous epithelium, the tunica media a few or single layer of smooth muscle cells and the tunica adventitia is quite this, and may megre with surrounding tissue.

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Arteries and veins are exposed to different pressures of blood flow. These differences are reflected in the structure of the vessels. Arteries experience a pressure wave as blood is pumped from the heart therefore the walls of arteries are much thicker than those of veins. In addition, the muscular tunica media is much thicker in arteries than in veins. As a result, arteries have a more uniform, circular shape. Veins are not exposed to pressure waves, therefore the vessel walls of veins are thinner than arteries and do not have as much tunica media. The tunica media is smaller in relation to the lumen than in arteries. Veins are more irregular in shape.(Hall, 2015; Torres-Vázquez et al., 2003)

Vascular endothelial cells in the resistant arteries are constantly exposed to the dynamic changes of blood flow (Fig. 2). The hemodynamic forces can be resolved into three components: (1) shear stress, the tangential frictional force acting at the endothelial cell surface, (2) hydrostatic pressure, the perpendicular force acting on the vascular wall, and (3) cyclic strain, the circumferential stretch of vessel wall (Hsiai & Wu, 2008).

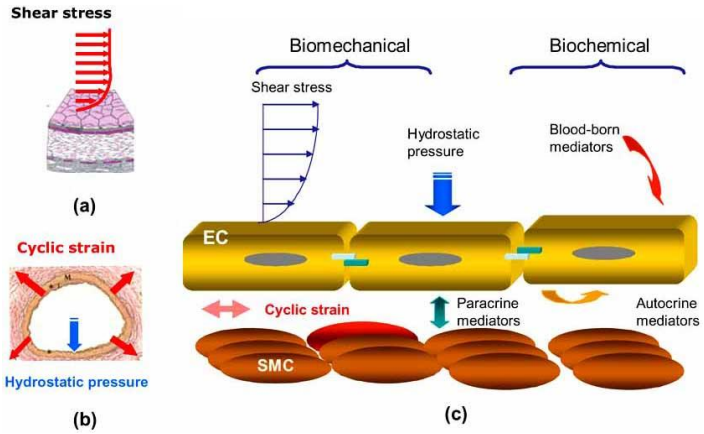


Figure 2.

Figure from (Hsiai & Wu, 2008)

Hemodynamic forces acting on blood vessels

Diagram of hemodynamic forces acting on endothelial cells and smooth muscle cells in the blood vessel wall. (a) Fluid shear stress which is the tangential frictional force by virtue of blood viscosity, acts on endothelial cells mainly. (b) Cyclic strain exerts a circumferential stretch on arterial wall in response to cardiac contraction. Hydrostatic pressure acts perpendicularly on endothelial cells. (c) Endothelial cells are constantly

exposed to both biomechanical and biochemical stimuli, which modulate endothelial functional phenotype. The biochemical stimuli include hormones, growth factors, cytokines that may be delivered via the blood or via autocrine or paracrine mechanisms (Hsiai & Wu, 2008).

II./1. Hemodynamic control of vessel wall biomechanics

It has long been established that the conduction and wave forming/conducting/dissipating functions are determined by the geometrical and elastic properties of vessels. These characteristics, however may adapt to a set of physiological and pathological factors (Monos, 1986).

Short term adaptation

While alterations in tissue composition are usually seen in long term adaptation, short term adaptation or regulation of the vessel wall is most commonly achieved through setting of the myogenic tone of the vessel. Changes in geometrical and elastic properties are key elements in this process (Monos, 1986). The diameter of the vessel is determined by transmural pressure as well as by the elastic and contractile elements of the wall. Vessel tone (which may be measured by comparing vessel diameters in an active and passive state) is set by several factors.

The intrinsic tone, which can be observed on isolated vessel segments is modulated by the endothelium and by metabolic, endorine, neural and hemodynamic factors. All these factors therefore play a role in the control of peripheral resistance (Hall, 2015; Mulvany & Aalkjaer, 1990).

Myogenic tone is considered to be a key factor in the local regulation of blood flow (Bayliss, 1902)(Figure 3.). Myogenic response is defined as active vessel contraction to an increase of intravascular pressure (Bayliss, 1902; Kuo, Chilian, & Davis, 1991; Kuo, Davis, & Chilian, 1991; Meininger & Davis, 1992; Rajagopalan, Dube, & Canty, 1995) Mulvany and Aaljaker (Mulvany & Aalkjaer, 1990) described that this myogenic response is regulated by stretch or pressure response, mostly in small arteries and arterioles (<500 μm and <100 μm, respectively). Elevation of cytoplasmic Ca^{2+} ion

DOI:10.14753/SE.2020.2305

levels, due mechanically sensitive Ca^{2+} membrane channels have been described to be involved in these mechanisms (Hill, Yang, Ella, Davis, & Braun, 2010).

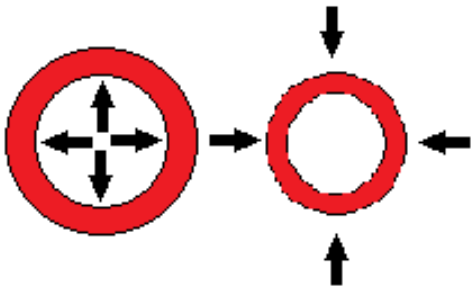


Figure 3.
Myogenic tone - active vessel contraction to an increase of intravascular pressure

Myogenic response may be studied in cannulated and pressurized vessel segment preparations in vitro. This way other variables can be controlled (neurohumoral, metabolic factors etc.) (Kuo, Davis, & Chilian, 1988; Meininger & Davis, 1992). Resistance artery control of local tissue flow, partially due to the myogenic tone is very effective according to the Hagen-Poiseuille law. In a thin, rigid tube, if flow is laminar the intensity of flow (Q) is directly proportional to the pressure difference between the points of the tube (P1-P2) and also proportional to the fourth power of the inner radius (r). In analogy with the Ohm's law used in electrodynamics, we can compute hydrodynamic resistance, and applying the Hagen-Poiseuille law, $R=(P1-P2)/Q=8xL\eta/\pi r^4$. Thus vascular resistance is directly proportional to the length of the vessel and the viscosity of blood, and is inversely proportional to the fourth power of the radius of the vessel. In consequence, a key factor in the acute regulation of blood flow is vessel diameter (Fonyó, 2011; Hall, 2015). As the intensity of flow changes in correspondence with the fourth power of the radius of the vessel wall, minor alterations in diameter lead to significant changes in flow. Every factor that influences the smooth muscle tone and reactivity of vessels may have short-term regulatory effects on hemodynamic adaptation (Monos, 1986). Flow autoregulation is implemented at the level of precapillary resistance arteries.

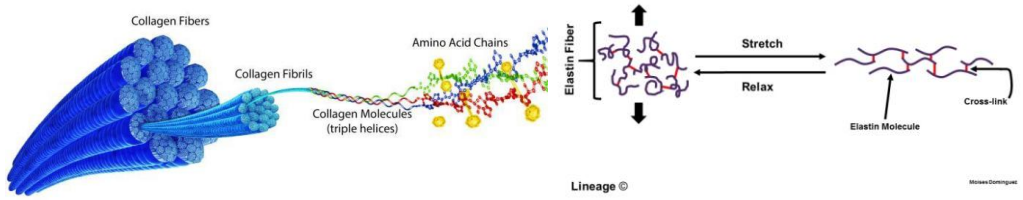
In addition to pressure and wall stretch, flow, which, in the arteries is pulsatile flow, also induces both long and short term reactions within the vessel wall. Release of vasoactive agents and immediate alterations in vessel diameter will be resulted (Cronenwett & Johnston, 2014). In case the endothelium is damaged there is a direct action of blood flow shear stress on the smooth muscel cells and fibroblast on the vessel wall. This may come from secretion, laminar, pulsatile, and oscillating flow shear stresses. These stresses may lead to alterations in alignment, contraction, proliferation, apoptosis, differentiation, and migration in the remaining cells of the vascular wall. Smooth muscle cells, and fibroblast both posses a high degree of plasticity that allows large scale yet reversible changes within the cell in response to alterations in local environmental factors. This is why smooth muscle cells, and fibroblasts may play a crucial role in vascular repair and remodeling (Shi & Tarbell, 2011). In a physiological state they are arranged in distinct patterns within the vessel wall. There is evidence that different types of mechanical stimuli regulate vascular cell morphology. Fluid shear may lead to the orientation of the endothelial cells parallel with flow (Chiu, Usami, & Chien, 2009). Laminar shear stress may induce perpendicular alignment of the smooth muscle cells. The alignment of smooth muscle cells semmed to dependend on and cytoskeleton-based mechanisms (Lee, Graham, Dela Cruz, Ratcliffe, & Karlon, 2002). Pulsatile strain and shear stress resulted in a circumferential pattern alignment of the smooth muscle cells. It has been found that non-uniform blood flow causes the smooth muscle cells to align perpendicular to luminal flow (parallel to transmural flow) and migrate toward the lumen, while uniform shear stress does not significantly affect smooth muscle cell orientation and migration. Circumferential alignment of the smooth muscle cells alloes the blood vessels to better resist tangential stresses induced by blood pressure (Shi & Tarbell, 2011).

Long-term adaptation

It has long been established that the alterations in tissue composition due to changes in hemodynamic forces may be part of physiological adaptation mechanisms, but also can be pathological processes (Monos, 1986). It is also known that the vascular wall is composed of elastic and of non-elastic components, and all these determine its biomechanical properties (Mulvany & Aalkjaer, 1990). Strain produced by stress

appears instantaneously in purely elastic body. The materials, where there is time gap before the strain appears are defined as viscoelastic (Milnor, 1972). The main building components of the vessel wall are elastin, collagen and smooth muscle cells. Wall composition will play a central role in determining the elastic response of the vessel in response to intraluminal pressure changes.

Elastin (Figure 4.) is a highly elastic biological material, a protein that allows many tissues to resume their shape after stretching or contracting. For elastin, an almost linear stress-strain relationship is characteristic. Collagens (Figure 4.) are astructural proteins found in a variety of tissue in abundance. The vital importance of collagen as a scaffold demands a manifold of essential characteristics, including thermal stability, mechanical strength, and the ability to engage in specific interactions with other biomolecules. The collagen molecule consists of helically wound chains of amino-acids. These helices are woven (Shoulders & Raines, 2009) into microfibrils, which weave together into subfibrils and fibrils. Due to this structural characteristic, this "waviness" the stress—strain relationship shows a very low stiffness at small stretch ratios. The stiffness increases fast once the fibers are deformed into straight lines.



a, b,

Figure 4.

Main contractile and force-bearing structural proteins of the vascular wall

(a) collagen, (b) elastin.

(https://www.tes.com/lessons/jezmMDoMqDP_kw/connective-tissue-collagen, https://step1.medbullets.com/biochemistry/102079/elastin,

Any transmural pressure within vessel exerts a force on the vessel wall that would tend to rip the wall apart were it not for the opposing forces supplied by the muscle and connective tissue of the vessel wall. This force is equal to the product of the transmural pressure and the vessel's inner radius, and it is defined as circumferential or

tangential wall tension. The relationship is defined by the Law of Laplace. The Law of Laplace applies directly only to cylinders with thin walls. Blood vessel walls are sufficiently thick, so in vascular mechanics the tangential wall stress is preferably used (Rhoades & Bell, 2012). Tangential wall stress of the vessel (σ) is determined by transmural pressure (P), vessel inner radius (r_i) and wall thickness (h). $\sigma=Pr_i/h$ (Laplace-Frank equation). Changes in wall thickness therefore lead to alterations in wall stress. Blood vessels that have thick walls relative to their radius are able to withstand higher pressure than vessels with small ratios because in the former wall stress is lower. Not pressure, but but wall stress is what must be overcome to contract a blood vessel (Rhoades & Bell, 2012).

Blood vessels are constantly exposed to mechanical forces in the form of cyclic strain and shear stress. The main source of cyclic strain is blood pressure as radial and tangential forces in the vessel wall work to counteract the intraluminal pressure. Hemodynamic forces have long been recognized as key modulators of protein synthesis, cell morphology migration, differentiation and proliferation. The Laplace-Frank equation describes the tension per unit of thickness of the wall, which represents the stress exerted on the wall in the circumferential direction. Circumferential stress affects all cell types in the vessel wall, whereas shear stress principally acts on the endothelium. Pulsatile flow also plays a role in the long term adaptation of the vessel wall. Chronic changes in mechanical forces lead to vascular remodeling and adaptive alterations in vessel shape and composition over time (Cronenwett & Johnston, 2014)

Vascular remodeling may occur as a result of changes in pressure, radius or blood flow. Smooth muscle cell hypertrophy and elevation in collagen and elastin production have been shown to accompany increased circumferential stress (Prado & Rossi, 2006). The opposite has been demonstrated as well, that a decrease in circumferential wall stress leads to wall atrophy (Cronenwett & Johnston, 2014). Studies have shown that when the diameter of a vessel increases, the number of lamellar units and the overall thickness of the vessel wall increase also in order to maintain circumferential wall stress. In elastic arteries the adaptive response physiologically works to normalize tensile stress.

In a tube with rigid, inflexible walls the volume of fluid is the same independent of the pressure difference between the inside and the outside of the tube, however blood

vessels are viscoelastic. Consequently, the volume contained within them is a function of both the pressure difference across their wall (transmural pressure - defined as the difference of pressure inside versus outside the vessel wall) and the degree of flexibility/elasticity of the vessel wall itself (Rhoades & Bell, 2012). Studies have revealed that average circumferential (tangential) wall stress increases with increased pressure and vessel size (Bérczi, Tóth, Kovách, & Monos, 1990; Monos, 1986; Monos & Kovách, 1980; Szekeres et al., 1998).

Young's modulus, also known as the elastic modulus, is a measure of the stiffness of a solid material. Constant elastic modulus (independent of strain) characterizes the linear elastic solid materials. This modulus defines the relationship between stress (force per unit area) and strain (proportional deformation) in a material (Bergel, 1961). The vessel wall is not a linear elastic solid material: the stress/strain ratio increases with increasing strain. Elastic modulus thus can be defined for a short range of the stress/strain relationship for given values of stress (in case of vessels, intraluminal pressure) only. Such moduli are called incremental elastic moduli and are plotted against stress or pressure (Monos 1986). Vascular stiffness is often expressed by the circumferential incremental elastic modulus, computed from the equation $E_{inc} = \Delta P / \Delta\, r_o \times 2r_i^2 \times r_o / (r_o^2 \times r_i^2)$, where E_{inc} is the incremental elastic modulus, r_i is the inner radius, r_o is the outer radius, and $\Delta\, r_o$ is the change in the outer radius in response to an intraluminal pressure change of P. When the parameters defined at specific pressure levels, different values may be expected at different pressure levels because pressure-diameter relationships of vessels are nonlinear (Hayashi & Naiki, 2009).

One problem with compliance is that different-sized vessels can not be compared. For example, a large stiff -walled vessel may have a higher compliance value than a tiny flexible vessel. For this reason, the percentage increase in volume for a given increase in pressure may be used as a means of comparing distensibility between vessels and segments of the vasculature of different sizes. This value is called vascular distensibility $D = \Delta V / V_o \Delta P$ (Rhoades & Bell, 2012).

The composition of the vessel wall determines its elasticity. From the elastic parameters used distensibility will depend both on vessel geometry and wall elasticity, while elastic modulus, especially it is expressed as a function of wall stress will be an inherent property of the wall material (Hayashi, 2003).

DOI:10.14753/SE.2020.2305

II./2. Humoral control of the biomechanical properties of the vessel wall

There are a myriad of agents that have an effect on the vascular system. These may be direct physical forces, chemical agents, hormones, paracrine substances, and receptor-mediated hormonal and neurotransmitter agonists. These agents may have an effect on the endothelium or on the vascular smooth muscle. The vasoconstriction/relaxation may be receptor mediated or non-receptor mediated.

Vasoconstrictors: Non–receptor-mediated

Calcium (Ca^{2+}): During vascular smooth muscle contraction actin and myosin filaments slide over one another. Compared with skeletal muscle, smooth muscle contracts very slowly, develops and maintains high forces for long periods of time. Also it uses a relatively low amount of adenosine triphosphate (ATP) in the process. This contraction is controlled by intracellular free calcium ion concentration. The calcium may enter the smooth muscle cells via voltage-gated calcium channels, receptor-dependent and mechanosensitive calcium channels, and by release from the sarcoplasmic reticulum. It is released from the endoplasmic reticulum by certain agents, IP_3 being the most important secon messenger with such an effect. Calcium binds to calmodulin, which in turn activates myosin light chain kinase to initiate crossbridge attachments (Fonyó, 2011; Hall, 2015).

Potassium (K^+): Potassium causes membrane depolarization, this in turn results in the activation of calcium channels.

Sodium (Na^+): Decreased transmembrane sodium gradient leads to decreased activation of plasma membrane sodium/calcium exchange and increased intracellular calcium levels. The Na^+/ K^+ pump is responsible for the base membrane potential in cells. The inward movement of sodium ions and the outward movement of potassium ions are passive and the reverse movements against the electrochemical gradients require the activity of an ATP-driven Na^+/ K^+ pump. Maintenance of a normal electrical environment in the cell requires that the Na^+/ K^+ ATP-ase. The Na^+/ K^+ pump is regulated by several factors including the intracellular sodium concentration and the neuromediators norepinephrine and acetylcholine. The Na^+/ K^+ ATP-ase alters the membrane potential and influences vessel function in an essential way.(Hall, 2015; Vassalle, 1987)

Increased transmural pressure: Increased transmural pressure leads to the activation of stretch-dependent calcium channels. Alteration of vessel wall tension may lead to a myogenic response that does not require the presence of the endothelium. It is accompanied by a membrane depolarization and an increase of the intracellular Ca2+ concentration, which depends largely on an influx of extracellular calcium via voltage-operated calcium channels.(Schubert & Mulvany, 1999)

Vasoconstrictors: Receptor-mediated

Norepinephrine (NA): Norepinephrine binds to either alpha or beta adrenergic receptors. Alpha receptors are divided into subtypes α_1 and α_2; beta receptors into subtypes β_1, β_2, and β_3. All of these function as G protein-coupled receptors, meaning that they exert their effects via a complex second messenger system.

Norepinephrine is a powerful vasoconstrictor. When the sympathetic nervous system is activated (through stress or exercise) the sympathetic nerve endings release norepinephrine into the tissues. This surge of norepinephrine has a stimulative effect on the heart, contracts the veins and arterioles. Through a dual control system norepinephrine may have an effect on tissues not only through direct simulation of the nerves, but through the indirect effects of circulating norepinephrine. (Hall, 2015) Norepinephrine binds to α-adrenoceptors on the cell membrane, which is linked to a membrane-bound regulatory protein, heterotrimeric G-protein (guanine nucleotide binding protein) with q/11 type alfa subunits. This activates phospholipase C, leading to hydrolysis of membrane-bound phosphatidyl inositol bisphosphate to yield inositol triphosphate and diacylglycerol. Inositol bisphosphate then causes the release of calcium from the sarcoplasmic reticulum, which ultimately leads to smooth muscle contraction reduction in vessel radius and thus increase in vascular resistance. Norepinephrine plays an important role in the regulation of myocardial perfusion, as has an effect on the vascular tone of coronaries (Quillen, Sellke, Banitt, & Harrison, 1992). There are both α and β receptors present on coronaries. α receptors cause vasoconstriction and β receptors vasodilation. The α1 receptors and α_2 receptors found on coronary vascular smooth muscle cells cause vasoconstriction through smooth muscle contraction. There are α_2 receptors on endothelial cells, these cause vasodilation through the release of nitric-oxide (NO) (Bassenge & Heusch, 1990; Furchgott &

Vanhoutte, 1989). There are β_2 receptors on coronary resistance arteries, here norepinephrine causes vasodilation through increasing cyclic adenosine monophosphate (cAMP) levels in vascular smooth muscle cells (Bassenge & Heusch, 1990; Hall, 2015)

Acetylcholine (Ach): Acetylcholine is a parasympathetic neurotransmitter. It activates a range of muscarinic M receptors. In endothelial cells it may activate M1, M2, or M3 receptors, which opens plasma membrane calcium channels, activates phospholipase C and increases the production of IP3 and Diacylglycerol (DAG). Most of the effect of acetylcholine in vessels is endothelium dependent. If the endothelium is damaged, acetylcholine induced vasodilation (see next section) turns into vasoconstriction in isolated aorta preparations.(Nasa, Kume, & Takeo, 1997; Tangsucharit et al., 2016)

Serotonin may cause vasoconstriction through the activation of 5-hydroxytryptamine (5-HT_2) receptors which open receptor-operated plasma membrane calcium channels. The constrictor action of the serotonine may be due to a direct activation of vascular smooth muscle, augmentation of the action of other endogenous vasoconstrictors such as catecholamines, angiotensin II and the release of norepinephrine from adrenergic nerves. (Ayme-Dietrich, Aubertin-Kirch, Maroteaux, & Monassier, 2017)

Vasopressin: Vasopressin is a powerful vasoconstrictor in the body when applied in higher concentrations. It is formed in nerve cells in the hypothalamus, is transported to the posterior pituitary gland, and from here it is secreted into the blood. It is a key factor in fluid volume control under physiological conditions. Direct vascular control is a less important function of vasopressin in physiological concentrations.

Angiotensin II (AngII): Angiotensin II is one of the most powerful vasoconstrictor substance known. It causes significant constriction of the small arteries and arterioles. It thereby plays an important role in determining total peripheral resistance, and increases arterial pressure. It activates angiotensin II receptors, and induces G protein-dependent signaling pathways (IP_3 /DAG pathways). IP3 binds to its receptor on sarcoplasmic reticulum, opening a channel that allows calcium influx into the cytoplasm. Ca^{2+} then binds to calmodulin and activates the myosin light chain kinase, which in turn phosphorylates the myosin light chain and enhances the

interaction between actin and myosin, causing smooth muscle cell contraction (Mehta & Griendling, 2007).

Endothelin: Endothelin is yet another potent vasoconstrictor. It may be found in the endothelium of the vasculature. The most typical stimulus for release is damage to the endothelium. Subtypes of endothelin receptors have been identifies, certain subtypes mediate vasoconstriction while other receptors mediate both vasoconstriction and vasodilatation. ET_A is a subtype for vasoconstriction found in the smooth muscle cells. It activates receptors, which stimulate of phospholipase C, formation of IP_3, and release of calcium from the sarcoplasmic reticulum.(La & Reid, 1995)

Thromboxane A2 (TxA2) is an extremely potent vasoconstrictor (Yamada et al., 2003) derived from the metabolism of arachidonic acid. (Scornik & Toro, 1992) Thromboxane (TxA2) is a vasoactive agent. It is produced in response to a myriad of stimuli, from the cell membrane. There are different types of receptors that mediate the effects of thromboxane along with prostaglandin on coronary vascular smooth muscle. The high-affinity (kD: > or = 1 nM) Prostaglandin H2 PGH2/TxA2 receptors mediate the contractile actions of TxA2. Through different G-proteins these receptors activate intracellular signal transduction pathways which control intracellular calcium level Adenosine triphosphate (ATP)-dependent K-channels are regulated by TxA2 and may be involved in the determination of coronary smooth muscle tone. (Schrör, 1993). TxA2 (Yamada et al., 2003) also has an effect on vascular smooth muscle, as it stimulates mitogenic growth.

Vasodilators: Direct

Nitric oxide (NO): Agents that control tissue blood flow locally typically only dilate only the small resistance arteries as they can only reach these, and not the larger caliber vessels upstream. Even so, when blood flow increases in the microvascular area, it leads to secondary mechanisms that have a vasodilatative effect on larger arteries. The endothelium in the small resistance arteries synthesizes substances that induce vasodilation. The key vasodilator substance is endothelium derived relaxing factor (EDRF). This mainly consists of nitric oxide. This has a very short half-life in the blood stream - only 6 seconds (Cocks, Angus, Campbell, & Campbell, 1985). Rapid flow of blood through the vessels causes shear stress on the endothelium, which through the

mechanical stretch leads to a significant increase in the release of nitric oxide, which leads to vasodilation of the vessels. This is fortunate because this mechanism causes vasodilation in the upstream arterial blood vessels whenever microvascular blood flow increases downstream. Without this mechanism the effectiveness of local blood flow control would be significantly decreased as a significant part of the resistance to blood flow is in the upstream small arteries. NO stimulates guanylate cyclase of the vascular smooth muscle, formation of cGMP, calcium removal and hyperpolarization. It also inhibits voltage-gated calcium channels.(Johns, 1991)

Hyperkalemia: Vascular smooth muscle membrane is depolarized in highly hyperkalemic solution and a contraction will be the result. However, in in vivo conditions, local hyperkalemia will mostly result vasodilation.

Magnesium: An increase in magnesium ion concentration causes powerful vasodilation because magnesium ions inhibit smooth muscle contraction. This is thought to be a direct action of magnesium, independent of NO.(Teragawa, Kato, Yamagata, Matsuura, & Kajiyama, 2001)

Vasodilators: Receptor-mediated

Epinephrine: Epinephrine leads to vasodilation through β_2 receptor-mediated activation of adenyl cyclase resulting in the formation of cAMP (cyclic adenosine monophosphate).

Adenosine: Adenosine is produced within the myocardial cell. It plays a key role in the local metabolic regulation of coronary circulation. Adenosine acts on purinergic receptors. A_1 receptor subtypes are found in the myocardium, and A_2 receptor subtypes are found in the vasculature. Adenosine acts through receptor activation of ATP-dependent potassium channels. This leads to membrane hyperpolarization and closure of voltage-gated calcium channels. The vascular A_2 receptors found on vascular smooth muscle cells stimulate the formation of cAMP. This leads to vasodilation in the coronaries. It also leads to hyperpolarization resulting from opening of potassium channels. Adenosine is also an endothelium-independent vasodilator (Kuo, Davis, & Chilian, 1995)

Histamine (H): Histamine acts on the H_2 receptor as avasodilator. H_2 receptors are spread throuout the resistance arteries and may be found on vasuclar smooth muscle

cells. Vasodilator effect is mediated through cyclic Adenosine monophosphate (cAMP); H_1 receptors may be found on endothelial cells, and their stimulation leads to the formation of NO (Ebeigbe & Talabi, 2014).

Bradykinin (BK): The group of agents called kinins are potent vasodilators. They are small polypeptides that are split away by proteolytic enzymes from alpha2-globulins in the plasma or tissue fluids. One of the key enzymes is kallikrein. It is present in the bloodstream and the tissue fluids in its inactive form, and may be activated by a myriad of factors, such as inflammation, chemical or physical effects. When it is activated, kallikrein acts on alpha microglobulin to release a kinin called kallidin. Kallidin is then converted into bradykinin by tissue enzymes. Bradykinin has a short life, only a few minutes, as it is inactivated by carboxypeptidase or by the converting enzyme, which activates angiotensin. Kallikrein enzyme is deactivated by the the kallikrein inhibitor. Bradykinin causes vasodilation and increased capillary permeability (Hall, 2015). BK has been shown to play a role in the physiological regulation of coronary microcirculation. BK is produced in both the myocardium and in the vascular tissue. BK may act on different receptors. Receptors may be found either on the endothelium or on vascular smooth muscle cells. Bradykinin causes endothelium-dependent vasodilation on constitutive B2 receptors located on endothelial cells of the vascular wall (Okamura & Toda, 1989), and vasoconstriction on smooth muscle cells, which is mediated by B1 receptors. The effects of bradykinin are thereby usually a combination of an endothelium–dependent vasodilator and a direct smooth muscle- dependent vasoconstrictor effect. The endothelium-dependent action of bradykinin through the activation of nitric - oxide synthase (Kuo, Chilian, & Davis, 1991). There is also evidence, that bradykinin has an NO-idependent vasodilation effect which may be mediated by prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) (Okamura & Toda, 1989). There is a body of evidence to support that bradykinin is a potent vasodilator on coronary arteries of different species and humans both on isolated vessels and heart preparations, such as in situ (Dézsi, Szekeres, & Schiszler, 1995; Rajagopalan et al., 1995). Bradykinin induced vasodilation appears to be a key mechanism in coronary microcirculation (Kuo et al., 1991; Rajagopalan et al., 1995).

DOI:10.14753/SE.2020.2305

Comparison of metabolic control mechanisms in coronaries and veins

Metabolic control mechanisms in coronary blood flow.

Myocardial metabolic demand is matched by an increase in coronary blood flow under physiological conditions. This process appears to be largely regulated by local humoral agents (Muller, Davis, & Chilian, 1996)(Figure 5.).

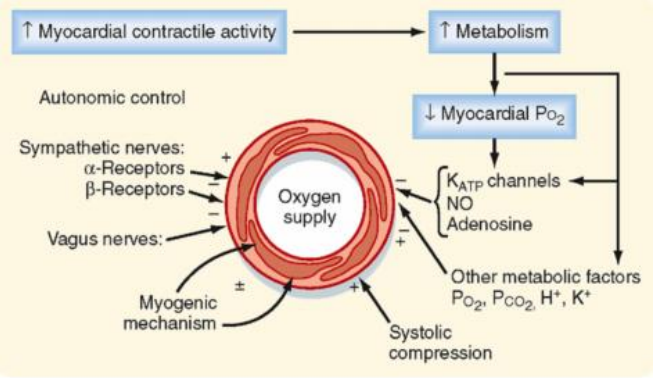


Figure 5.

Control mechanisms of coronary blood flow. Humoral agnets play a paramount role in the regulation of coronary blood flow.

(Figure- http://www.sci.utah.edu/~macleod/bioen/be6000/notes/L12-control-circ.pdf)

Adenosine – considerations in the coronary arteries. Adenosine is a substance that plays a cardinal role in the regulation of coronary blood flow adaptations during rapid changes in cardiac performance (Bassenge & Heusch, 1990). Adenosine is produced within the myocardial cell, when myocardial metabolism increases. It diffuses to the vessels, leading to vasodilation (Bassenge & Heusch, 1990). There is evidence to suggest, that small coronary arteries are the primary target for the effects of adenosine (Bassenge & Heusch, 1990; Kuo et al., 1995; Muller et al., 1996). When the myocardium becomes "underperfused" due to a rise in metabolic demand, hypoxia, hypercapnia, and acidosis also contribute to vasodilation. Metabolism becomes anaerobic, lactic acid is released and acidosis develops (Hall, 2015). Other agents, such as prostaglandins and bradykinin may also be released, and may contribute to the vasodilation. The activation of ATP sensitive potassium channels may also be an

important regulatory mechanism in the humoral control of microvascular resistance (Muller et al., 1996). Neural stimuli may have both direct and indirect effects on coronary microcirculation. Norepinephrine from the sympathetic nerves act directly through the release of neurotransmitters on the coronaries. Indirect effects are caused by decreased or increased activity of the heart (Hall, 2015). Therefore sympathetic neurotransmitters can elicit either vasoconstrictor or vasodilator effect, depending on the presence of receptors. In the coronaries we find both α and β receptors with the predominance of β receptors (Juhász-Nagy & Szentiványi, 1973). There are also α2 receptors in the endothelium, which are responsible for the release of NO and therefore have vasodilatative effects.

Endothelium dependent control mechanisms

The endothelium is the active participant of more than one regulatory mechanism (Fiugre 6.). One of the most important vasodilator agents is produced by and released from the endothelium. It is the endothelium derived relaxing factor (EDRF) which is composed principally of nitric oxide. Blood flow in the vessels causes shear stress, leading to contortion of the endothelial cells leading to an increased release of nitric oxide. (Bassenge & Heusch, 1990; Furchgott & Vanhoutte, 1989; Kuo et al., 1991; Kuo, Davis, et al., 1990)

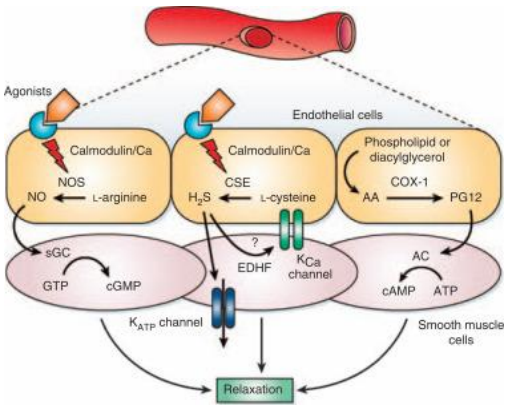


Figure 6.

Figure from (Wang, 2009)

Molecular basis for endothelium-dependent vasorelaxation

NO is produced from the endothelium by NO synthase (NOS), cystathionine γ-lyase (CSE), and cyclooxygenase-1 (COX-1), respectively. They are released from the endothelium and act on the adjacent smooth muscle cells to induce relaxation. NO and H2S are also produced in smooth muscle cells. AA - arachidonic acid; EDHF - endothelium-derived hyperpolarizing factor. Prostacyclin (PGI2) is produced from the endothelium through cyclooxygenase-1, and binds to specific receptors in smooth muscle cells and activates adenylate cyclase. Thus, increased cAMP levels in smooth muscel cells relax the cells ((Wang, 2009)).

Furchgott and Zawadki (Furchgott & Zawadzki, 1980) demonstrated that acetylcholine relaxation in isolated arteries is endothelium dependent. Therefore the agent was named endothelium derived relaxing factor (EDRF). Endothelium dependent relaxation of blood vessels is produced by several substances (i.e. acetylcholine, ADP, bradykinin, histamine etc.). NO stimulates guanylate cyclase of the vascular smooth muscle, with the resulting increase in cGMP activating relaxation. Other relaxing factors include prostaglandin I_2 and endothelium derived hyperpolarizing factor (EDHF) (Bassenge & Heusch, 1990; Furchgott & Vanhoutte, 1989; Shepherd & Katusić, 1991). Several agents (acetylcholine, bradykinin, histamine, ATP) acting on endothelial receptors may release endothelium derives relaxing factors causing vascular relaxation (Bassenge & Heusch, 1990; Furchgott & Vanhoutte, 1989) The direct action of these agents on smooth muscle cells may cause vasoconstriction. The resulting vascular effect depends on the equilibrium of vasodilator and vasoconstrictive effects. If the endothelium is damaged vasoconstriction may occur by the stimulation if otherwise vasodilator agonists (i.e. acetylcholine) (Bassenge & Heusch, 1990).

Humoral control of veins

While both arteries and veins are composed of similar cellular components, there are substantial differences. In terms of biological activity of the endothelium arteries and veins differ from each other (de Sousa et al., 2005) (Figure 7.).

DOI:10.14753/SE.2020.2305

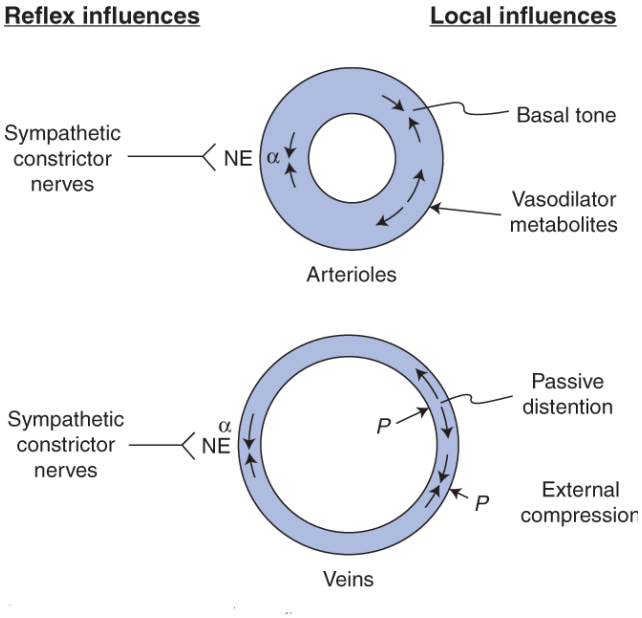


Figure 7.
Figure from David E. Morman Cardiovascular Physiology 8th Edition
As seen from the figure due to the structural and hemodynamical differences between arteries and veins, there are differences in regulations as well.

During experiments regarding arterial and venous biomechanics usually the endothelium-dependent vasodilator response is also tested (Haas et al., 2007). This response may be elicited by infusing substances that promote synthesis or release of NO from the endothelium. Bradykinin is one of these substances. Venous endothelial cells express constitutive B_2 kinin receptors. These receptors may be activated by bradykinin amongst other substances. These receptors activate phospholipase C, leading to an increased level of intracellular calcium, which in turn stimulates NO synthesis. Here, the effect of bradykinin enhancing endothelium-dependent vasodilatation through the release of NO (Dachman, Ford, Blaschke, & Hoffman, 1993).

Previous studies where acetylcholine was administered to elicit endothelium-dependent vasodilator response demonstrated that high-dose infusions of acetylcholine may cause vasoconstriction. There has been more than one study, focusing on the dose-dependent and endothelium-dependent dilator effect of acetylcholine in veins. They suggested that vasodilation mediated by acetylcholine in veins occurs only via NO, similarly to arteries (Vallance, Collier, & Moncada, 1989).

Several studies have demonstrated that both acetylcholine and bradykinin can be used for evaluation of endothelium-dependent venodilation (Rabelo et al., 2008).

Alpha 1 adrenoreceptors that have been described to be present in several venous segments, such as vena cava, saphenous vein, pulmonary vein etc. promote vasoconstriction (Muramatsu, Ohmura, & Kigoshi, 1995).

II./3. Biomechanical remodeling of resistance arteries in hypertension, sex differences, effects of sex hormones

The effect of hypertension on the geometric and biomechanical parameters of resistance arteries.

Hypertension is a well established risk factor for several cardiovascular diseases. Clinically hypertension is defined as systolic blood pressure higher than 140 mmHg and/or diastolic pressure higher than 90 mmHg for systemic arteries (Bergan et al., 2008).

The rise in blood pressure affects the structure and morphology of the vessel wall. The mechanical properties and therefore the contractility of the vessels may also be altered (Hayashi & Naiki, 2009)

Small resistance arteries play a key role in the control of blood pressure. These segments are responsible for the most significant decrease of hydrostatic pressure along the circulatory system. Peripheral resistance small arteries are defined as having a lumen diameter of <350 μm and arterioles as having lumen diameter of <100 μm. These two types of vessels are major contributors to total peripheral resistance. According to Poiseuille's law resistance is inversely proportional to the forth power of the radius, consequently small changes in lumen - either functional or structural - lead to significant alteration in arterial resistance along these vessel segments (Laurent & Boutouyrie, 2015; Mulvany & Aalkjaer, 1990; Michael John Mulvany et al., 1996; Rizzoni & Agabiti-Rosei, 2012).

Living tissue may adapt to mechanical demands by altering structure, geometry and biomechanical properties. Mechanical stress is a key factor. In blood vessels the most relevant stresses are: tangential stress induced by blood pressure (circumferential

direction), wall shear stress caused by blood flow, and axial stress elicited by axial elongation (Hayashi & Naiki, 2009). These forces will induce the long-range adaptive processes that affect the geometry (lumen size and wall thickness), tissue composition and elasticity of the resistance vessels. In addition, we can not neglect the nonhemodynamical neural and humoral effects, which are also altered in case of hypertension (epinephrine, norepinephrine, angiotensin II, etc.)

Small Artery Remodeling in Essential Hypertension

According to several studies some of the most essential findings in resistance arteries in case of essential hypertension are vasoconstriction, eutrophic remodeling with increased medial thickness: lumen ratio, altered distensibility and a decrease in the vasodilation reserve (Laurent & Boutouyrie, 2015; Mulvany & Aalkjaer, 1990; Mulvany et al., 1996; Rizzoni & Agabiti-Rosei, 2012)

Increased systemic vascular resistance (SVR), and small artery remodeling are typical findings in established essential hypertension (Eftekhari et al., 2012). It has been described that small artery structure is predictive of cardiovascular events (Mathiassen et al., 2007; Rizzoni et al., 2003). In hypertension, a typical biomechanical manifestation of arterial wall adaptation is hypertrophy. This adaptation has several advantages. First of all, it restores circumferential wall stress (tangential stress) at in vivo operating pressure to a normal value. It also optimizes arterial stiffness (Fridez et al., 2001; Hayashi & Naiki, 2009). Through these mechanisms in hypertension arterial elasticity is gradually optimized at in vivo working pressures. In hypertension, an increase in vascular tone is seen (Fridez et al., 2001; Mulvany & Aalkjaer, 1990). Eutrophic inward remodeling may be typical in small arteries (Mulvany, 2008; Mulvany & Aalkjaer, 1990, Intengan et al. 1999; Rizzoni & Agabiti-Rosei, 2012). When inward eutrophic remodeling occurs, a thickening of the media, a reduction in lumen and outer diameter, and increased medial thickness: lumen ratio are seen. This occurs without significant alteration to the overall amount of wall tissue or media cross-sectional area (Mulvany et al., 1996; Rizzoni & Agabiti-Rosei, 2012)(Figure 8.).

DOI:10.14753/SE.2020.2305

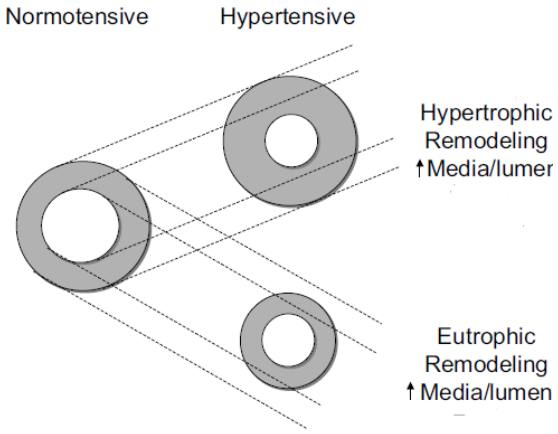


Figure 8.

Figure from (Schiffrin, 2004)

The cross-sectional area of the media, will remain normal in eutrophic remodeling. However in hypertrophic remodeling. the cross-sectional area of the media will be increased. The lumen diameter is decreased in remodeled small arteries, and the exterior diameter may be reduced in eutrophic remodeling.

In eutrophic remodeling, the outer diameter and lumen are both reduced, however the cross-section area of the media is maintained. Although the media cross-section does not undergo hpyertrophy, the smooth muscle cells themselves rearrange around a smaller lumen. This in turn leads to an increase in media width. Because the lumen diameter is reduced, the media–lumen ratio is increased, which should not be confused with hypertrophy (Schiffrin, 2004).

According to the Laplace-Frank equation (circumferential wall stress, $\sigma=MBP\times R/h$, where MBP is mean BP, R is the radius, and h is the wall thickness), if the same amount of wall material is organized around a smaller lumen without net cell growth, it is an effective way to normalize circumferential wall stress.

In contrast to some types of secondary hypertension, where hypertrophic remodeling may be seen, we see inward eutrophic remodeling in the early stages of essential hypertension (Rizzoni et al., 2000). As essential hypertension reaches later stages, it has been theorized that eutrophic remodeling may turn into hypertrophic (Mulvany et al., 1996) In essential hypertension, inward eutrophic remodeling may

present a protective mechanism against the rise in blood pressure, thereby preventing the rise in circumferential wall stress at the level of arterioles and capillaries which are ill equipped to withstand such loads. Another typical finding in essential hypertension is a decrease in the vasodilation reserve (the ability to increase blood flow with maximal vasodilatation) (Laurent & Boutouyrie, 2015).

Vascular smooth muscle cells are activated by hypertension (Hayashi & Naiki, 2009). Smooth muscle cells play a role in vascular remodeling in hypertension. Along with collagen and elastin, smooth muscle cells are cardinal structural elements in the vascular wall, and may therefore be essential factors in hypertensive remodeling. The changes in vascular tone and contractility seen in hypertension are linked to smooth muscle cell activity. Vascular smooth muscle cells are regarded as the sensory and effector elements in the adaptation processes, and it is thought that they are activated during the early stages of arterial adaptation to hypertension (Schiffrin, 2004).

Structural changes along the small arteries are accompanied with changes in myogenic tone. At higher pressure loads, increased myogenic tone reduces lumen diameter (Izzard, Rizzoni, Agabiti-Rosei, & Heagerty, 2005). This plays an important role in the autoregulation of blood flow, and the regulation of capillary pressure.

Resistance arteries have the greatest capacity for myogenic response. This response to increased circumferential wall stress is inversely related to the diameter of the vessel (Allen et al., 1997). This way circumferential wall stress is optimized at normal pressure loads (Prewitt et al., 2002). Even though increased wall stress is a stimulus for growth, a myogenic response can prevent this process despite an increased blood pressure. By preventing growth, eutrophic remodeling is maintained. Therefore in case of impaired myogenic tone, as seen in certain types of secondary hypertension, the rise in wall stress would not be counteracted, and thus hypertrophy develops (Schofield, Malik, Izzard, Austin, & Heagerty, 2002). Therefore, the maintained myogenic properties of the vessels play an essential role in determining further structural changes in hypertension. Beside altered myogenic tone, vasoconstriction related to chronic neurohumoral stimuli (contributing to induction of hypertension) may also play a role in the adaptation mechanisms seen in hypertension.

As noninvasive ultrasonographic measurement techniques advanced, it became possible to measure wall thickness, diameter in human patients accurately even in

smaller vessels (Hayashi & Naiki, 2009), which is a prerequisite for calculating biomechanical properties. Significantly larger intimal medial thickness was found in hypertensive patients compared to the normotensive group, the internal diameters were less affected. Hypertensive patients had an increased ratio of wall thickness to internal diameter, increased elastic modulus and lower vascular compliance compared to normotensives. There is a decrease in the elastin to collagen ratio which seems to be connected with changes of elastic modulus (Berry & Greenwald, 1976). According to these studies the restoration of normal wall stress by hypertrophy in hypertension in animals may also be observed in human hypertensive patients.

There is also a strain hypothesis regarding hypertensive remodeling (Takamizawa & Hayashi, 1987), according to altered strain is more important than altered stress in inducing the remodeling process (Guo & Kassab, 2004). Remodeling of the ECM adjusting it to elevated myogenic tone of the smooth muscle should explain the morphological hypertensive remodeling according to that theory.

Coronary microvascular dysfunction characterized by abnormal structure and function of the coronaries has been linked to systemic hypertension (Hayashi & Naiki, 2009). Although several studies have investigated these fields, the pathogenic mechanisms underlying coronary microvascular remodeling in hypertension are incompletely understood (Mancini et al., 2013).

The role of inflammation in hypertension

Hypertension is associated with inflammation; however, whether inflammation is a cause or effect of hypertension is not well understood. Human and animal studies have suggested that inflammation leads to the development of hypertension, and oxidative stress and endothelial dysfunction and other potential proinflammatory conditions such as activation of the sympathetic nervous system, aging, and elevated aldosterone may play a role (Figure 9.).

DOI:10.14753/SE.2020.2305

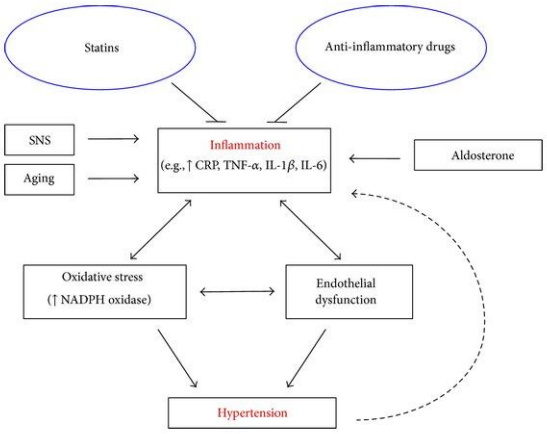


Figure 9.

Figure from (Dinh, Drummond, Sobey, & Chrissobolis, 2014).

This figure demonstrates the relationship between inflammation and hypertension and the contributing factors involved. According to this theorem anti-inflammatory drugs and statins may be effective antihypertensive due to their anti-inflammatory properties

CRP can stimulate monocytes to release proinflammatory cytokines such as interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and tumour necrosis factor alpha (TNF-α) and also endothelial cells to express intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1. This in effect will further promote inflammation.CRP is considered the inflammatory marker with the strongest association with hypertension. It has been demonstrated in numerous clinical trials that hypertensive patients commonly have increased plasma CRP levels.

Experimental studies have focused upon the pathophysiological role of individual T-cell subsets, dendritic cells, TNF-α, IL-17, MCP-1, and IL-6, an chemokins in hypertension. They have found that innate and adaptive immunity both play a significant role in the pathogenesis of hypertension. Specific immune cell types, cytokines, toll like receptors, and components of inflammasomes may all pose novel targets for antihypertensive therapy (De Miguel, Rudemiller, Abais, & Mattson, 2015) (Table 1.).

DOI:10.14753/SE.2020.2305

Table 1.

Table from (De Miguel et al., 2015).

Table showing the components of the immune system that may play a role in the development of hypertension. Possible mechanisms, and potential targets for therapy.

Type of Mediator	Description	Potential Application as a Target in Hypertension
Regulatory T-cells (Tregs)	T cell subtype characterized by the ability to suppress inflammatory signaling; proposed to be protective, where deficiency of Tregs leads to autoimmune disease	- Increasing the presence or functionality of Tregs may reduce oxidative stress, increase NO bioavailability, block immune cell accumulation, and protect against hypertension
Th17 cells	T cell subtype that produces IL-17; pro-inflammatory and exacerbate tissue damage and disease	- Blunting Th17 signaling may alleviate inflammation and target organ tissue damage associated with hypertension
Dendritic cells (DCs): - Classical DCs - Plasmacytoid DCs - Monocyte-derived inflammatory DCs - Langerhans cells	Bone marrow-derived antigen presenting cells (APCs) that play a key role in modulating the inflammatory response by distinguishing between self- and non-self antigens; induce the activation of T-lymphocytes	- Circulating precursor DCs as biomarkers to predict the development of cardiovascular disease - Development of isoketal scavengers to attenuate immunogenicity of DCs and hypertension
Immunosenescent $CD8^+$ cells	T cells characterized by shortened telomeres, loss of CD28, gain of CD57 expression, and increased production of inflammatory cytokines and chemokines	- Biomarker and potential therapeutic target in hypertensive patients
Chemokines and cytokines - TNF-α - IL-17 - MCP-1 - IL-6 - CD40L	Activated and recruited immune cells produce these inflammatory mediators, which determine the local inflammatory response	- Implicated in development and maintenance of hypertension - Blockade of these inflammatory pathways may decrease infiltration of immune cells, inflammation and blood pressure
Toll-like receptors (TLRs: TLR1-13)	- Family of receptors that trigger pro-inflammatory signals in response to microbial structures or DAMPs released by injured tissues. - Potential molecular link between innate and adaptive immune responses in cardiovascular disease	- Targeting TLRs may modulate the inflammatory cascade at an earlier point to help control disease more effectively

A single nucleotide polymorphism in the gene SH2B3 encoding the lymphocyte adaptor protein, LNK has been shown to play a role in the development of hypertension. Loss of LNK leads to profound inflammation in renal and vascular tissues as well as increased IFNγ leading to hypertension and end-organ damage (Dale & Madhur, 2016).

The role of Angiotensin II in vascular remodeling

Angiotensin II is greatly implicated in vascular remodeling of small-resistance arteries. A characteristic change seen in Angiotensin II induced hypertension is

increased medial thickness to lumen ratio. This may result from a reduced outer diameter. In this case the lumen is narrowed without net growth (eutrophic remodeling). The media to lumen ratio may also increase, or a thicker media may be formed (hypertrophic remodeling). Increased arterial stiffness is a hallmark of structural alteration typical of angiotensin II induced hypertension. (Intengan & Schiffrin, 2000; Neves et al., 2004; Neves, Virdis, & Schiffrin, 2003)

Changes in the extracellular matrix (including collagen type I and III elastin and fibronectin) may also be seen. The overall stiffness of the arterial wall depends on the ratio between distensible components (elastin) and the less distensible components (collagen, fibronectin) (Intengan & Schiffrin, 2000). Elevations in collagen and fibronectin content and decreased elastin have been shown in the media of small arteries from AngII-infused animals. (Brassard, Amiri, & Schiffrin, 2005; Neves et al., 2004; Neves et al., 2003). It has been demonstrated in immunohistochemical and histochemical assays that angiotensin II has the ability to increase collagen type I and fibronectin content and decrease the amount of elastin in small resistance arteries (Brassard, Amiri, & Schiffrin, 2005; Neves et al., 2003)

This suggests, that in Angiotensin II induced hypertension smooth muscle cells are not the only elements of adaptation, but other cells, such as fibroblasts and/or myofibroblasts may play a role in adaptation. The adaptation of the extracellular matrix is fundamental in stiffening the arterial wall. Collagen plays a key role in this process, as it is found in abundance in the vascular wall. Fibrinectin may accumulate in the media of the small resistance arteries. The process of fibrosis may be influenced by increased activity of local growth factors such as transforming growth factor β, platelet-derived growth factor and other growth factors, such as insulin-like growth factor and basic fibroblast growth factor. (Intengan & Schiffrin, 2001)

Angiotensin II binds to receptors on the vascular smooth muscle cells. Angiotensin type 1 receptor (AT_1 receptor) is a G-protein coupled receptor, and has been described to mediate the cardiovascular effects of angiontensin II (Singh & Karnik, 2016). This receptor is predominantly expressed in cardiovascular cells, such as vascular smooth muscle cells, and activates various signalling molecules, including G-protein-derived second messengers, protein kinases and small G-proteins (Higuchi et al., 2007). AT1 receptor signalling is mediated through G-proteins, G-protein

independent β-arrestin, reactive oxygen species, non-receptor type tyrosine kinases, small G-proteins, transactivation of receptor tyrosine kinases. Furthermore, interacting scaffold, mechanical stress, heterodimerization; and signalling through phosphorylation, desensitization, and internalization may also be involved. It has been described that non-physiologocal activation of AT_1 receptor may lead to a number of pathologies including cardiovascular remodeling and hypertrophy, vascular inflammation and atherosclerosis, endothelial dysfunction, oxidative stress, extra cellular matrix deposition, angiogenesis (Singh & Karnik, 2016). The AT_1 receptor activates tyrosine kinases, epidermal growth factor receptor, platelet-derived growth factor receptor, and insulin-like growth factor-1 receptor, and nonreceptor tyrosine kinases, such as c-SRC. As a result, activation of NAD(P)H (nicotinamide adenine dinucleotide phosphate) oxidase may occur, resulting in intracellular generation of reactive oxygen species, which influences redox-sensitive signaling molecules, such as mitogen-activated protein kinases, transcription factors, and matrix metalloproteinases (Schiffrin & Touyz, 2004; Tabet et al., 2008).The attachment of the extracellular matrix to the smooth muscle may also be affected via the adhesion molecules that mediate the anchoring of vascular smooth muscle cells to ECM components (Intengan et al., 1999).

Sex differences in hypertension

Sex differences in hypertension have been described, and are at least in part thought to be due to the effects of estradiol and testosterone (Barton et al., 2012). One of the effects of testosterone is an increase in angiotensin levels (Yanes et al., 2009). It has been described, that vasoconstrictors and vasodilators have different effects in the the presence of male and female hormones. Chromosome-linked genetic differences have been assumed to be a factor contributing to sex differences in hypertension (Ely et al., 2010). Differences have also been reported in therapy and blood pressure control (Thoenes et al., 2010).

Sex differences in the early stages of hypertensive vascular adaptation in the heart and in the intramural small coronary arteries that are fundamentally responsible for the blood supply of the heart muscle have not been studied thoroughly. The literature is also scarce regarding sex differences in the biomechanical adaptation, and changes in the pharmacologic reactivity of the vascular wall of intramural coronaries in

angiotensin induced hypertension. Considering that sex differences in cardiovascular disease are well documented differences in biomechanical adaptation and alteration of the range of pharmacologic reactivity in angiotensin II (AngII) hypertension may also be present as well.

The role of sex hormones in hypertension

We may conclude that hypertension leads to adverse remodeling and vasomotor alterations in coronaries. It is well established that chronic hypertension leads to hypertrophic remodeling of the vessel wall, dysfunction of the endothelium, and changes in smooth muscle reactivity. The characteristics of remodeling are determined by several factors, including hypertensive stimuli, sex, and hormonal effects. Alterations in vasoconstriction and/or vasodilation are detectable in most cases (London & Safar, 1996; Mulvany, 2002).

Following the onset of menopause blood pressure levels increase and become similar to those in men suggesting an important role of sex hormones in the regulation of blood pressure. This evidence suggests that sex hormones influence the cardiovascular system; however, these influences are still poorly understood. Sex hormones are able to modulate blood pressure by acting on important systems as cardiovascular, renal, and neural. They can have complementary or antagonistic actions. For example, testosterone can raise blood pressure by stimulating the renin-angiotensin-aldosterone system, whereas estrogen has been associated with decreased blood pressure. The reduced cardiovascular risk observed in females has been attributed to the beneficial effects of estrogen on endothelial function. Estrogens physiologically stimulate the release of endothelium-derived vasodilator factors and inhibit the renin-angiotensin system (dos Santos, da Silva, Ribeiro, & Stefanon, 2014).

The role of sex hormones in the development of hypertension in older women is still controversial. Some studies in postmenopausal women have shown that transdermal hormone replacement therapy reduced mean blood pressure in normotensive women, yet, other studies found no effect on resting blood pressure. Studies have shown castration to prevent the increase in blood pressure in males and ovariectomy to exacerbate the increase in blood pressure in females. It has been suggested that the underlying mechanisms of the sex differences in the development of hypertension and

estradiol's suggested protective effects involve multiple end organs including the peripheral vasculature, renal function and brain regions important for central regulation of sympathetic outflow. Circumventricular organs are structures in the brain characterized by their extensive vasculature and highly permeable capillaries. The sensory organs include the area postrema and the subfornical organ. Estradiol inhibits firing of neurons in these circumventricular neurons and inhibits their activation by circulating peptides. At the level of the nucleus tractus solitarii, estradiol can directly modulate neuronal activity, baroreceptor afferent information integration and ultimately arterial baroreflex function. Estradiol also has effects on sympathetic pre-motor neuron drivers and is able to inhibit the activity of these neurons involved in driving sympathetic outflow. The inhibition of the neurons may be how estradiol protects from some forms of neurogenic hypertension. Estrogen may also act to modulate gene function and protein expression of specific proteins such as the AT1 receptor and NOS (Hay, Xue, & Johnson, 2014).

Experimental studies on sex differences in the developmental programming of hypertension indicate that development insults exert sex-specific programming of nephron number and blood pressure in males and females. The exact mechanism(s) involved remain unclear, but appear to be multi-factorial. Potential mechanisms by which a developmental insult differentially programs the long-term control of blood pressure may be due to the influence of the hormonal milieu on the renin angiotensin system, due to innate sex differences in production of reactive oxygen species or endothelin, or impacted by increased susceptibility that occurs with age and the development of age-dependent increases in adiposity leading to activation of the sympathetic renal nerves. The fetus also exhibits innate sex differences in expression of the intrarenal renin angiotensin system which may or may not reduce nephron number. (Ojeda, Intapad, & Alexander, 2014).

The role of androgens in cardiovascular risk is not clear. There are observations that suggest that androgen deficiency in man might contribute to the development of cardiovascular disease and hypertension. Epidemiological studies have found that there is a high prevalence of low testosterone levels in men with coronary heart disease.There is also evidence available to siggest that testosterone and other androgens have

protective effects on cardiovascular and may play important roles in the acute regulation of vascular function. Some studies have demonstrated that testosterone exerts beneficial effects on cardiovascular function by inducing rapid vasorelaxation of vascular smooth muscle(dos Santos et al., 2014).

Testosterone deficiency has been shown to increase cardiovascular risk, as well as testosterone having been shown to increase cardiovascular risk. Among the men with testosterone levels under 300ng/dL an increased risk of heart attack and strokes in men who received testosterone prescriptions compared to those who didn't (Miner, Barkin, & Rosenberg, 2014).

The specific effects of hypertension on intramural coronaries in women has been documented in one of our previous studies (Mátrai et al., 2010) however, little is known the intramural coronary arteries in the left ventricle are of the utmost importance concerning target organ damage in hypertensive and ischemic heart disease. It is technically demanding to dissect these coronary branches, which may contribute to the fact that there is more data available on peripheral branches and epicardial coronaries (Schiffrin, Park, Intengan, & Touyz, 2000). Intramural arteries are rarely investigated in vitro (Nádasy et al., 2001).

Compared with epicardial coronaries intramural vessels demonstrate unique flow conditions. This is hypothesized to be the effect of the characteristic vascular bed created by the muscle environment (Toyota et al., 2005).

The effects of ovariectomy and estrogen therapy on vascular remodeling have been reported in normotension (Mericli et al., 2004). As women have been shown to be less vulnerable to cardiovascular risk, it is fair to hypothesize that hormones such as estrogen may help counterbalance some of these effects. There is minimal data available on the effects of ovariectomy and estrogen therapy in menopausal hypertension induced by angiotensin II (AngII).

DOI:10.14753/SE.2020.2305

II./4. The effects of venous hypertension on the geometrical and biomechanical characteristics of the venous wall.

The endothelial cells are subjected to shear stress of the flow, which is also much less in veins than in arteries. As we have stated above, living tissue may adapt to mechanical demands by altering structure, geometry and biomechanical properties. Mechanical stress is a key factor. In blood vessels the most relevant stresses are: tangential stress induced by blood pressure (circumferential direction), wall shear stress caused by blood flow, and axial stress elicited by axial elongation. (Hayashi & Naiki, 2009) Gaining information regarding how cells recognize different levels and modes of stress and how they respond to these may be a significant step in understanding vascular adaptation mechanisms. Endothelial cells, for example used to be considered a uniform passive cell population serving as a physical barrier between blood and tissue, now they are recognized to be quite "heterogeneous". Endothelial cells may differ widely in morphology and function along the vasculature. Endothelial cells of the resistance arteries or arterioles are longer and narrower than those in small veins or venules. One of the major roles of arteriolar endothelial cells is the control of vascular tone, whereas post-capillary venular endothelial cells are the primary site of leukocyte trafficking. While arterial endothelial cells are long and narrow or ellipsoidal,as is appropriate of their alignment in the direction of blood flow, venous ones are short and wide, as blood flow rates in the venous circulation are significantly lower than in the arterial circulation. Shear stressen differ in the arterial and venous segments as well. Physiological venous and arterial shear stress levels are typically 1–5 dynes/cm2 and 10–40 dynes/cm2, respectively (dela Paz & D'Amore, 2009).

Depending on the type and magnitude of stress, endothelium responds to shear stress through various mechanisms. Exposure of vascular endothelium to shear forces in the the normal value range stimulates endothelial cells to release agents such as prostacyclin, NO, calcium, thrombomodulin etc. How the mechanical forces are detected and transformed into signals that stimulate intravascular processes remains is not yet described. Models of mechanotransduction included a localised model, where the mechano-receptor, is considered to be located in cellular membrane. Possible channels also located in membrane, such as those of potassium, sodium and calcium,

may respond to shear stress alteration. Another model is the decentralised model, where shear stress forces acting on a cell's surface are transmitted through a cytoskeleton, allowing the activation of several mechano-receptors within the cell. Integrines connected with cytoskeleton have been related to this ofrm of emchanoception (Papaioannou & Stefanadis, 2005).

As we have seen earlier, hypertrophy is a typical vascular adaptation to hypertension. This has several advantages, i.e. restoration of circumferential wall stress, optimizing arterial stiffness (Hayashi & Naiki, 2009). Similar alterations may also occur in the venous wall.

Venous hypertension appears in chronic venous diseases, i.e. valvular dysfunction, iliofemoral deep vein thrombi. In these cases venous hypertension is accompanied by varicosities, edemas, pigmentation etc. Chronic venous insufficiency is a combination of reflux and obstruction.

Neglén (Neglén & Raju 1995) observed that the femoral and popliteal veins, which are exposed to venous hypertension in the patient and have venous insufficiency due to reflux/obstruction, were less compliant, than those seen in healthy patients. Monos et al, (Monos, Contney, Cowley, & Stekiel, 1989; Monos et al. 2007) studied the biomechanical effects of chronic venous hypertension. The venous hypertension was induced by exposing rats to a head up tilt for 2 weeks, pressure-diameter relations and wall thickness were studied in vitro using videomicroscopy and angiometry. In the head up tilt group the outer diameter increased, and wall distensibility decreased compared to the non-tilt group. No difference was found in wall thickness between the groups. Augmented myogenic response, with increased mass of the venous smooth muscle cells in the venous wall due to increased passive diameter were found. Hayashi (2003) induced venous hypertension in the femoral vein in rabbits, by constriction of the external iliac vein. Biomechanical properties of the femoral vein were studied. In venous hypertension after one week wall thickness was increased thereby circumferential wall stress decreased to control level. Stress was kept at normal up to 4 weeks. In venous hypertension, vascular tone contractility increased, while elasticity and compliance remained normal. These results are reminiscent of those seen in arteries in response to hypertension, indicating that not only arteries but veins also attempt to

operate against hypertension. Monos et al. found (Monos & Csengödy 1980), that venous arterialization occurs in venous grafts as adaptation to elevated blood pressure.

After ligation of the left anterior descending coronary vein in swine, (Choy, Dang, Molloi, & Kassab, 2006) found that wall area and wall thickness to radius ratio increased significantly due to venous hypertension. Due to the fact that the vessels are embedded in the myocardium to a certain degree, the remodeling was circumferentially and axially nonuniform because it is tethered to the myocardium to different degrees. In this case, the remodeling served to restore circumferential wall stress to normal levels (Hayashi & Naiki, 2009). To retain wall stress at normal levels in chronic hypertension wall thickening, increase in vascular tone and contractility occur. These remodeling phenomena in veins are similar to those observed in arteries exposed to hypertension. (Hayashi & Naiki, 2009; Raffetto & Khalil, 2008).

It has been shown, that venous hypertension plays a role in the pathogenesis of varicose veins (Raffetto & Khalil 2008). Increased expression of matrix metalloproteinases has been demonstrated in varicose veins. Matrix metalloproteinase-2 reduces the contraction ability of the vein. The exact relation between venous pressure, MMP expression and venous dysfunction is unclear. It has been proposed, that chronic venous hypertension leads to an overexpression of matrix metalloproteinases and decreased contractility, which in turn promotes venous dilation. (Raffetto & Khalil, 2008) Even less is known on the effect of altered flow on venous remodeling. (Hayashi & Naiki, 2009)

II./5.Mechanisms leading to the development of varicose veins

Varicosity is a common pathology on the lower extremities. Clinically it is usually characterized by excessive dilation and tortuousity of the subcutaneous venous system (Aunapuu & Arend, 2005). The primary cause for development of varicosity has been thoroughly studied, but not yet established. Evidence from the literature suggests that vein valve dysfunction and hydrostatic venous pressure play a significant role in the development of this disease (Raffetto & Khalil, 2008). Valve-reflux has been demonstrated to precede venous dilatation (Schultz-Ehrenburg, Weindorf, Matthes, & Hirche, 1992), but it has also been shown, that venous dilation may precede venous

reflux, and it has been suggested that valvular dysfunction may be an epiphenomenon of venous dilation (Gandhi et al., 1993; Kirsch et al., 2000; Raffetto & Khalil, 2008).

There seems to be a consensus that one key factor in the development of subcutaneous venous varicosities is chronically elevated venous pressure in the lower extremities. Such arises due to gravitational effects determined by lifestyle and will be further exaggerated by valvular incompetence. Flow disturbances, inherited connective tissue weakness, obesity and local inflammation contribute to the pathomechanism. (Bergan et al., 2008; Fegan & Kline, 1972; Gemmati et al., 2009; Lee, Evans, Allan, Ruckley, & Fowkes, 2003).

There is data to suggest that alterations in the extracellular matrix proteins may lead to connective tissue changes prior to valvular insufficiency (Gandhi et al., 1993). Certain matrix metalloproteinases (MMP-1, -2, -3, -9, -12 and -13) have been demonstrated an increase in expression / activity in the venous wall and in the plasma of patients with varicose veins (Jacob et al., 2002; Woodside et al., 2003). The expression of MMP-1 and -9 has been demonstrated not only in the adventitial fibroblasts, but in the medial smooth muscle and in the endothelial cells also (Woodside et al., 2003). A significant increase in MMP-1, -2, and -9 has been found in varicosity patients, where thrombophlebitis developed. These alterations in the level of matrix metalloproteinases may lead to remodeling of the extracellular matrix, and thereby inducing alterations in the mechanical properties of the vein wall. These mechanisms may play a role in progression of the disease (Kowalewski et al., 2004). Increased expression or matrix metalloproteinase-2 may occur in response to mechanical stretch/pressure on the endothelium (Milkiewicz & Haas, 2005).

II./6. Development of varicose veins, biomechanical alteration in varicosity

Depending on the population studied, (differences include: age, race and gender, methods of measurement and disease definition) the prevalence of varicose veins may reach 56% in males, and 73% in females (Beebe-Dimmer, Pfeifer, Engle, & Schottenfeld, 2005; Robertson, Evans, & Fowkes, 2008). The definition of the disease in these studies ranges from self reporting by the study participants, or patient recall of their diagnoses to standardized physical examination. The Framingham Heart Study showed the incidence rate of varicose veins in women to be higher, than in males (2.6%

vs.1.9%) (Raffetto et al., 2010).The San Diego Population Study was a cross-sectional study of 2,211 men and women from different ethnical backgrounds, and it showed varicose veins in 28% of the adult female population compared with in 15% of the adult males (Raffetto et al., 2010)

The veins in the affected region become dilated and tortuous (Aunapuu & Arend, 2005). Signs of degeneration may be observed in the vein wall (Stauffer et al., 2010). Diabetes, obesity, smoking and age have been implicated as risk factors for the development of varicose veins. It has not been established what the primary cause is in the development of lower limb varicosity, but vein valve dysfunction an hydrostatic venous pressure have been implicated as major contributing factors to this to the progression of this disease (Pascarella & Schönbein, 2005; Raffetto & Khalil, 2008; Raffetto et al., 2010).

Gravitational stress, hemodynamic disturbance (Fegan & Kline, 1972) genetic factors, (Molnár et al., 2013), local inflammation, (Woodside et al., 2003) have been implemented in the development of varicose veins. The pathomechanisms underlying the development of varicose vein is still inconclusive.

Age and pregnancy have been shown to be definite risk factors for varicosity Genetic link and past history of deep vein thrombosis are supported by good evidence as risk factors for varicosities (Wright & Fitridge, 2013). However the evidence regarding other risk factors is not conclusive. The difficulty with study design regarding prolonged standing is exact measurement criteria, though Working in a standing position has been shown to be associated with subsequent hospitalization due to varicose veins for both men and women (Tüchsen, Krause, Hannerz, Burr, & Kristensen, 2000). Other implicated risk factors include prolonged standing, obesity, diet, smoking, etc. Obesity, which has been proposed to be a primary cause of lower limb varicosity has been shown to be an aggravating factor only by some studies (Lin, Zhang, Sun, Ren, & Liu, 2015; London & Nash, 2000). The development of varicose veins is commonly attributed to vessel wall degeneration (Rooke & Felty, 2014). Regulatory genes of mediators of the inflammatory reaction and collagen production have been shown to be up-regulated and down-regulated, respectively in patients suffering from chronic venous insufficiency (Markovic & Shortell, 2013). Venous hypoxia has been thought to be a causative agint in the development of varicose veins,

as hypoxia activated leucocytes and endothelium lead to the release of mediators regulating vein wall remodelling similar to those observed in varicosities (Lim, Gohel, Shepherd, Paleolog, & Davies, 2011).

More unusual secondary causes of varicosities may include traumatic arterio-venous fistul, iliac vein thrombosis, Klippel Trenaunay syndrome, and another suffered arterio-venous malformations (Bhatti et al., 2013). Some articles suggest that the development of varicose veins insufficiency is preceded by and associated with the remodelling of the venous wall. They emphasize that a rise in venous pressure is sufficient to promote varicose remodelling of veins by augmenting wall stress and activating venous endothelial and smooth muscle cells (Pfisterer, König, Hecker, & Korff, 2014). There is evidence to support that there is an association between higher estradiol levels and varicosity or increased venous distensibility(Ciardullo et al., 2000). Estradiol/free testosterone ratios were studied in men and result suggest that estradiol/free testosterone ratio is associated with varicose veins in male patients .

Others suggest that varicose veins result from failure of valves in the superficial veins leading to venous reflux and vein dilatation is superseded by the hypothesis that valve incompetence follows rather than precedes a change in the vein wall. This in turn would mean that tha vein wall has inherent structural weakness in varicose veins, which leads to dilatation and separation of valve cusps so that they become incompetent. The fact that dilatation of varicose veins is initially distal to the valve supports this theory (London & Nash, 2000). Intimal thickness, intimal fibrosis, and total thickness and intimal/total thickness ratio were highest in venous clinical severity score 0, 1 in chronic venous insufficiency, suggesting that the vessel wall undergoes changes before varicose veins become apparent (Dhanarak & Kanchanabat, 2016). It has been described in several articles that valve-reflux preceeds dilatation, (Schultz-Ehrenburg, Weindorf, Matthes, & Hirche, 1992), while in other publication the opposite sequence has been show. (Gandhi et al., 1993; Delia Kirsch et al., 2000; Kirsch, Wahl, Böttger, & Junginger, 2000; Raffetto & Khalil, 2008). Our opinion is that valve insuficciciency will induce dilation and valve incompetence in the retrograde direction while flow induced fast dilation can be an other reason of dilation with valve insufficiency. There are clinical studies in the literature that describe venous insufficiency occurring without axial reflux of the superficial, deep or perforator veins, (Labropoulos et al., 1999) There

is also evidence to support the fact that alterations in the extracellular matrix, i.e. in the extracellular matrix proteins may precede valve insufficiency (Gandhi et al., 1993) Studies have also confirmed alteration in matrix metalloproteinases in the venous wall of patients suffering from varicosities.

We may state that substantial biochemical alterations occur in the varicous vein wall. Susceptibility of the venous wall for stretching is strongly dependent on smooth muscle cells and connective tissue fibres content of the vessel wall.

Vascular smooth muscle cells are highly specialized cells, that contract and the produce the extracellular matrix components of the vessel wall. Smooth muscle cells in the vessel wass express a range of contractile proteins, ion channels and signaling molecules. Depending on circustances a variety of smooth muscle cell phenotypes may be found. Previous studies have distinguished the spindle-shaped contractile and epithelioid-shaped synthetic and proliferative phenotypes (Chen, Qin, Wang, & Zhang, 2015)(Figure 10.).

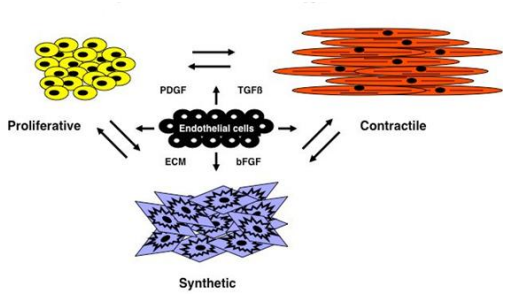


Figure 10.

(Figure from https://www.nationwidechildrens.org/%7Bffa9d737-80c9-4edf-a491-5f2222197032%7D)

Prolifetive and contractile subtypes of smooth muscle cells

Varicose veins have thickened vessel walls, considered to be the result of the dysregulation of the synthesis of extracellular matrix proteins.NELIN is an F-actin-associated protein found in veins. The protein mediates cell motility and is important in the process of cell migration and adhesion. SM22α is a calponin-related protein ans is a marker of a mature smooth muscle cell. In a study tarcking these two proteins in

varicose vein, it was concluded that varicose vein smooth muscle cells transformed from a contractile to a synthetic phenotype with varicosity (Chen et al., 2015).

There is also evidence from clinical studies showing, that venous insufficiency may occur without axial reflux (Labropoulos et al., 1999).

Incompetent venous valves lead to reflux, and this mechanism has been portrayed as a major contributor to many phenomena seen in chronic venous dysfunction (Bergan et al., 2006). Stretching, thinning and tearing of the valves have been described. Some studies found hypertrophy and alterations in collagen and the endothelium in chronic venous disease (Corcos et al., 2000). In this kind of patient, the number of valves in a given segment may be reduced, and degeneraed valve stumps may appear (Ono, Bergan, Schmid-Schönbein, & Takase, 1998). Depending on the population studied, (differences include: age, race and gender, methods of measurement and disease definition) the prevalence of varicose veins may reach 56% in males, and 73% in females (Beebe-Dimmer, Pfeifer, Engle, & Schottenfeld, 2005; Robertson, Evans, & Fowkes, 2008).

Experiments centered on inducing venous hypertension showed that reduced flow may trigger inflammatory processes such as adhesion of leukocytes to the endothelium and increase in free radicals. Under normal flow conditions shear stress induced by blood flow, acting through the endothelium may inhibit inflammatory processes, through several mechanisms (Garin & Berk, 2006). In an atherosclerosismodel physiologic fluid shear stress has been demonstrated to decrease TXNIP (Thioredoxin-Binding Protein) expression. This results in increased TRX (Thioredoxin) binding to ASK1 (Apoptosis signal-regulating kinase 1). This in turn inhibits cytokine activation of the JNK (Jun N-terminal kinase) pathway. JNK plays a critical role in death receptor-initiated extrinsic as well as mitochondrial intrinsic apoptotic pathways. In turn such proinflammatory events such as VCAM1 (Vascular cell adhesion protein 1) expression wil be prevented. This shows that normal flow prevents the activation of inflammatory pathways. Inhibition of mitogen-activated protein kinase inflammatory pathways reduction in the expression of adhesion molecules and activation of antioxidant mechanisms have also been described. (Chiu et al., 2003). Shear stress may also act not only through the endothelial cell, but also directly on the leukocytes leading to mechanisms like downregulation of adhesion

molecules such as CD18 as well as detachment of leukocytes from the endothelial wall. This explains how low-flow conditions and reduced shear stress may trigger processes of inflammation (Bergan et al. 2008).

However histological evidence of macrophage invasion, and new cell formation in association with a newly developed venous collateral system is hard to come by in the literature.

In the vein ligation model, where venous hypertension was created by ligating a large venous branch demonstrated that elevation of pressure in the vein lead to an increased number of leukocytes in and around the venous wall (Hahn et al., 2000).

Experimental elevation of venous pressure in the leg was achieved in our laboratory by keeping rats in tilted tube-cages for a chronic period, which doubled venous pressure and resulted in several massive cytophysiological alterations (Lóránt, et al., 2003; Monos, Bérczi, & Nádasy, 1995; Monos et al., 1989; Monos et al., 2007; Raffai et al., 2008) in the venous wall. However, applied alone, it failed to induce varicous morphological remodeling (Lóránt et al., 2003). Meanwhile, a wider use of Duplex scan and high resolution ultrasonographic devices in phlebological practice revealed a substantial flow disturbance in the majority of varicous networks. (Delis, 2005; Labropoulos, Tassiopoulos, Bhatti, & Leon, 2006)

From the above we may conclude that there is evidence to suggest that chronic flow alterations induce morphological remodeling processes in the affected vessels (Baeyens & Schwartz, 2016; Carmeliet, 2005; Dumont, Loufrani, & Henrion, 2007). Unfortunately, despite the emerging significance of flow disturbances in the development of pathological varicose morphology, there is a lack of experimental studies addressing the flow induced remodeling processes of the venous side of the circulation.

The role of inflammation in the development of venous network collaterals

Experiments focusing on the induction of venous hypertension by creating an arterio-venous fistula showed that this leads to an immediate increase in venous pressure and the dilation of the veins affected by the created fistula. The appearance of reversed flow did not occur immediately but through the course of several weeks. Progressive alterations in valve morphology were also observed including disappearance of venous valves in certain segments over time. The valves that were

subjected to increased pressure had an increased number of granulocytes, monocytes/macrophages, and T-lymphocytes, and elevated levels of inflammatory markers. Over the course of several weeks, alteration in the venous structure such as thickening of the wall and fibrosis were also observed (Bergan et al., 2008). As previously seen in the previous occlusion model, there is evidence in these venous hypertension models as well, that normal blood flow conditions (laminar flow, unidirectional) act as an anti-inflammatory trigger through the endothelial cells, If flow decreases, becomes turbulent, or changes its direction, it may trigger an inflammatory response (Li, Haga, & Chien, 2005). Increased pressure itself has been described to have a pro-inflammatory effect (Pauletto & Rattazzi, 2006). Triggered by increased pressure and decreased flow, the venous valve may show signs of histological changes. The inflammatory response may lead to structural weakness and damage of the venous valves (Bergan et al., 2008; Hahn et al., 2000).

Recent evidence suggests that processes of inflammation and angiogenesis are interconnected, especially in human pathologies. This may be one of the mechanisms that play a role in the development of venous network collaterals. While research typically focuses on understanding and exploiting the role of angiogenic factors and vascular cells on new blood vessel formation, the activity of the immune system is being increasingly recognized to impact vascular formation and adaptation (Kwee & Mooney, 2015). Newly formed blood vessels enable the continuous recruitment of inflammatory cells, which release a variety of proangiogenic cytokines, chemokines, and growth factors and further promote angiogenesis. Recently, this concept of reciprocity of angiogenesis and inflammation has been expanded to include oxidative stress as a novel mechanistic connection between inflammation-driven oxidation and neovascularization. Pathological angiogenesis appears to be closely associated with inflammation and inflammation-generated oxidative stress. A number of inflammatory cells, including neutrophils, eosinophils, mast cells, natural killer cells, macrophages, and dendritic cells, are involved in inducing and promoting angiogenesis (Kim, West, & Byzova, 2013).

As we see the extracellular martix of the vessel wass is significantly altered in varicose veins. The expression and the activity of matrix metalloproteinase 1, -2, -3, -9, -12 and -13 may be increased (Kowalewski et al., 2004; Woodside et al., 2003). The

increase in matrix metalloproteinase 1 and 9 has been shown to increase not only in the fibroblasts of the adventitia but in the smooth muscle cells of the media and in the endothelium also (Woodside et al., 2003). Evidence suggests that matrix metalloproteinase -1-2 and 9 are significantly elevated in the venous wall affected by varicosity and thrombophlebitis. It has also been suggested that this increase in matrix metalloproteinases leads to significant remodeling of the extracellular matrix of the wall, which in turn may alter the biomechanical characteristics of the venous wall (Kowalewski et al., 2004). Matrix metalloproteinases may therefore contribute to progression of venous disease.

Inflammatory processes have been described to play a role in the development or varicose disease. Acute and chronic inflammation is associated with changes in microvascular morphology and function. Under physiological conditions endothelial cells maintain a nonthrombogenic, nonreactive surface at the interface between blood and tissue. Should proinflammatory mediators be triggers, the endothelium may be a majot contributor in the generation of the inflammatory response (Pober & Sessa, 2014). In animal models venous hypertension was induced by venular occlusion/ligation and creation of an arteriovenous fistula. The data from these studies suggest that inflammatory process may be triggered by the alteration of flow and pressure in the venous system. Inflammatory processes may lead to valve insufficiency characteristic in venous disease (Bergan et al. 2008). Studies have revealed that proteinases released by inflammatory infiltration cells, may be responsible for the venous wall hypertrophy. Inflammatory infiltration cells lead to the activation of matrix metalloproteinase activation and dissolution of collagen in theextracellular matrix. Endothelial cells may also be activated, and may release transforming growth factor-β (TGF-β), which leads to the migration of smooth muscle cells proliferation and migration into the intimal layer. There is some evidence to suggest that altered hemodynamics and abnormal blood flow are are causes of valvular damage in varicosity (Kucukguven & Khalil, 2013).

DOI:10.14753/SE.2020.2305

II./7. Morphology, geometry and the development of network collaterals

With improved ultrasonographic devices in clinical use, more significance is attributed to blood flow alterations, preceeding or accompanying chronic venous disease of the lower extremities in addition to pressure changes. Animal experiments showed that occlusion of the auricular vein in mice led to alteration in vein morphology resembling the early stages of varicosity in humans (Pfisterer, König, Hecker, & Korff, 2014). The branching patterns, geometry and morphological alteration of the vascular system are quite complex. There is morphometric data in the literature regarding the vascular trees of several segments i.e., coronary, pulmonary, skeletal muscle vessels, mesentery, omentum, and conjunctiva. It would appear based on these studies, that these biological trees obey a set of scaling laws that are based on the hypothesis that the cost of construction of the tree structure and operation of fluid conduction should be minimized (Hahn et al., 2008). Blood, air, urine, bile etc. is conducted within the body structures by channels forming „biological trees". Energy expenditure is necessarily required for the conduction of any of the above through the biological tree structure. There are frictional losses along the route. When the branches of the biological tree have greater diameters, these frictional losses may be minimized.

There is of course an energy cost associated with the construction and maintenance of a large diameter branch (Aunapuu & Arend, 2005; Revellin, Rousset, Baud, & Bonjour, 2009) Muray described a compromise between frictional and metabolic cost expressed as cost function. Murray's law is the formulation of the minimum energy hypothesis. Murray's law is expressed as: $Q= kD^3$ where Q and D are volumetric flow rate and diameter of a vessel segment, respectively, and k is a proportionality constant. Murray's law proposes a universal, invariant exponent of 3.0 for all trees, where flow along the course of the tree structure is laminar. It has been proposed (Kassab, 2006) that this constant may vary between 2.33–3.0 depending on whether the flow is turbulent (2.33) or laminar (3.0).

Genetic programming plays a cardinal role in the development and design of biological trees. This genetic program may, however be influenced by many environmental factors. These factors are often related to the particular biological needs of the target tissue of the biological tree. So subsequent growth and morphological and

geometric alterations and remodeling along these biological trees may be seen. The changes observed along the course of the branches may be indicative of the functional, biological or metabolic demands affecting the tissues. This may be seen as adaptation to the environment and a selection and the survival of the valuable tree structure design. The factors determining geometric design are multiple. It may be stated that Murray's law may not be applicable to many biological trees in the human body. Therefore an extension of Murray's law has been proposed, where the exponent is not a universal constant (Kassab, 2006; Revellin, Rousset, Baud, & Bonjour, 2009). As blood is composed of multiple components, it has complex rheological characteristics. It has been demonstrated that blood may be considered a non-Newtonian fluid, as it demonstrates such characteristics as shear-thinning, viscoelasticity, yield stress, etc. Evidence suggests that the vascular system remodels to maintain wall shear stress within a physiological range (Revellin, Rousset, Baud, & Bonjour, 2009). This theory is based on Murray's law, which underlines the principle of minimum work and states that the third power of the radius of the parent vessel is the same as the sum of the third power of the radii of the daughter vessels.

The laws that govern the development of vascular tree structures include scaling relationships between length and overall vascular volume of the tree; lumen diameter and blood flow conditions in the branches; diameter and length of the vessel branches. (Hahn et al., 2008) It is required by Murray's law that the exponent of the diameter-flow rate relation be equal to 3.0, but in the case of vascular trees, the ratio of metabolic to viscous power dissipation of the tree of interest also plays a role (Kassab, 2006).

The literature is scarce regarding documentation of newly developed bypassing collaterals that formed as a result of the stricture and the highs pressure-low flow conditions in a venous network.

DOI:10.14753/SE.2020.2305

III. Aims of study

The vasculature is no longer regarded as a network of elastic tubes, but a complex, highly sensitve and highly adaptive system.Biomechanical remodeling may occur as a consequence of many factors (hemodynamic, endocrine and others), and the remodeling itself may be varied. Our aim was to study hemodynamic and endocrine factors in the biomechanical remodeling of the vascular wall. Our intention was to focus on vessel wall remodeling in diseases that have a large adverse impact on the well-being of the population. Therefore our research centered on coronary pathologies in hypertension and the possible effects of sex hormones of coronary pathologies. The other field chosen was the development of varicosities. Coronary pathology in hypertension and varicosities are widespreaddiseases and deeper understanding of underlying mechanisms may contribute to more targeted therapeutic options in the future

The following questions were intended to be answered by this study:

III./1.Is there a difference between the sexes regarding vascular remodeling in the coronary resistance arteries of the rat following AngII induced hypertension?

III./2. How does ovariectomy and hormone replacement affect this remodeling in the intramural coronaries, which play a cardinal role in cardiovascular pathophysiology?

III./3. How does the increase in venous pressure and decrease of venous flow effect the geometrical and biomechanical characteristics of the saphenous vein main branch in chronic main branch stricture model in rats?

III./4. Is there evidence of collateral network formation following partial clipping of the main branch of the saphenous vein of the rat?

III./5. Is there evidence of macrophage invasion and new cell formation at the clipping site?

DOI:10.14753/SE.2020.2305

IV. Methods

For better cohesion, we start this section with a summary table of the experiments performed (Table 2.).

Table 2.

Summary table of animals. First series included chronic Angiotensin II infused, Sprague Dawley rats male vs. female, and the left descending coronary was studied via angiometry under the induced hypertensive conditions. The second series included chronic Angiotensin II infused Sprague Dawley rats, where one group was ovariectomized, one group was ovariectomized and given estrogen therapy, and the third, "control hypertensive group" underwent a sham abdominal operation, the left descending coronary was studied via angimoetry under the induced hypertensive conditions. In the third series, the saphenous vein, and it's tributaries were studied in a low-flow high-pressure model. Videomicroscopy studies were performed on the first group, hemodynamic measurements were made on the second group. The third group underwent preparation with Batson#17 Corrosion Kit, and plastic casts were prepared to study the developed vanous collateral system. The fourth and firth group were sacrificed at 4 and 8 weeks respectively and angiometry was performed. Histopathology was performed in the sixth and final group.

Chronic Angiotensin II infused animals					
male	female				
10	10				
Chronic Angiotensin II infused animals					
hypertensive controls -female	ovariectomy - female	estrogen therapy - female			
11	11	11			
Clipped veins					
Videomicroscopy	Hemodynamic measurements	Batson 17 cast	Angiometry 4 weeks	Angiomerty 8 weeks	Histopathology
6	12	12	12	8	13

DOI:10.14753/SE.2020.2305

IV./1. Chronic Angiotensin II infusion

Chronic Angiotensin II was used used in a study designed to gain a deeper understanding of the sex differences in the early mechanisms of vascular adaptation of the intramural small coronary arteries. The protocol for angiotensin-dependent hypertension has been reported in detail (Várbíró et al., 2001; Várbíró et al., 2000). Previous studies reported that this subcutaneous dose led to chronic blood pressure elevation after 2 to 3 weeks without acute pressure effects, which is why we chose this model to study early hypertensive vessel alterations and sex differences. An osmotic minipump was implanted into Sprague-Dawley rats (Figure 12). The osmotic minipump implanted was from Alzet (2ML4, Durect Co, Cupertino, California) containing AngII acetate (Sigma-Aldrich Co, St. Louis, Missouri and Budapest, Hungary). Pentobarbital (Nembutal®, Phylaxia-Sanofi, Budapest, Hungary) was used for anesthesia. After surgical intervention, long-term 100,000 IU of penicillin (TEVA-Biogal® (Retardillin, TEVA-Biogal, Debrecen, Hungary) was administered intramuscularly to prevent infection. The pump remained in situ for 4 weeks, infusing a dose of 100 ng/kg/min Angiotensin II acetate. Four weeks later, the animals were sacrificed, and the intramural coronary arteries from the, were prepared.

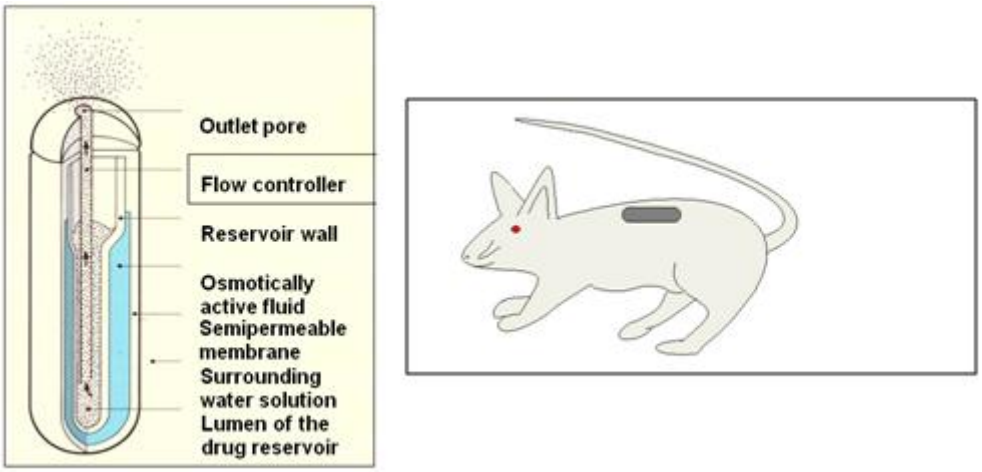


Figure 12.

Osmotic minipum implanted subcutaneously to release AngII to induce hypertension.

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The left coronary branch, which is fundamentally responsible for the blood supply of the heart was chosen to be studied. The pharmacologic reactivity and biomechanical properties of the dissected and isolated left anterior descendent coronary (approximately 200μm in diameter) branches were cannulated with plastic microcannulas at both ends, and studied in vitro in a vessel chamber (Figure 13.). The inner and outer diameter of the coronaries were measured by microangiometry, and spontaneous tone, wall thickness, wall cross-sectional area, tangential stress, incremental distensibility, circumferential incremental elastic modulus, thromboxane agonist-induced tone, and bradykinin-induced dilation were calculated.

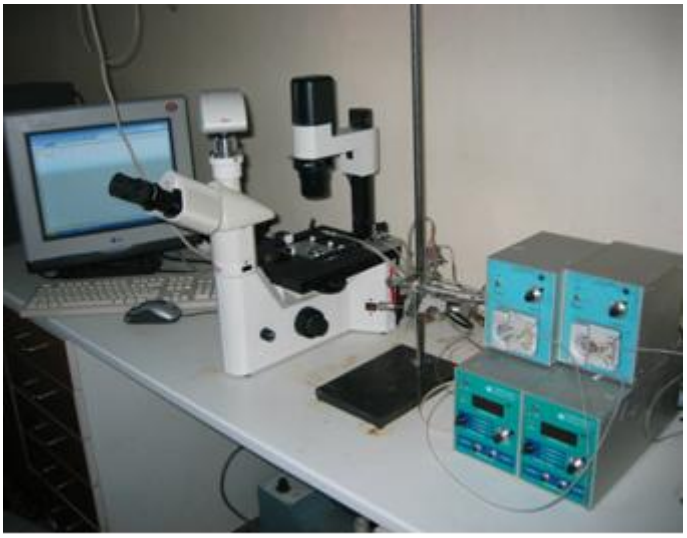

Figure 13.

Vessel chamber and tissue bath set-up used for microangiometry. Images from the monitor of the set-up depicting vessel segments set up for analysis.

Sex differences between Male vs. Female Animals

A total of 20 sexually mature, 2-month-old, age matched Sprague-Dawley rats were used (10 virgin females and 10 males; Charles River Laboratories, Wilmington, Massachusetts), weighing 220 to 250 g (females) and 280 to 320 g (males) at the beginning of the study. The females and males were both subjected to subcutaneous implantation of an osmotic minipump performed as described above.

No medical or surgical complications were observed. Conventional rat chow and tap water were provided ad libitum. The investigation conformed to the Principles of Laboratory Animal Care National Institutes of Health publication No. 85-23 (revised 1985) with the Euroconform Hungarian Law on Animal Care (XXVIII/1998) and was approved by the institutional Animal Care Commission (institutional review board approval: 22.1/2960/003/2009).

Ovariectomy and estrogen therapy

We designed a study to analyze the effects of ovariectomy and estrogen therapy in a rat model of menopausal hypertension induced by angiotensin II (AngII). A total of 33 sexually mature, virgin female Sprague- Dawley rats were used (Charles RiverLaboratories, Wilmington, MA, Semmelweis University Budapest), weighing 210 to 240 g at the beginning of the study. All of these rats were subjected to subcutaneous implantation of osmotic minipump as described above- We investigated intramural coronaries taken from ovariectomized rats (n=11) that received chronic AngII treatment to induce hypertension, and compared the results with those found in rats that were also given estrogen therapy following the ovariectomy (n=11). The ‘‘hypertensive control’’ group (n=11) underwent an abdominal sham operation, and received AngII.

No medical or surgical complications were observed. Conventional rat chow and tap water were provided ad libitum. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and was accepted by the University’sAnimal Care Commission and Hungarian authorities.

DOI:10.14753/SE.2020.2305

IV./2. Partial chronic occlusion (‘‘clipping’’) of the saphenous vein in the rat

We designed a study to better understand factors that play a role in the development of varicosities. We induced a combined flow-pressure disturbance in the saphenous system of the rat by performing chronic partial clipping of the main branch. In vivo hemodynamical, in vitro biomechanical and quantitative histological testings were undertaken.

Experiments were performed on male Sprague–Dawley rats weighing 273±40 (mean±SD) g at the beginning of the experiments. All rats were anesthetized as described above. During a microsurgical operation, the most proximal segment of the main branch of the saphenous vein was dissected at a distance of 10mm from its confluence with the deep femoral vein. A longitudinally slit 4mm long semi-rigid, thick-walled piece of a plastic tube with a lumen diameter of 500 µm was placed around the vein (Figure 14.). This did not close the vessel but prevented any further increase in diameter as the animal grew.

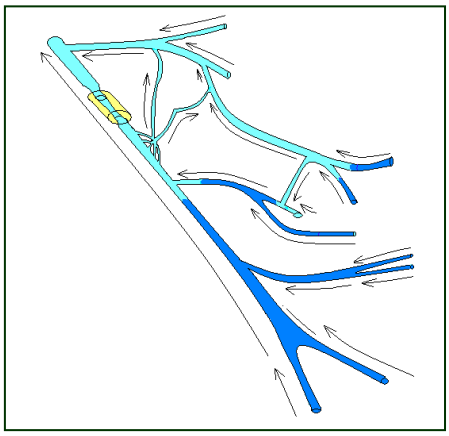

Figure 14.

Chronic partial clipping of the saphenous vein main branch, inducing a flow-pressure. The clip fixes the diameter of the saphenous vein at 500µm

DOI:10.14753/SE.2020.2305

Measurement protocols

Following the induction of AngII hypertension and the partial cclusion of the saphenous vein, a series of testing was undertaken. Pressre-angiometry was performed for all dissected segment (both coronaries and venous segments), and for venous segments videomicroscopy, hemodynamic measurements, Batson 17 casting, and histologisal examination were performed also (Figure 15. and 16.).

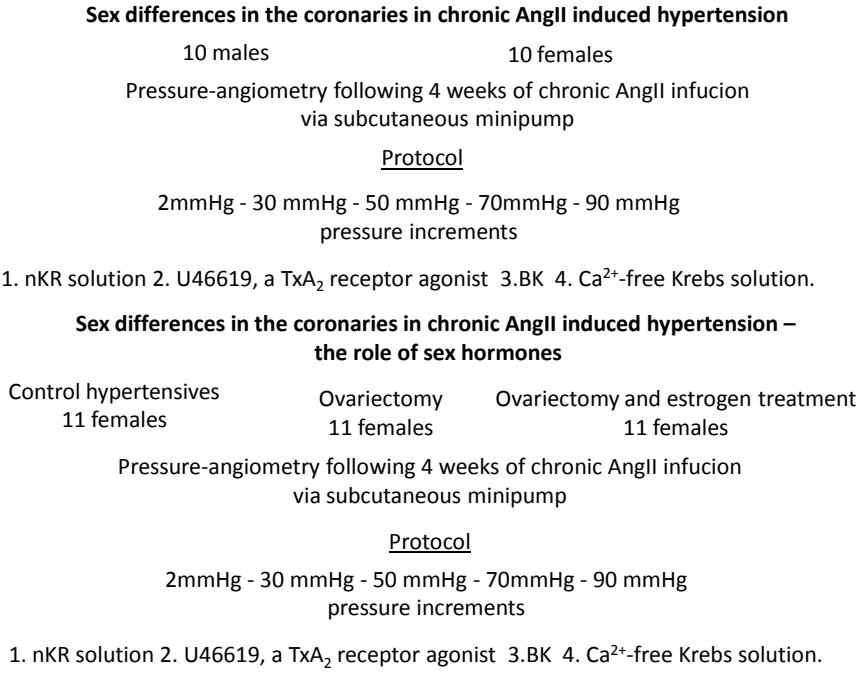

Figure 15.

This Figure depicts the pressure angiometry protocols performed for all animals that had undergone subcutaneous implantation of a minipump to induce hypertension via AngII.

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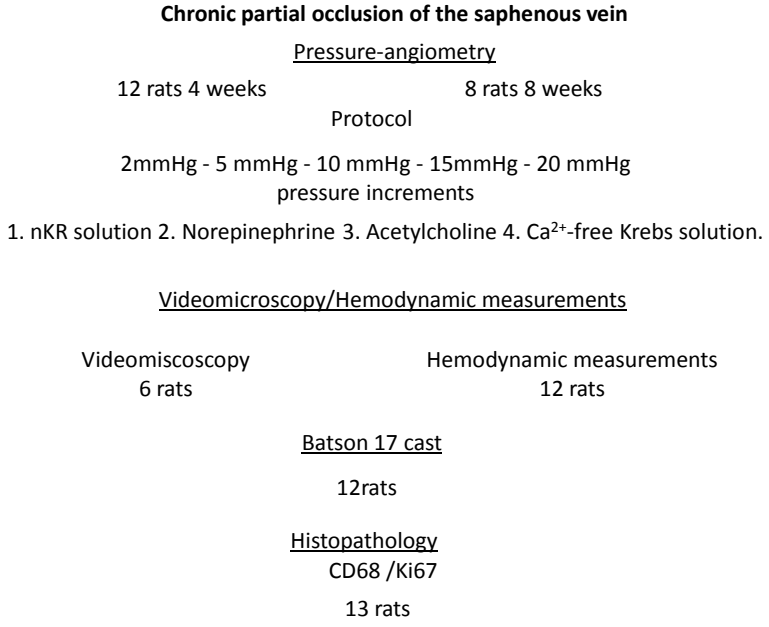

Figure 16.

This figure shows the test performed on vensous segments.

IV/3. In vitro pressure-angiometry

In Vitro Mechanical and Pharmacological Reactivity of the Intramural Coronary Artery

After 4 weeks of treatment, all specimens that were implanted with an osmotic minipump (male vs female groups and control hypertensive vs. ovariectomy vs. estrogen groups) that released Angiotensin II were re-anesthetized as described above. Blood pressure was measured directly by cannulating the carotid artery. The heart was removed through the chest and placed into cold, oxygenized, normal Krebs-Ringer (nKR) solution. The 200 μm secondary branches of the left anterior descending coronary artery were isolated, as previously reported byour research group (Mátrai et al., 2010; Nádasy et al., 2001; Várbíró et al., 2006). Aftre the segment was mounted in a tissue bath as described above both cannulas were connected to pressure-servo systems (Living Systems, Burlington, Vermont, US), and the arteries were pressurized under no-flow conditions. The glass-bottomed tissue bath was positioned in the light path of a microscope.

DOI:10.14753/SE.2020.2305

The outer diameter of the arteries was measured by microangiometry. A magnified picture of the vessel was taken with a Philips LDH 0702/20 video camera and a monitor (Philips Computer Monitor 80; both Royal Philips Electronics, Amsterdam, Netherlands). Two light markers connected to a microcomputer were used to measure the inner and outer diameter of the vessel segment (Figure 17.). Diameter measurements were taken at incremental pressure levels.

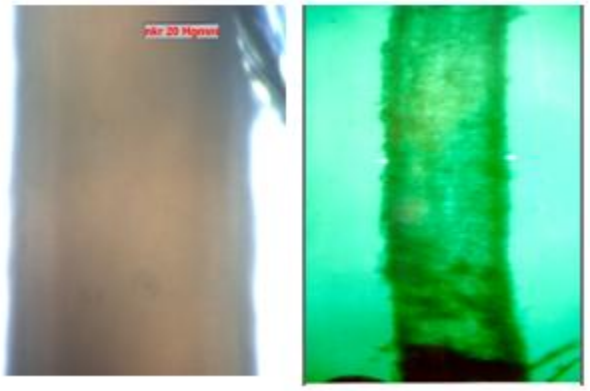

Figure 17.
Images from the monitor of the pressure-angiometry set-up. Light markers are used to measure inner and outer diameters of the vessels.

The intraluminal pressure was measured at both sides of the segments with a Gould pressure transducer. The pressure and diameter signals were digitized by an analog/digital converter (PCL 7/8, Advantech Corporation, Milpitas, California) and transmitted to an IBM Pentium PC computer (IBM, Armonk, New York) for data storage and further processing.

Materials for coronary artery studies

The composition of the normal Krebs-Ringer solution (nKR) used in these in vitro studies was (in millimoles per liter) sodium chloride (NaCL) 119, potassium chloride (KCl) 4.7, sodium phosphate (NaH_2PO_4) 1.2, magnesium sulfate (MgSO4) 1.17, sodium bicarbonate ($NaHCO_3$) 24, calcium chloride ($CaCl_2$) 2.5, glucose 5.5, and EDTA 0.034. The calcium (Ca^{2+})-free Krebs solution contained (in millimoles per liter) NaCl 92, KCl 4.7, NaH_2PO_4 1.18, $MgCl_2$ 20, $MgSO_4$ 1.17, $NaHCO_3$ 24, glucose 5.5,

ethylene glycol tetraacetic acid 2, and EDTA (Ethylenediaminetetraacetic acid) 0.025. The temperature of the solution was maintained at 37°C, and it was bubbled with 5% carbon dioxide, 20% oxygen, and 74% nitrogen, which stabilized the pH at 7.4.

U46619 was used as vasoconstrictor agent. Alternative Name: 9,11-Dideoxy-9alpha,11alpha-methanoepoxy prostaglandin Chemical Name: (5Z)-7-[(1R,4S,5S,6R)-6-[(1E,3S)-3-Hydroxy-1-octenyl]-2-oxabicyclo[2.2.1]hept-5-yl]-5-heptenoic acid Biological Activity. U46619 is a PGH2 (TxA_2) analog, wich stimulates TP receptor-mediated, but not other prostaglandin receptor-mediated responses. U46619 has been described to be a selective TxA2-mimetic, and is a useful substance it studying the effects of TxA_2 (Coleman, Humphrey, Kennedy, Levy, & Lumley, 1981).

U46619, a thromboxane A_2 (TxA_2) receptor agonist, and bradykinin (BK) were obtained from Sigma-Aldrich Co. The drugs were freshly prepared in nKR solution on the day of the experiments.

Angiometry protocol for coronary arterioles - sex differences male vs. female groups and ovariectomy vs. estrogen replacement vs. control hypertensive groups

The coronary arteries were allowed to equilibrate for 30 minutes at 50mmHg intraluminal pressure in nKR solution. After this incubation, the pressure was decreased to 2mmHg, and then increased first to 30 mmHg, then up to 90 mmHg in 20 mmHg in increments. The steady-state diameter was measured at each step. The pressure load cycle was repeated with U46619, a TxA_2 receptor agonist (at a concentration of 10^{-6} M), and then with BK (at a concentration of 10^{-6}M), both administered into the superfusion saline solution with continuous flow, separately, one after another, allowing 10 minutes of incubation with each drug. Finally, the passive diameter was obtained in Ca^{2+}-free Krebs solution. The segments were incubated for 20 minutes, and then the intraluminal pressure was increased in the previously described increments; the passive diameter of the arteries was obtained at each pressure level.

Venous segment protocols

In vitro biomechanical testing of the main branch was performed as follows. Just distal to the clip, a side-branch free segment of the main branch was dissected and

excised (12+8 rats, with four and eight weeks after chronic occlusion, respectively), both from the strictured and from the contralateral, non-strictured (control) sides. The length of the removed (dissected) cylindrical segments varied between 8 and 12 mm. The identical segments of the contralateral side served as controls. The cylindrical venous segments were mounted on 500 μm diameter glass cannulas, immersed in thermostated saline and stretched to their in vivo length in the glass-bottomed tissue bath of the pressure angiometry setup.

Protocol of in vitro angiometry of venous segments

The normal-Krebs Ringer (nKR) solution was bubbled with a gas mixture of 95% oxygen and 5% carbon dioxide. After a 30-min incubation period in nKR solution at 5 mmHg intraluminal pressure, pressure was increased in a stepwise manner, to 2-5-10-15-20 mmHg. Then at 5mmHg intraluminal pressure, 10 μmol/l norepinephrine was added to the tissue bath and the measurement cycle was repeated to test contractility of the segments. With the norepinephrine still in the bath, 5.5 μmol/l acetylcholine was added to study endothelial dilation. Experiments were terminated by recording the characteristic pressure-diameter curves in Ca^{2+}-free nKR solution (to test the passive state).

IV./4. Intravital videomicroscopy and hemodynamic measurements

Intravital videomicroscopy was performed to demonstrate the newly developed bypassing collaterals following partial clipping of the saphenous vein in the high-pressure low-flow venous model. After four and eight weeks, the animals were reanesthetized as described above. In vivo videomicroscopic studies of the microsurgically dissected specimens were performed on six rats (n=6) (Figure 18.) to visualize the morphology of the collateral venous network. To test the effects of chronic partial occlusion on the hemodynamics, in separate sets of rats (n=12) venous pressure was measured in anesthetized animals through a microcannula (around 200 μm outer diameter) introduced into the saphenous vein main branch, distal to the level of the stricture, through a popliteal side branch. It was attached to a Braun pressure head and a low-pressure mainframe. Fifty microliter saline stained with methylene blue was

injected into the vein and venous flow was determined by following the movement of the stained bolus on a long stretch of the saphenous vein by serial pictures (1 frame/s) by intravital microscopy (Figure18.). Similar measurements were made on the control sides.

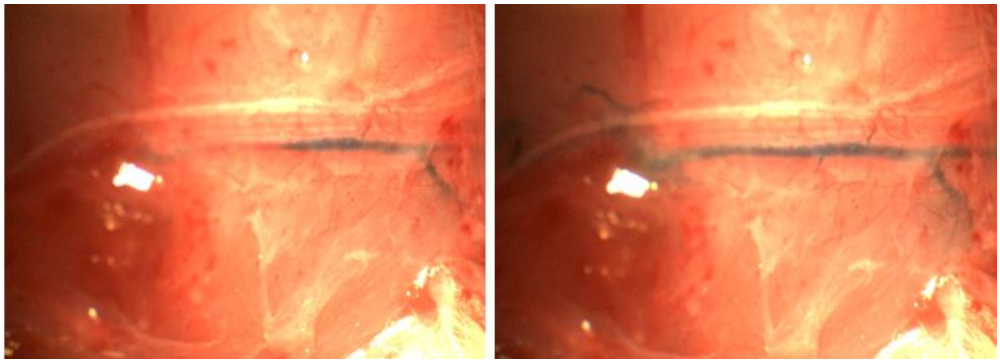

Figure 18.
Intravital videomiscroscopy of the venous segment. The stained bolus, used to determine flow is demonstrated.

IV/.5. Histology and immune-histology of the main branch following chronic partial clipping of the saphenous branch in high-pressure low-flow venous model

In a separate series, the same segments and surrounding tissue were removed for histological studies after four and eight weeks of clipping (n=13), and analog specimens from contralateral sites served as controls. Histological samples were stained with hematoxylineosin (nuclei) and resorchin-fuchsin (elastica) and immune-histological staining was performed for CD68 (macrophage invasion), Ki67 (cellular division activity), and smooth muscle actin (SMA, contractile protein).The source of the antibodies was the R&D Systems Inc. Secondary antibodies were visualized with the DAB technique. The Ventana Benchmark XT Immune-Automat System was used for the immunehistochemical staining. This ensured identical staining procedures and made the sections comparable with each other. For embedding the specimens tissue array technique was used. Sections were scanned with the 3D Histech Pannoramic 250 Scanner, and selected high magnification (pixel sizes 0.31 μm) RGB pictures (0–255 levels) were analyzed with the Leica QWin image analysis software. The green (for RF) and blue (DAB) levels were measured in a radial line using the ‘‘Measure profile’’ function, starting at the luminal surface and going radially in an outward direction

DOI:10.14753/SE.2020.2305

(Figure 19.). The colors (green for RF and blue for DAB with methylene blue background) were chosen after careful preliminary evaluation of color histograms.

Distribution of the green color on RF stained sections was measured radially from the endothelial surface toward the adventitia. Earlier color analysis histograms were showing that green color is suppressed by the magenta color of this dye: more dense elastic tissue will be marked by lower green levels. Similar measurements were made for smooth muscle actin immunohistochemistry densities (DAB) in blue color.

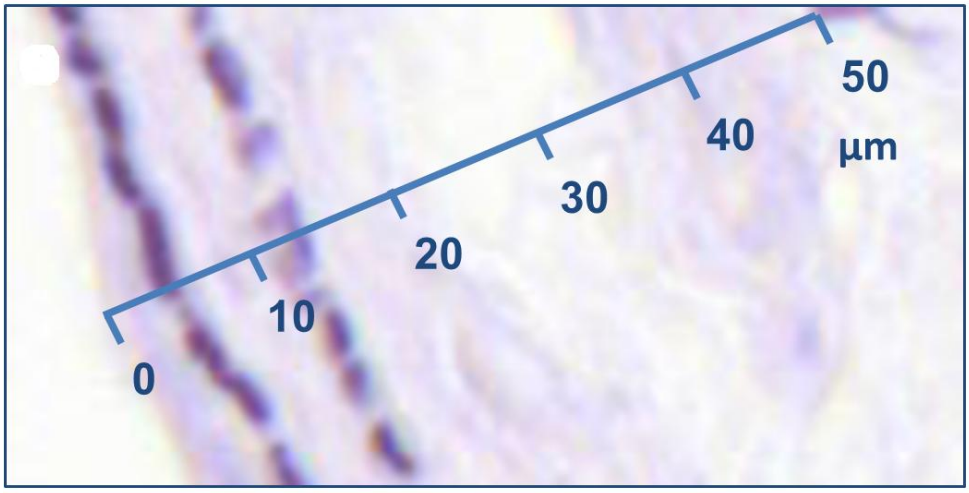

Figure 19.

Reorganization of the elastic membrane was quantitatively demonstrated by the green color density measurements made in the radial direction outwardly from the luminal surface (the magenta color of the resorcin-fuchsin stain suppresses the green component).

IV./6. Batson 17 plastic casts

Batson 17 plastic casts were prepared in the high-pressure low flow venous chronic partial clipping model, to visualize the newly developed collateral network of the saphenous vein through a popliteal side branch vein. In Nembutal anesthesia, it was possible to fill that collateral system from the direction of the saphenous vein main branch using the Batson #17 Anatomical Corrosion Kit. It is used for ambient temperature preparation of exact anatomical corrosion specimens by injection of liquid plastic. After hardening, the tissue is corroded away by caustic 10% potassium hydroxide yielding a durable, scientifically exact model for anatomical study (n=12)(Figure 19.).

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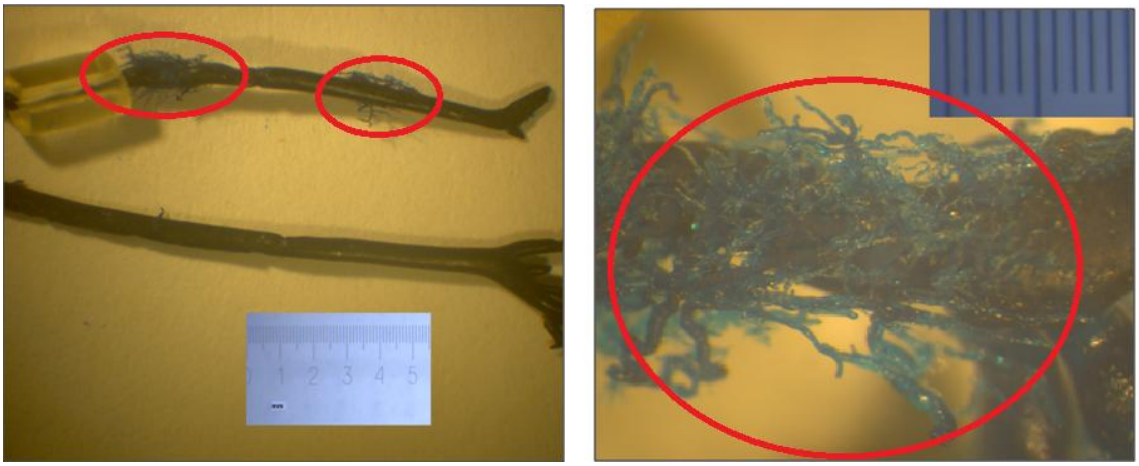

Figure 19.
Batson 17 plastic cast revealing the newly developed rich collateral system of the saphenous vein on the clipped side.

This procedure is used to produce scientifically exact, multi-colored specimens for anatomical study, comparative demonstrations and for quantitative scanning electron microscopy studies. It is based on a procedure originated by Professor Oscar Batson of the Department of Anatomy at the University of Pennsylvania. The kit consists of partially polymerized monomer, a catalyst, and a promoter to allow curing at room temperature after injection, and pigments are supplied. Batson 17 plastic cast solution for injection was prepared according to specifications for injection and curing, and all materials were stored according to manufacturer specifications. Soft tissues were corroded away from the specimens according to the maceration process described by manufacturer.

IV/7. Biomechanical calculations for all vessel segments

From the original calibrated pressure-diameter plots, the following geometric and biomechanical parameters were computed for each intraluminal pressure level. The tangential stress was computed according to the Laplace equation, $\sigma = P \times r_i/h$ where σ is the tangential (circumferential) wall stress, P is the intraluminal pressure, r_i is the inner radius, and h is the wall thickness ($h = r_o - r_i$, where r_o is the outer radius). The incremental distensibility was $D_{inc} = \Delta V / V \Delta P$, where D_{inc} is the incremental distensibility and ΔV is the change in vessel lumen volume relative to the initial volume of V in response to a pressure change of ΔP.

The circumferential incremental elastic modulus was computed from the equation $E_{inc} = (\Delta p / \Delta r_o) \times 2r_i^2 \times r_o / (r_o^2 \times r_i^2)$, where E_{inc} is the incremental elastic modulus, r_i is the inner radius, r_o is the outer radius, and Δr_o is the change in the outer radius in response to an intraluminal pressure change of Δp.

The spontaneous tone of the vessels was expressed as active strain, quantified for each intraluminal pressure level: $T_{nKR} = (r_i Ca^{2+}\text{-free} - r_i nKR) / r_i\ Ca^{2+}\text{-free}$, where $r_i\ Ca^{2+}$-free and r_i nKR are the inner radii measured in Ca^{2+}-free Krebs solution and in nKR solution, respectively. The TxA_2-induced tone and BK-induced tone in nKR solution was also expressed as active strain, quantified for each intraluminal pressure level as follows: $T_{BK} = (r_i Ca^{2+}\text{-free} - r_i\ BK)/r_i Ca^{2+}\text{-free}$, where $r_i Ca^{2+}$-free and r_i BK are the inner radii measured in calcium-free Krebs solution and BK, respectively. In active strain parameters the size of the vascular lumen does not influence the value (%) of vascular reactivity. BK-induced relaxation compared with tone in nKR solution was calculated with the following formula: BK relaxation $= (r_i\ BK - r_i\ nKR)/r_i\ nKR$.

Statistical analysis

For statistical analysis, the data measurements were compared by 2-way ANOVA. The in vitro parameters, plotted as a function of intraluminal pressure between groups, were compared by 2-way ANOVA. Paired comparisons for treatment groups were made for the curves. As a post hoc test, Tukey‘s test was used. t Tests were applied for discrete parameters (e.g., body weights). Paired data were compared with paired t-tests. A P value 0.05 was uniformly accepted as a significant difference. The data were presented as means (SEM).

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V. RESULTS

V./1. Sex differences in the remodeling of rat coronary resistance arteries in angiotensin II hypertension

Main Biological Parameters

Following Angiotensin II treatment mean arterial pressure did not differ significantly between females (131±5 mmHg) and males (134±7 mmHg) (Table 3.). The heart weight index - meaning the relative weight of the heart, calculated per 100 g body mass - was greater in hypertensive females than in males ($P<0.001$; Table 3.).

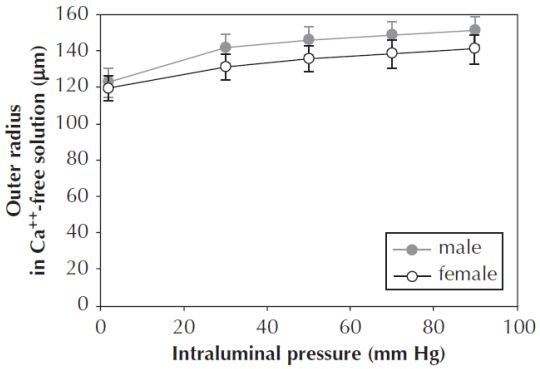
Table 3.

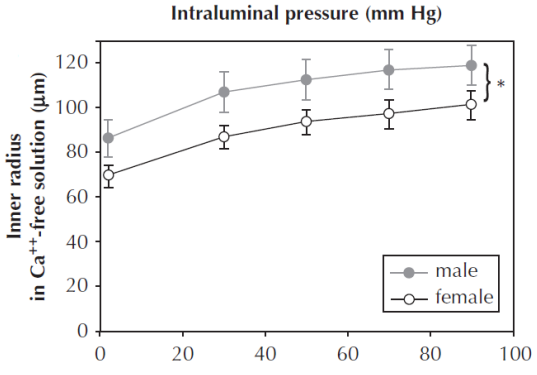
*After 4 weeks of AngII treatment, no difference was found in blood pressure between the male and female hypertensive groups. The relative heart weight of the hypertensive females was greater compared with hypertensive males. Two-way ANOVA tests between the groups are shown, ***P< 0.001.*

Characteristics	AngII–Treated Females	AngII–Treated Males
Blood pressure, mmHg	131±5	134 ±7
Heart weight, g/100 g body mass	0.387 ±0.009***	0.306 ±0.006

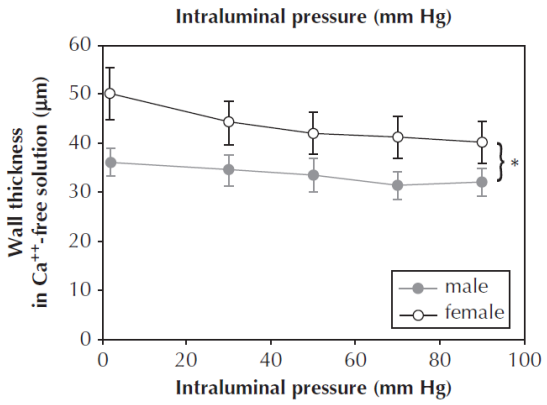
In the passive state no significant difference was found between the outer radii of males and females (measured in Ca^{2+}free solution) (Figure 20.a). In hypertensive females, we observed significantly smaller inner radii (Figure 20.b) and greater wall thickness (Figure 20.c) than in males ($P<0.05$). The difference in cross-sectional areas (meaning the overall amount of wall material) did not reach the level of statistical significance between the two sexes, being 23.4 ±3.5 10^3 μm^2 in males and 27.0±4.5 μm^2 in females.

DOI:10.14753/SE.2020.2305





(b)



(c)

Figure 20.

Geometric properties of the intramural coronary resistance arteries from male (n=10) and female (n=10) rats following 4 weeks of AngII treatment. (a) The values of the outer radius as a function of intraluminal pressure measured in the passive condition (in calcium [Ca^{2+}]-free Krebs solution) male vs. female following 4 weeks of AngII treatment. (b) The values of the inner radius as a function of intraluminal pressure measured in the passive condition (in Ca^{2+}-free Krebs solution) male vs. female

following 4 weeks of AngII treatment. (c) The values of the wall thickness as a function of intraluminal pressure measured in the passive condition (in Ca^{2+}-free Krebs solution) male vs. female following 4 weeks of AngII treatment.

*Data are expressed as the means (SEM) values. The significance levels of 2-way ANOVA tests between the 2 groups are shown (factors: sex, intraluminal pressure). *P<0.05.*

Sex Differences in Vessel Biomechanics

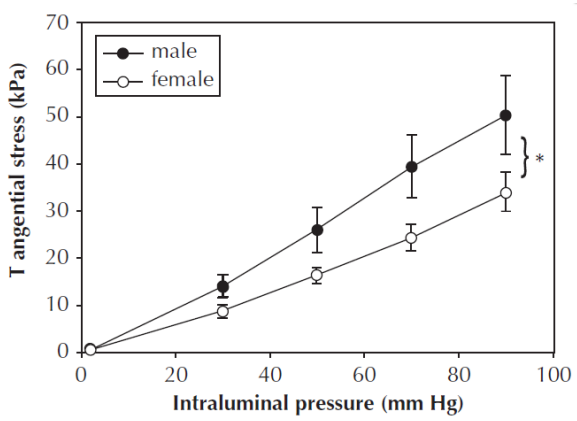


Figure 21.

*The biomechanics of coronary arteries from male (n=10) and female (n=10) rats following 4 weeks of angiotensin II treatment. The tangential wall stress of rat intramural coronary resistance arteries (male vs. female) as a function of intraluminal pressure in the passive condition (in calcium [Ca^{2+}]-free Krebs solution) following AngII treatment. Data are expressed as means±SEM values. The significance levels of 2-way ANOVA tests between the 2 groups are shown *P< 0.05.*

Tangential wall stress values, wich shows the mechanical loading of the vessel were significantly higher in males than in females (Figure 21.); P< 0.01).

DOI:10.14753/SE.2020.2305

V./2. Remodeling of rat coronary resistance arteries in angiotensin II hypertension. Effects of ovariectomy and ovariectomy combined with estrogen replacement

Mean arterial pressure

The mean arterial pressure of the control hypertensive group was 130±5 mmHg, the ovariectomized hypertensives had pressures of 134±6 mmHg, and the ones given additional estrogen replacement therapy was 142±5 mmHg. No significant differences were found.

Biomechanical parameters

The following table shows the summary of biomechanical parameters calculated for the AngII, AngII+ovariectomy, AngII+ovariectomy +estrogen therapy (Table 4.).

Table 4. Biomechanical parameters for the following three groups are shown: Angiotensin II; Angiotensin II treatment + ovariectomy [OVX]; Angiotensin II + ovariectomy [OVX]+ estrogen therapy). Parameters were calculated for the three groups at intraluminal pressure of 50 mmHg as this correlates with in vivo transmural pressure. Relative heart weights were normalized for 100 g of body weight.

Parameter	Ang II (n=11)	Ang II- Ov (n=11)	Ang II - Ov – Estr (n=11)
Tangential wall stress (kPa)	16,32 ± 1,75	18,33 ± 2,58	19,15 ± 2,50
Distensibility (1/kPa)	0,0288 ± 0,0079	0,0282 ± 0,0093	0,0375 ± 0,0087
Elastic moduli (lgPa)	5,48 ± 0,15	5,50 ± 0,10	5,29 ± 0,14
Cross section area (μm^2)	30137 ± 3795	25586 ± 2596	29550 ± 3801
Wall-to-lumen ratio (1/ µm)	0,461 ± 0,050	0,428 ± 0,050	0,409 ± 0,048
Relative heart weight (g/100g bw)	0,386 ± 0,009	0,365 ± 0,021	0,390 ± 0,011

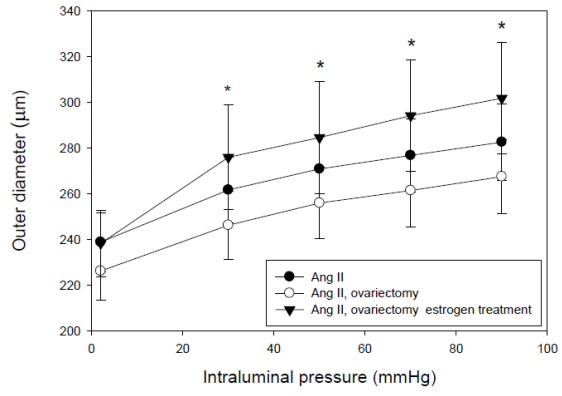
No difference was found in terms of wall stress, distensibility (Table 4.), and elastic moduli among the three groups (“control” Angiotensin II; Angiotensin II+ovariectomy [OVX]; Angiotensin II+ovariectomy [OVX]+ estrogen therapy) (Table 4.).The cross-sectional areas and wall to lumen ratios of vessel wall did not differ among the three groups (Table 4.).

Vessel geometry of intramural coronary arterioles was analyzed following ovariectomy and ovariectomy plus estrogen replacement on AngII treated hypertensive

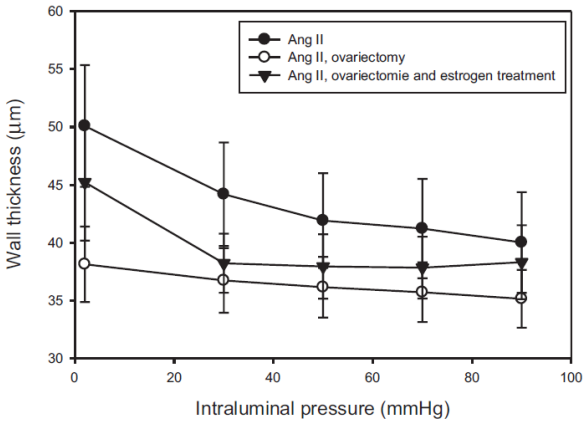
DOI:10.14753/SE.2020.2305

female rats. Ovariectomy reduced the lumen from 270±14μm to 254±14μm, while estrogen treatment not only restored lumen diameter but resulted in even higher values than in control AngII treated rats 284±24 μm. (Ovariectomized significantly smaller than not ovariectomized or ovariectomized plus estradiol replaced, $p<0.05$).

We found similar results regarding the outer radius (Figure 22.a). However the differences between the groups regarding wall thickness did not reach the level of statistical difference in our series (Figure 22.b).



(a)



(b)

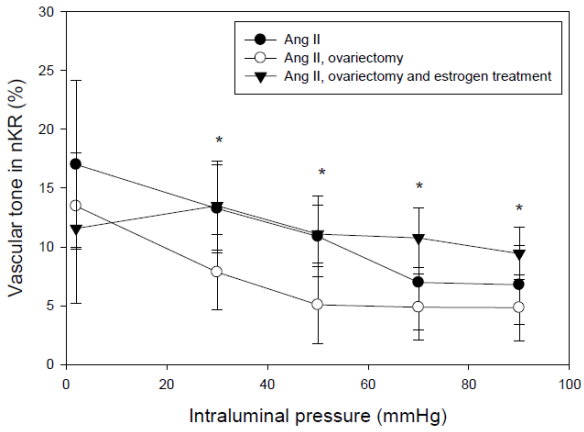
Figure 22.

(a)Outer radius values from the control angiotensin II-treated group (AngII; full circles, n=11), angiotensin II treated plus ovariectomized group (OVX; empty circles, n=11), and angiotensin II treated, ovariectomized and estrogen –replaced (full triangles, n=11) animals. (b)Difference in wall thickness did not reach the preset level

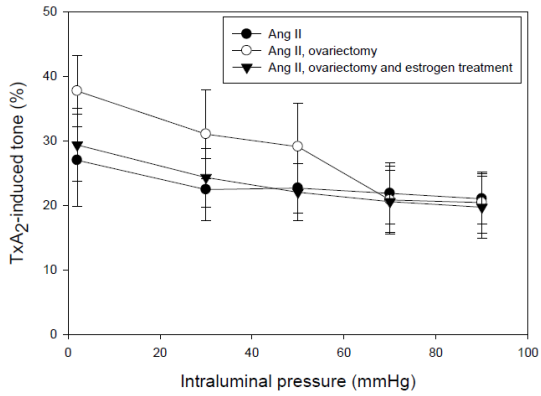
of statistical difference between the groups. Mean ±SEM values. Asterisk indicates statistical significance (P<0.05) between ovaryectomized groups with nonovariectomyzed and ovariectomized plus estrogen replaced groups.

Effects of ovariectomy, and ovariectomy plus estrogen therapy on the contractility of hypertensive intramural coronary arterioles

In our series spontaneous myogenic tone was higher in the estrogen treated group compared with the ovariectomized group (Figure 23.a) There was no difference in U46619-induced tone between the groups (Figure 23.b).



(a)



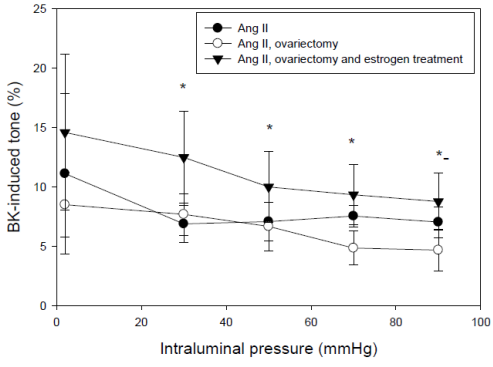
(b)

Figure 23.

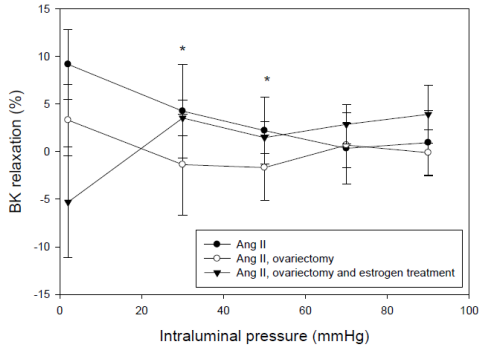
(a) Myogenic (spontaneous) tone, (b) TxA_2-induced tone of rat intramural coronary arterioles as a function of intraluminal pressure; values from the control angiotensin II-treated group (AngII; full circles, n=11), angiotensin II treated plus ovariectomized group (OVX; empty circles, n=11), and angiotensin II treated, ovariectomized and

estrogen-replaced (full triangles,n=11) animals. Mean ±SEM values. Asterisk indicates statistical significance (P<0.05) between control AngII and estrogen-treated groups versus OVX group. Spontaneous myogenic tone was higher in the estrogen-treated ovariectomized group compared with the ovariectomized group without hormone replacement. There was no statistically significant difference in U46619-induced tone between the groups.

Following application of bradykinin to the tissue bath remaining tone was significantly higher both in non-ovariectomized and in the estrogen-treated ovariectomized groups compared with the ovariectomized animals without estrogen hormone replacement. Estrogen+Ovariectomy+AngII, 11.1 ±2.1%, AngII 9.9±2.8%, and Ovariectomy+AngII 6.6±2.0% at P=50 mmHg in BK-induced relaxation. Comparing with spontaneous tone, there was no significant relaxation in the Ovariectomy+AngII and the AngII group; however, estradiol treatment restored nitric oxide (NO)-dependent, BK-induced relaxation to the hypertensive control level (Figure 24.a,b).



(a)



(b)

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Figure 24.

Bradykinin (BK)-induced tone of the rat intramural coronary arterioles was expressed as active strain and a function of intraluminal pressure (10^{-6}M BK). Values from the control angiotensin II-treated group (AngII; full circles, n=11), ovariectomy (OVX; empty circles, n=11), and estrogen-treated (E; full triangles, n=11) groups are shown. Mean SEM values. Asterisk indicates statistical significance (P<0.05) between estrogen-treated group versus OVX group in respect of BK-induced tone (a) and control AngII and estrogen-treated groups versus OVX group in respect of BK relaxation (b). Remaining tone was significantly higher in the estrogen-treated and control AngII hypertensive group compared with the ovariectomized animals in BK-induced relaxation. Comparing with spontaneous tone, there was no significant relaxation in the ovariectomized group; however, estradiol treatment restored nitric oxide-dependent, BK-induced relaxation to the hypertensive control level.

V./3. The effects of high-pressure low-flow conditions induced by chronic stricture of the main branch of the saphenous vein on the hemodynamics of the saphenous main branch. Biomechanical alterations of the main branch following partial clipping of the main branch of the saphenous vein

The effects of high-pressure low-flow conditions induced by chronic stricture of the main branch of the saphenous vein saphena on the hemodynamics of the saphenous main branch

In accordance with our expectation chronic partial occlusion of the saphenous vein induced a substantial pressure rise was observed in the main branch. Measurements were taken in anesthetized animals in the supine body position and we found that in comparison to the control side venous pressure doubled, $p<0.001$ with two-way ANOVA (Figure 25.a)

The rise in pressure was accompanied by a drastic drop in the flow values of the main branch. After eight weeks of occlusion, control side flow of 3.5±1.4 µl/s dropped to a mere 0.65±0.18 µl/s at the side of the stricture, $p<0.001$ with two-way ANOVA (Figure 25.b). Reduction of blood flow seems to be induced by diversion of blood from main branch toward the retrograde filling collateral system.

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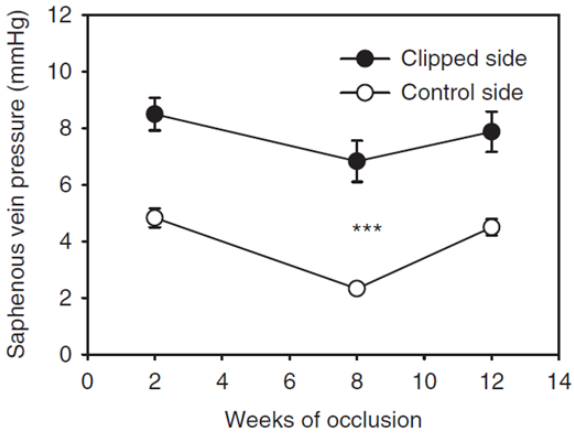

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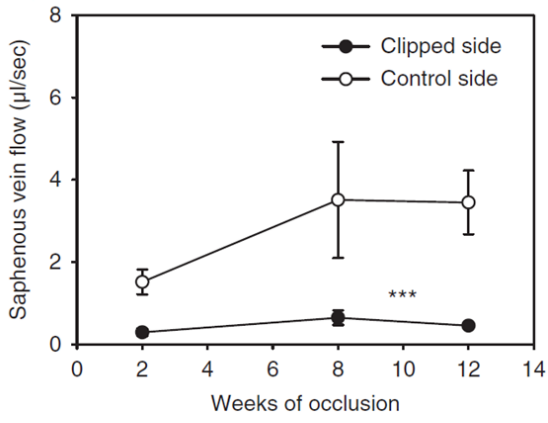

(b)

Figure 25.

*Main branch of the saphenous vein was narrowed to 500µm for 4-8-12 weeks, contralateral saphenous veins served as controls. Black circle clipped side, empty circle – control side. (a) Saphenous vein pressure plotted against weeks of occlusion. Venous pressure doubled compared to control side. (b) Saphenous vein flow plotted against weeks of occlusion. At eight weeks of occlusion, control side flow of 3.5±1.4 µl/s dropped to 0.65±0.18 µl/s on the clipped side. ***p<0.001, significant difference between marked groups, according to one- and two-way ANOVA tests*

Biomechanical alterations of the main branch following partial clipping of the main branch of the saphenous vein.

A reduction in diameter of the clipped segments in comparison with their contralateral unclipped controls was found in our series (Figure 26.). At intraluminal

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pressure of 10mmHg, the relaxed outer diameter of clipped segments reduced to 642±29 µm in comparison with 764±24 µm of the control side ($p<0.001$ with the paired t-test). Corresponding values following eight weeks of clipping were 613±30 µm and 734±25 µm ($p<0.01$ with paired t test).

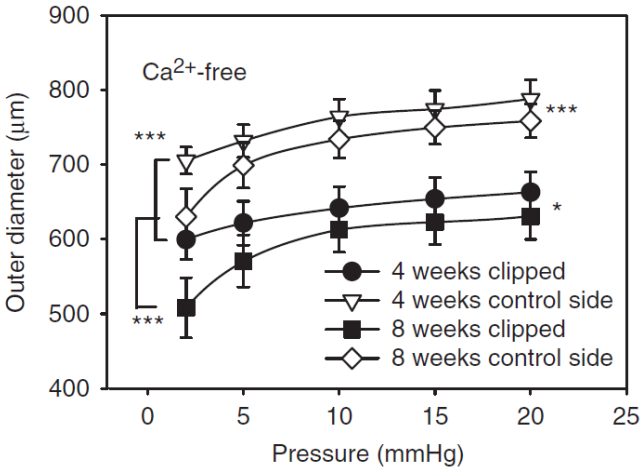
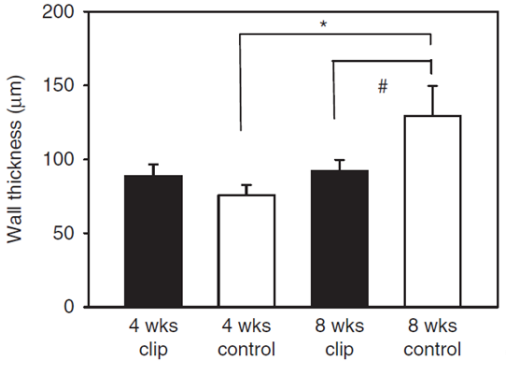


Figure 26.

Hemodynamically induced biomechanical remodeling of the saphenous vein main branch. Relaxed diameter clipped and control saphenous vein segments following 4 weeks and 8 weeks of clipping plotted against pressure in calcium-free medium.

Following eight weeks of clipping, the wall thickness values of the clipped segments were significantly reduced compared to those found in the contralateral side (Figure 27.a). This in turn leads to reduced wall mass values on the clipped side (Figure 27.b).



(a)

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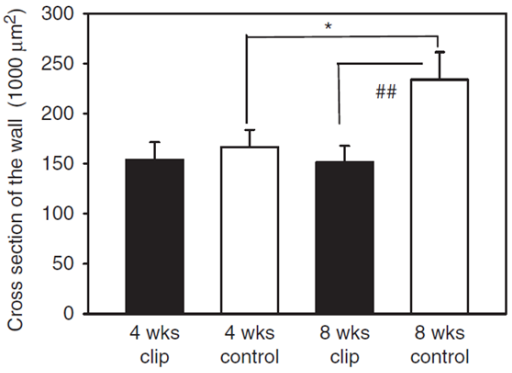

(b)

Figure 27.

Hemodynamically induced biomechanical remodeling of the saphenous vein main branch. Cross section areas (a) and wall thickness (b) of clipped and control saphenous vein segments following 4 weeks and 8 weeks of clipping at 10 mmHg in Ca^{2+}-free medium.

Low-stress elastic modulus decreased between weeks 4 and 8 in controls however this reduction was less on the clipped side. When elastic moduli were plotted against wall stress, after four weeks, the low stress modulus increased and the high stress modulus decreased in obliterated segments (Figure 28.). Their values were at 0.5 kPa wall stress 4.36±0.30 vs. 3.65±0.22 and at 1.5 kPa wall stress 4.58±0.15 vs. 4.88±0.20, for clipped and control sides, respectively (logarithmic values in lgPa, statistically significant with ANOVA, $p<0.05$).

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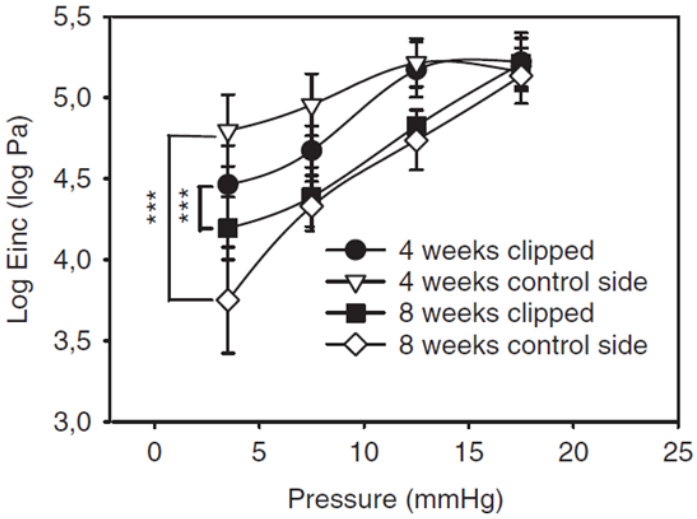

Figure 28.

Hemodynamically induced biomechanical remodeling of the saphenous vein main branch. Log elastic modulus plotted against intraluminal pressure of clipped and control saphenous vein segments following 4 weeks and 8 weeks of clipping.

Alterations in contractility of the main branch following partial clipping of the main branch of the saphenous vein.

We found that the induced low flow–high pressure remodeling massively reduced contractility. Spontaneous tone was found to increase between weeks 4 and 8 in control segments ($p<0.01$), however this increase was missing in the clipped side. As a result, after eight weeks, clipped segments exhibited much smaller spontaneous tone than control ones (Figure 29.a)($p<0.01$). Similar observations were found regarding maximal contraction induced by norepinephrine (Figure 29.b). After four weeks of partial occlusion the reduction in contractility reached the level of statistical significance ($p<0.05$). In addition, reduced endothelial dilation capacity was found in venous segments after eight weeks of occlusion (Figure 29.c) ($p<0.05$).

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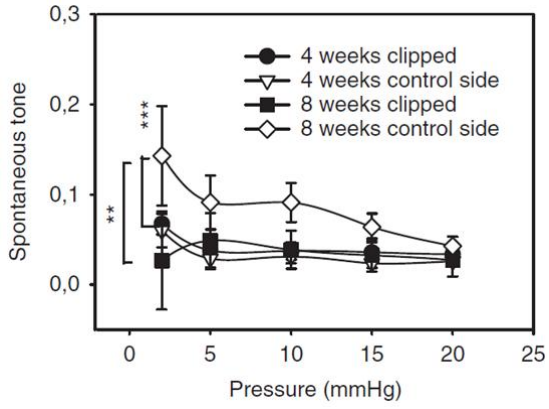
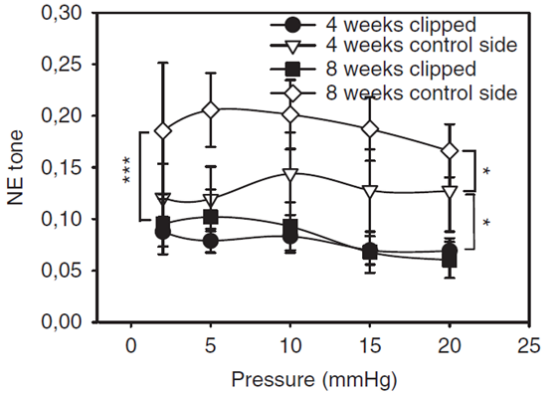


(a)



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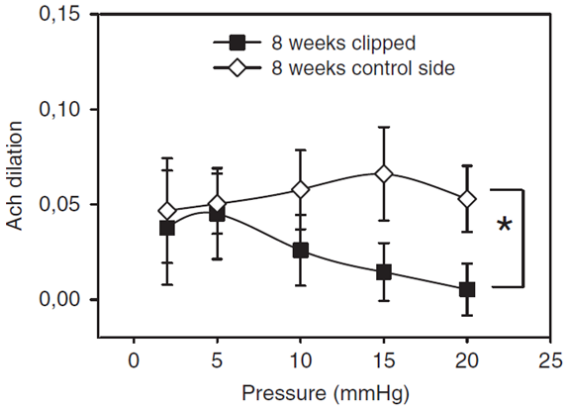

(c)

Figure 29.

*Hemodynamically induced biomechanical remodeling of the saphenous vein main branch. Clipped and control saphenous vein segments were analyzed following 4 weeks and 8 weeks of clipping. (a) Spontaneous tone as a function of pressure. (b) Norepinephrine-induced tone (10 μM/l) as a function of pressure. (c) Ach-induced (endothelial) dilation as a function of pressure. *p<0.05, **p<0.01, ***p<0.001, significant difference between marked groups, according to one- and two-way ANOVA tests. $^{\#}p<0.05$, $^{\#\#}p<0.01$, different by paired t-test.*

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V./4. The effects of high-pressure low-flow conditions induced by chronic stricture of the main branch of the saphenous vein saphena on the collateral system of the saphenous main branch

Geometry of the venous networtk following 4 and 8 weeks of partial obstruction of the saphenous vein

When incising the inguinal skin of the reanesthetized animals, a rich bypassing collateral system was found on the clipped side under video-microscopic examination. Brush-like conglomerates, consisting of several hundred small veins, running mostly parallel, were observed, and they were leading away from the affected main branch (Figure 30.a). Some of these branches bypassed the artificial stricture, and some of them dilated forming corrugated collaterals. We could prove that blood flow was leading away from the main branch via these newly developed veins by analyzing the direction of flow as they filled up and by the serial pictures made after injecting either methylene blue or saline into one of the popliteal side-branches of the saphenous vein. The collateral system could be filled from the direction of the saphenous vein main branch using Batson 17 plastic in a retrograde manner. On the contralateral unclipped veins, such retrograde filling side-branches were scarcely observed, and existing valves in the vicinity of the confluence effectively closed retrograde flow. Cross sections of such multiple bypassing side-branches could also be observed on the histological sections (30.b).

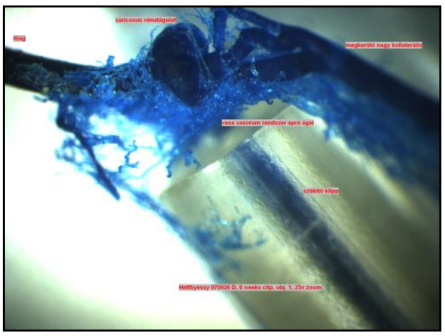

Figure 30. (a)

A rich bypassing collateral system was found on the clipped side under video-microscopic examination.

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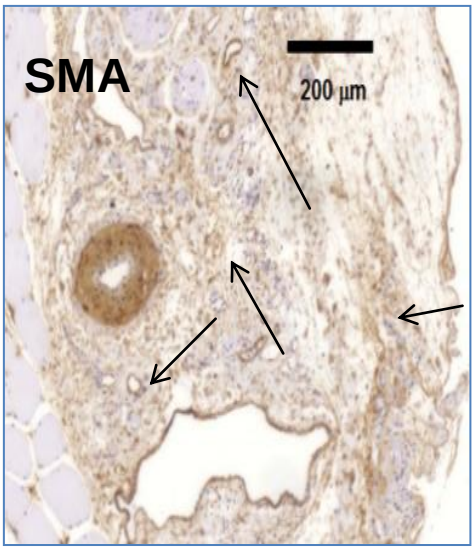

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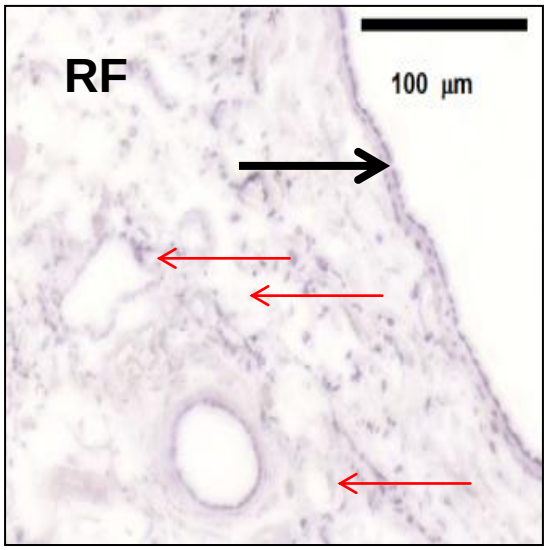

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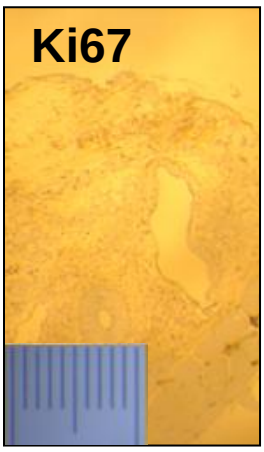

3.

4.

Figure 30.b

Histological samples

1. Network and wall (smooth muscle) remodeling. Note intensive smooth muscle actin (SMA,DAB, arrows) staining marking developed small collateral veins.

2. Network and wall (elastica) remodeling Resorcin-fuchsin staining. Note fragmented inner elastic membrane in main branch (black arrow) and weak staining in newly developed smaller collateral veins in the surrounding tissue red arrows).

3. Ki67 (cell division activity) in tissue area with intensive collateral vein neoformation.

4. CD68 (Macrophagic activity) in tissue area with intensive collateral vein neoformation

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V./5. The effects of high-pressure low-flow conditions induced by chronic stricture of the main branch of the saphenous vein on the histological characteristicsof the saphenous main branch

Inflammation, New cell formation

Histological analysis, CD68 staining proved the presence of macrophage invasion (Figure 31.a). With Ki67 stining there was evidence of new cell formation and at the occlusion site (Figure 31.b). The presence of a few (usually one or two) cells with cell division activity can be considered a normal feature – based on findings from the control side (Figure 31.c).

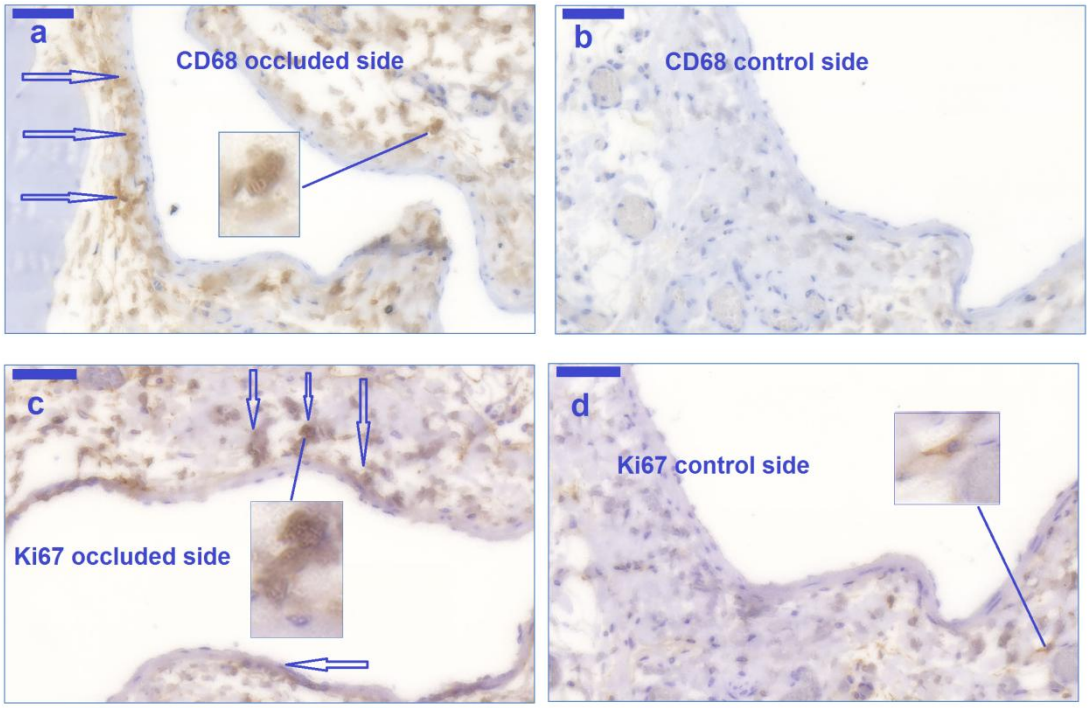

Figure 31.

Histological wall remodeling following four weeks of partial occlusion of the saphenous main branch. Immuno-histochemical staining. Arrows show positively staining structures. Bars, 50 µm. (a) CD68 (macrophage activity), clipped side; (b) CD68 control side. Macrophage activity was found to be much higher on the clipped sine. (c) Ki67 (cell division activity), clipped side; (d) Ki67, control side. Ki67 staining revealed an increase in new cell formation on the clipped side.

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Elasticity

Resorchin-fuchsin (RF) staining was performed to track alteration in the elastic component of the vessel wall revealed a massive remodeling of the elastic components in the wall. Quantitative image analysis was performed and we found that the inner elastic membrane became less condensed, and less organized in the clipped, remodeled segments (Figure 32.).

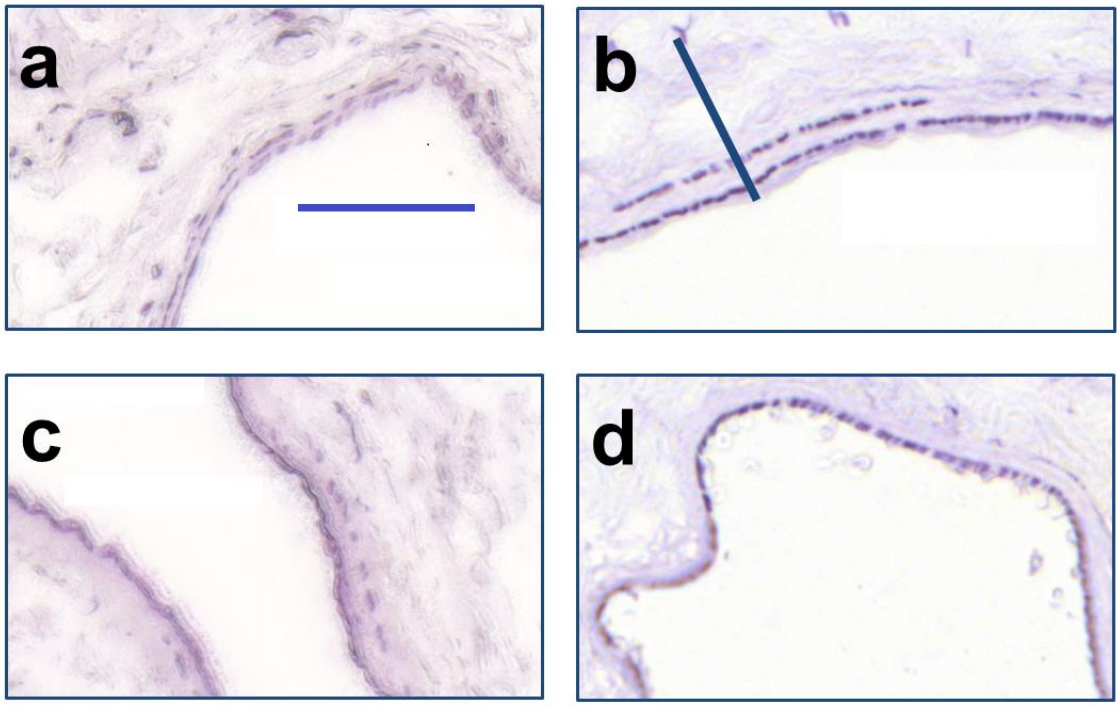
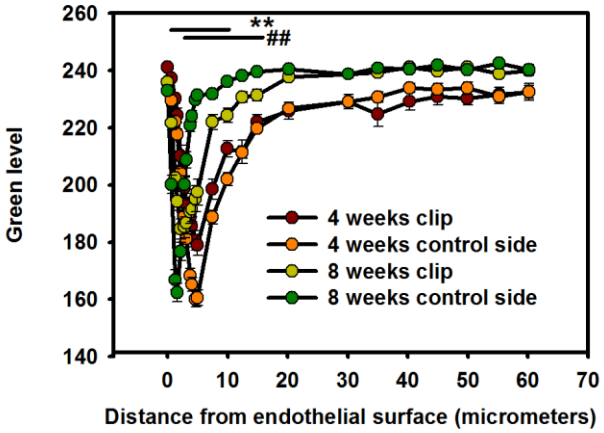


Figure 32.

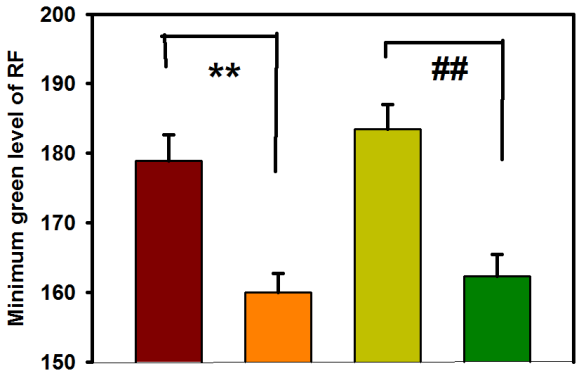
Remodeling of the elastic components after four and eight weeks of partial occlusion of the saphenous vein. (a to e) Resorcin-fuchsine-stained (RF) sections. (a) Four weeks occlusion clipped side; (b) four weeks control side; (c) eight weeks clipped side; (d) eight weeks control side. Bar, 50 mm (valid for a–d). Organized, condensed elastin became less condensed and fragmented on the occluded side.

Green density measurements did not show as low values in the vicinity of the endothelium of clipped side venous segments than in their unaffected contralateral controls (Figure 33.)($p<0.01$). This demonstrates a reduced compactness the inner elastic membrane on the clipped side. The most probable reason is that during

remodeling of the morphological lumen, the original inner elastic membrane should be at least partially digested away to ensure insertion of new elastic components.



(a)



(b)

Figure 33.

Remodeling of the elastic components after four and eight weeks of partial occlusion of the saphenous vein. (a) Results of RF elastica density measurements in the green color as a function of distance from the luminal surface. Lower green values represent higher elastica density. Each curve represents 5–8 rats, and 15–24 measurements were made on each venous segment. (b) Minimum values of the above density curves showing maximum inner elastic membrane densities. Color code identical with that of (a).

Another observation is that peripheral elastic elements diminish between four and eight weeks in controls. This process seems to be less effective in clipped segments (Figure 32.a-d, 33.a-b). Thus, clipped and unclipped segments were statistically

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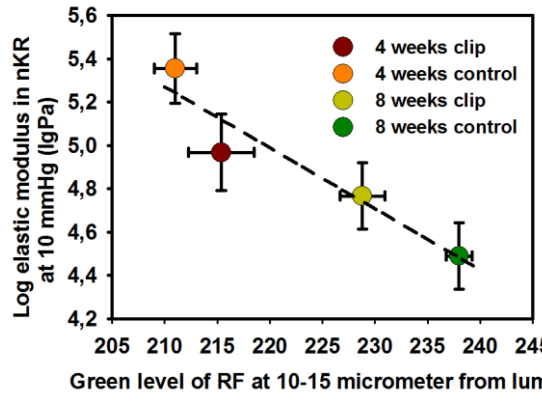
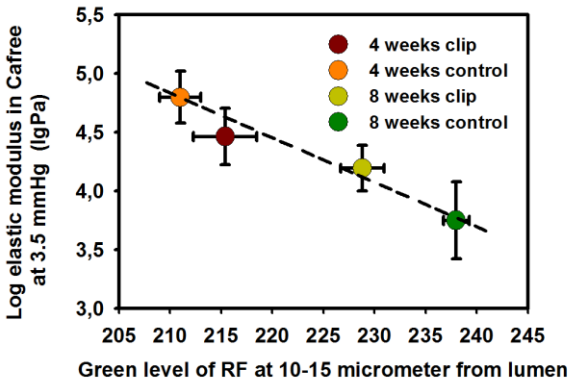
different ($p<0.01$) not only around their minimums, but in a distance of 0.3–10 μm (four weeks) and 3–17 μm (eight weeks) measured radially outward from the endothelial surface (on formaldehyde-fixed segments). The significance of elastic remodeling is outlined by tha fact that comparison of RF densities with measured elastic parameters revealed a close to linear function between elastic densities in the range of 10–15 μm from luminal surface and elastic modulus measured in spontaneous tone at 10 mmHg (Figure 34.a) or in the passive state at low pressure (Figure 34.b) $p<0.01$ for both).



(a)



(b)

Figure 34.

*Remodeling of the elastic components after four and eight weeks of partial occlusion of the saphenous vein. (a and b) Elastic moduli are plotted against RF green density in the range of 10–15 μm from the luminal surface. (a) Moduli of segments in the spontaneous tone at physiologically feasible higher pressure (10 mmHg). (b) Passive moduli at low pressures (2 mmHg). Statistical differences with one- and two-way ANOVAs are shown, **$p<0.01$ between clipped and controls at four weeks, $^{\#\#}p<0.01$ between clipped and controls at eight weeks. $^{\dagger\dagger}p<0.01$ statistical significance of the Pearson correlation (negative).*

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The applied low flow–high pressure hemodynamic challenge also induced a remodeling of the contractile elements: age-induced accumulation of the contractile protein in the inner medial layers was found to be less effective in clipped segments. (Figure 35.a-d).

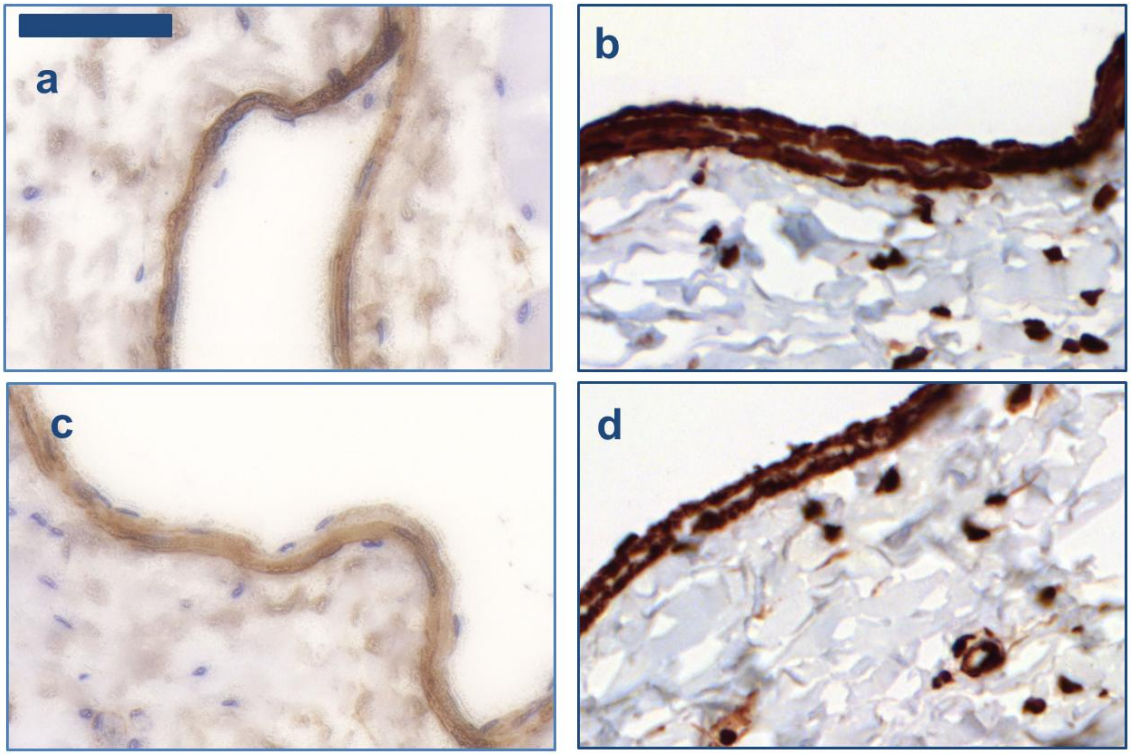

Figure 35.

a-d Remodeling of the contractile components after four and eight weeks of partial occlusion of the saphenous vein. Smooth muscle actin (SMA-DAB) immune-histochemistry. (a) Four weeks' occlusion; (b) four weeks' control; (c) eight weeks' occlusion; (d) eight weeks' control. We found that accumulation of the contractile proteins in the inner layers of the tunica media was less effective in clipped segments Bar, 50 mm (valid for a–d).

At the same time, both at week 4 and 8, there is more contractile protein present scattered in the outer layers of the media in the occluded segments. (Figure 37)($p<0.01$ with two-way ANOVA). The explanation seems to be that during morphological remodeling of the wall the area of contractile protein accumulation was less focused because of continuously changing mechanical stress.

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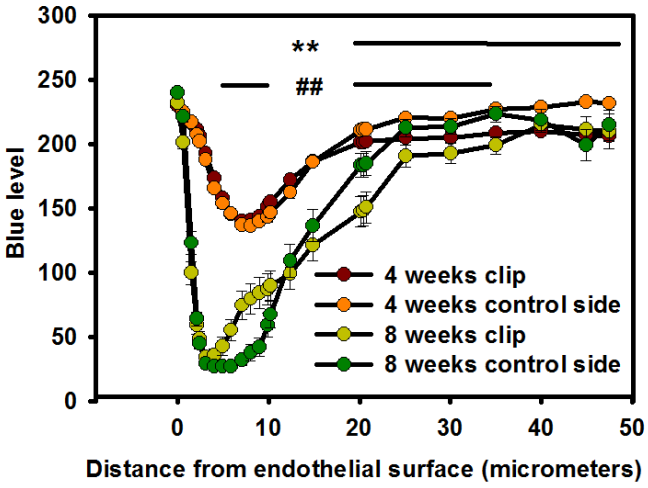

Figure 36.

*Density measured in blue color as a function of distance from the endothelial surface. At 4 and 8 weeks of occlusion, we found more contractile protein present scattered in the outer layers of the media in the occluded segments. **p<0.01 between clipped and controls at four weeks, ##p<0.01 between clipped and controls at eight weeks.*

DOI:10.14753/SE.2020.2305

VI. Discussion

Adaptive mechanism of the vasculature - which is a complex complex and highly versatile system – may be promted by many factors. Adaptation may take the form of biomechanical remodeling may occur as a consequence hemodynamic, endocrine and others factors. Remodeling machanisms vary depending on the specific segment, the hemodynamic feature altered altered (pressure/flow), and the endocrine enviroment, i.e. sex hormones.

Remodeling occurs along the vasculature in several diseases that have a large adverse impact on the well-being of the population. Coronary pathologies in hypertension affects the population in high numbers. A study of men and women aged 45 to 83 years showed the age-standardized prevalence of hypertension at baseline was 74.3% for men and 70.2% for women (Lacruz et al., 2015). Understanding the mechanisms through which coronaries adapt to hypertension is key in the possibility to impede deliterious effects of hypertension. Hypertension is a common pathological state in postmenopausal women; the geometrical, contractile, and mechanical properties of intraluminal coronary resistance arteries play a paramount role in hypertensive and ischemic heart disease. Judging from the statistics that show a definite difference in the degree to wich men and women are effected by coronary heart disease, it is sensible to suspect therole of sex hormones in the adaptation mechanisms of coronary arteries. As women are less effected my coronary heart disease the effects of female hormones, or lack of female hormones are important to understand in desiging research projects for therapy in the future. We have to recall here that intramural coronaries are mainly responsible for the blood supply of the heart. Such arteries are extremely difficult to examine in humans, therefore an animal model is paramount.

There is increasing evidence to suggest that sex hormones play a very diversified role in cardiovascular pathologies, and in turn in coronary remodeling. Limitation of our experiments are that estrogen therapy only was investigated, even though other sex hormones may play a role in the development or more importantly in the prevention of vascular pathologies in hypertension (i.e. progesterone). Another limitation of our experiments is that the specific effects of testosterone were not investigated. The strong point of our study is that it is a reliable animal model for the investigation of the elusive,

but pathophisiologically cardinal intramural coronaries. It is unique in stripping back to the basic biomechanical essence of sex differences and the role of sex hormonesin the remodeling of coronaries in hypertension. Through our experiments we may capture step-by-step the way the vascular wall adapt to pressure loading.

The adaptive mechanisms of the vasculature play a role in the development of another common disorder, varocosities. Varicosities are a widespresd disease, with prevalence reaching 56% in males, and 73% in females (Beebe-Dimmer, Pfeifer, Engle, & Schottenfeld, 2005; Robertson, Evans, & Fowkes, 2008). The significance of pressure load has been emphasized in the literature for a long time, however structural changes in the vessel wall, and flow-induced adaptations may also play a role in the development of varicosities. One of the strong suit of our experiments is that in provided a reliable animal model for the development of a collateral network reminiscent of that seen in the early stages of varicose disease. Another advantage is that this early stage remodeling prompted by a high-pressure low-flow environment was analyzed by a range of techniques (pressure-angiometry, morphological studies, histopathology etc.), thereby proviging us with a wide scope of information.

It is a well recognized fact that pressure will affect the vasculature both on the arterial and venous side. However we found that the remodeling that occurs as a result of a chronic rise in pressure differs based on the segment studied (arterial/venous), sex, and the hormonal environment provided. Different segment will demonstare different remodeling strategies in the face of pressure loading. Particulars will be discussed below.

Based on the literature and our experiments inflammation and inflammatory proteins are associated with vascular remodeling in both hypertension and varicose disease. It is not yet clear whether inflammation instigates, propagates, or accompanies vascular remodeling, however it appears, that they are linked. Limitation of our experiments is that inflamamtion was studies only in the setting of venous remodeling.

Following the structure that was followed in the Intrduction, Aims, Methods and Results section, the researched topics will be discussed in relevant sections also.

DOI:10.14753/SE.2020.2305

VI./1. Sex Differences in the Biomechanics of Intramural Coronary Arteries in Angiotensin II–Induced Hypertension

Chronic AngII treatment raised mean arterial pressure significantly, modeling the early stages for AngII-induced hypertension as expected. Adverse vascular changes have been demonstrated to follow (Mátrai et al., 2010).

In earlier studies we analyzed the potential biomechanical differences between intramural coronary resistance arteries of male and female rats and certain differences were found i.e. higher wall thickness and higher vascular contractility in males were associated with similar endothelial function and larger high-pressure elasticity compared to females (Mátrai et al., 2007).

In our current study we aim to understand the sex-related biomechanical adaptation in hypertension the same coronary segments. In females inward eutrophic remodeling and an increase in relative heart mass was found. However in males, we found an increase in wall strain with initial morphologic stability of the vessel wall. According to the data in the literature these alterations develop only following a longer period of hypertension.

When comparing the results of these two studies the higher wall thickness we found in AngII hypertensive female rats was the opposite of what we found in normotensives, in which males had thicker walls and, consequently, lower values of isobaric wall stress (Mátrai et al., 2007). There is limited data available on the biomechanical adaptation of this coronary segment Park et al (Park et al., 2008) found higher arterial elastance in hypertensive females than in males during physical exercise. Such differences disappeared when normalized to body surface in their study.

Sex chromosomes and therefore certain ganatic differences may also play a role in hypertension. Differences in the sympathetic nervous system and the renin - angiotensin system have been described to be linked to the Y chromosome. Angiotensin-converting enzyme inhibitors have also been described to be the most effective antihypertensive medications in females (Stimpel, Koch, & Oparil, 1998). We may draw hypothesize that in females AngII is the most important hypertensive stimulus. The underlying molecular mechanisms are the c-fos and c-jun pathways, which are linked to the estradiol and AngII reaction pathways. It has also been shown

that the expression of AT1R is decreased as an effect of estradiol (Gragasin, Xu, Arenas, Kainth, & Davidge, 2003; Koganti, Snyder, & Thekkumkara, 2012).

We found that ovariectomy led to an increase in blood pressure and a decrease in endothelium-dependent relaxation in AngII induced hypertension in female rats. The underlying causes includee alterations of nitric oxide and the renin-angiotensin system (Xu et al., 2008). Castration been shown to normalize increasing blood pressure in male rats, and ovariectomy led to increased blood pressure in females (Grigore, Ojeda, & Alexander, 2008). These phenomena were described to be mediated by the renin-angiotensin system, through testosterone (Ojeda et al., 2010). The effects of male sex hormones on the vascular system are controversial; it is now believed that testosterone dominantly affects the renin angiotensin system and increases sympathetic tone, leading to vasoconstriction and the progression of atherosclerosis (Kienitz & Quinkler, 2008).

In our current studies was found that wall thickness was greater in females, leading to lesser relative mechanical loading. In males the vessel wall was more rigid and the vessel wall showed less hypertrophy, which means that the mechanical loading was greater. In our series we found that optimizing mechanical loading was the primary adaptation mechanism in females, whereas preventing thickening of the wall was most important in males. From a biomechanical point of view the essence of this difference between the genders was that although hypertrophy of the wall to normalize wall stress was more apparent in females than in males, the dominant compensatory mechanism was an increase in wall rigidity, which was directly responsible for the blood supply of the heart muscle. In spontaneously hypertensive rats, Bonacasa et al (Bonacasa et al., 2008) observed decreased remodeling of the coronary arteries after methoxyestradiol treatment of oophorectomized females and intact males. Pressure load may also affect and damage the microvessel network of the heart more directly. This may explain certain aspects of the sex differences in the incidence of myocardial infarction as well as biomechanical differences found between the coronary arteries of males and females.

n our previous studies, as greater contracture could be evoked in normotensive males, (Várbiró et al., 2006) whereas no difference was found concerning spontaneous myogenic tone between the seyes (Mátrai et al., 2007). Thus hypertensive males “morphologically fix vasoconstriction,” whereas females reach an equilibrium via

remodeling (namely vessel wall hypertrophy, which compensates for greater pressure loading).

We believe that these differences can be attributed to different testosterone and estrogen levels (Grigore, Ojeda, & Alexander, 2008). Middle-aged and older men are under more pronounced endothelin receptor A vasoconstrictor control than women (Stauffer et al., 2010) Ca^{2+} current density was higher in whole cell clamps of vascular smooth muscle cells prepared from the coronary arteries of male miniature swine compared with those from female swine (Bowles, 2001).

In accordance with our findings isolated vascular smooth muscle cells from female rats have been shown to respond with less contraction to different stimulus than male rats (Ma et al., 2010). Endothelial nitric oxide release and vasodilation have been found to be stimulated by estradiol in coronary arteries, including cases of hypertension (Levy, Chung, Kroetsch, & Rush, 2009; Orshal & Khalil, 2004). Our studies revealed less remaining tone after BK-induced endothelial coronary dilation in long-term AngII–infused female rats than in males.

Short term - 10 days - AngII treatment in males has been shown to lead to upregulation of the NAD(P)H oxidase gp 67 phox subunit in the mesenterial vessels, resulting in mesenterial vascular dysfunction (Tatchum-Talom, Eyster, & Martin, 2005). In hypertension, the G protein coupled receptor kinase had partial effects on-adrenergic receptors in males and AngII receptors in females (Keys, Zhou, Harris, Druckman, & Eckhart, 2005).

In our model of single common etiology hypertension, AngII, age-matched animals, cardiac remodeling was observed in females as elevation in heart weight index, whereas this phenomenon did not reach the level of statistical difference in males. Based on unpublished data from our previous work we did not find gender differences in the normotensive state (0.32±0.02 g/100 g body weight in males vs. 0.32±0.02 g/100 g body weight in females); therefore, this difference in relative heart weight in hypertensive animals was caused by altered adaptation mechanisms in hypertension.We found that hypertrophic remodeling did not appear in the coronary arteries in femles but in cardiac muscle only.

The dominance of angiotensin-dependent mechanisms suggested that pharmacologic treatment might show sex differences as well. In our study of long term

DOI:10.14753/SE.2020.2305

AngII-induced hypertension, we found differences in the contractile reactivity of the intramural coronary small arteries. The extent of TxA_2-induced vasoconstriction was greater in the intramural coronary arteries of males than of females. Different segments of the coronary network showed different vasoconstrictor reactivity (Szekeres et al., 1998). Sex differences in the effects of TxA_2 have been described in epicardial coronary arteries. Secondary mesenterial arteries showed markedly stronger contractions in response to TxA_2 agonists than did the main branches (Nobe, Hagiwara, Nezu, & Honda, 2006).

VI./2. Estrogen therapy may counterbalance eutrophic remodeling of coronary arteries and increase bradykinin relaxation in a rat model of menopausal hypertension

It has been postulated however that menopausal hypertension may lead to different vascular adaptation mechanisms compared with those observed earlier in normotension (Mericli et al., 2004). In our study we focused on sexual steroid-related vascular adaptation in hypertension.

Geometry

In the early stages of AngII-induced hypertension unwelcome changes in vessel geometry, such as a decrease in coronary lumen have been described. In our series even though the lumen of the AngII+OVX group was significantly narrower, the cross-section of vessel wall did not differ compared to AngII only “hypertensive control” group. This is in accordance with eutrophic remodeling (Mulvany, 1999).

Estrogen has been proven to counterbalance adverse vascular effects of ovariectomy in certain regions, such as the hypothalamus (Szelke et al., 2008). An important observation of ours is that estrogen therapy leads to an increase in coronary vessel lumen despite maintained hypertension. The eutrophic wall remodeling seen in AngII hypertension was effectively counteracted by estrogen in our series.

DOI:10.14753/SE.2020.2305

Contractility

Key alterations were observed in our series regarding coronary contractility. Earlier work demonstrated that U46619 constrictions were elevated in the AngII hypertensive group compared with controls. Stabilization of this contracted lumen may be a possible mechanism responsible for the observed eutrophic remodeling (Mátrai et al., 2010).

In this series we did not observe a statistically significant difference in U46619-induced tone between the ovariectomized and hormone therapy groups (Figure 23.b). Spontaneous myogenic tone and remaining tone after BK-induced dilation however were higher in the control hypertensive and estrogen-treated groups compared with the ovariectomized group. This elevated spontaneous tone in vivo may allow for a greater functional range, and a greater capacity for vasodilation, and therefore may be considered to be a cardioprotective mechanism. From pathological and clinical point of view it is impressive that vasodilation to BK practically disappeared in the ovariectomy group, meaning that this key vasodilatative mechanism was practically lost. It is important to state, that it was retained in the estrogen therapy group.

Biomechanical parameters

In a previous study we found that tangential wall stress and elastic modulus decreased significantly in hypertensive animals compared with normal ones, especially at high-pressure levels (Mátrai et al., 2007). In the present series however no further difference was found in terms of wall stress, distensibility, and elastic moduli between ovariectomized and hormone therapy groups.

VI./3. Remodeling of the venous wall in high pressure-low flow conditions

Pressure as an etiological factor in the development of varicose veins has been discussed in detail in the literature (REFS). Little is known however on the effects of chronic altered flow conditions on the venous system of the lower limb. Our hemodynamic measurements showed a reduced flow and doubling of venous pressure in the strictured main branch (Figure 25. (a-b)). Based on the literature it would have been prudent to expect the dilation of the saphenous vein under these conditions. However

our studies unanimously confirmed that partial occlusion of the main branch of the saphenous vein did not lead to the expected varicosity-like pressure induced dilation, but instead morphological involution of the wall and of the lumen was observed. There was a significant decrease of the passive outer diameter of partially occluded segments in comparison to controls at weeks 4 and 8 alike (Figure 26.). We found that wall thickness (Figure 27.) and wall mass (Figure 27.) of the control side veins increased over the examined period of time however, this did not occur on the partially occluded side. The differences between occluded and non-occluded sides were statistically significant. Acting chronically the low–flow high pressure hemodynamic environment massively altered segmental geometry. After a combined biomechanical– histological study of the main branch, we can conclude that the wall had sufficient strength to withhold doubling of luminal pressure without any morphological distension. Our results suggest that these alterations are the result of chronic adaptation to reduced flow. Venous inflow into the main branch was directed toward the newly developed bypassing venous routes with ‘‘inverted flow’’ flow.

Such massive changes in wall geometry cannot be expected to occur without parallel changes in wall elasticity (Figure 28.) demonstrate that really that is the case. As measured by quantitative histochemistry, the structure of the inner elastic membrane of the wall of the occluded segments loosened and became less dense (Figure 32.) which corresponds with reduced passive elastic modulus at the physiologically relevant 1.5 kPa level wall stress. Elastic elements outward from the inner elastic membrane were also affected. A spontaneous reduction of such elements between weeks 4 and 8 is less effective. Our analysis suggests that the presence of fragmented dense elastic elements in the outward vicinity of the inner elastic membrane, at a distance of 10–15 μm from the endothelial surface (Figure 34.), is an important factor determining elastic modulus at low pressures in the passive state (Figure 34.) and also at higher pressure with some smooth muscle tone.

Another important observation of ours was reduced contractility. Both spontaneous tone and norepinephrine induced tone decreased (Figure 29.a-b). Actin density in the inner medial layers did not differ between clipped and non-clipped sides, and this cannot explain the observed significant reduction in the contractile force. The presence of more scattered smooth muscle elements in the outer layers of the venous

wall, however, marks that contractile elements are also affected by the low flow–high pressure remodeling process. There is an accumulation of the contractile proteins in the inner medial layers between weeks 4 and 8 even in controls with substantial elevation of contractile force. This process is less effective in clipped segments, or results in accumulation of scattered contractile elements in the outer layers without any improvement of contractility.

The almost total loss of endothelial dilation capacity after eight weeks of clipping (Figure 9.(h)) cannot occur without pathological consequences. According to analogies observed on resistance arteries, it can be connected with lowered flow and be responsible for lumen shrinkage. Such mechanism may play an essential role in the development of the human varicosity disease: venous branches occluded for longer periods by sustained isometric muscle contraction can be expected to shrink morphologically and also loose their ability to dilate at intermittent release of muscle tone. Flow will be diverted to pathologic direction by tsuch mechanisms.

VI./4.Early stage varicose disease

The relationship between pressure, flow and chronic venous disease has long been investigated (Bergan et al., 2008). Mechanisms such as turbulence shear force, (Fegan & Kline, 1972) increased wall tension, (Raffetto & Khalil, 2008) and inflammatory cascades have been suggested to lead to changes in vessel wall structure, and alteration in constriction/relaxation properties seen in varicose veins. More recently, a theory was introduced naming impending venous drainage, and outflow obstruction as the underlying cause of the formation of varices (Gaweesh, 2009; Neglén, Thrasher, & Raju, 2003).

To substantiante the relationship between pressure and the development of varicose veins an animal model was developed in our laboratory was based on the commonly accepted theory that the formation of varicose veins is mainly driven by pressure loading. Rats were submitted to chronic gravitational loading and during the course of this study several adaptive and pathological alterations were identified in the venous network. However, classic pathological remodeling characteristic for chronic venous disease with the appearance of reticular veins, teleangiectasias and varicose

dilations, torques or plaques was not observed (Lóránt et al., 2003; Monos et al., 1995; Monos et al., 1989; Monos et al., 2007; Raffai et al., 2008).

Observations on how vein structure, geometry, elasticity, and contractility are affected by varicose disease are divergent. An ultrasound study suggested that varicose vein sections may show dilatation of the lumen compared with the control with no change in the wall thickness itself. Wall thickness compensation in this case is by production of collagen instead of smooth muscle (Wali & Eid, 2001).

There is evidence to suggest that the formation of varicose veins may be due to the weakness of the vein wall as a result of significant increase in intimal and medial thickness and elevation of collagen content in the media with concomitant decrease of elastin content (Elsharawy, Naim, Abdelmaguid, & Al-Mulhim, 2007). The reduced amount and fragmented state of elastic fibers, (Kirsch et al., 2000) dysregulation of the synthesis of collagen I and III in smooth muscle cells, (Sansilvestri-Morel et al., 2001) and overexpressed aFGF in the wall via FGFR and the MAP kinase pathway may influence expression of enzymes involved in extracellular matrix metabolism (R. Kowalewski, Malkowski, Sobolewski, & Gacko, 2009) may also play a role in vein wall remodeling, leading to the described rigidity (Kirsch et al., 2000).

It has been suggested that smooth muscle cells derived from varicose veins are less differentiated and demonstrate increased proliferative and synthetic capacity than those derived from normal veins and may therefore contribute to the weakening capacity of the vein wall to resist pressure (Xiao, Huang, Yin, Lin, & Wang, 2009).

DOI:10.14753/SE.2020.2305

VII. Conclusions

Conclusions regarding differences between the sexes in terms of vascular remodeling in the coronary resistance arteries of the rat following angiotensin II induced hypertension

The early steps of angiotensin II–dependent hypertension evoked very different adaptation mechanisms in males and females. Vessel wall remodeling and an increase in relative heart weight in females was seen. Increased mechanical loading prevented remodeling in this stage in males.

Conclusions regarding the effects of ovariectomy and hormone replacement on the remodeling of intramural coronaries, which play a cardinal role in cardiovascular pathophysiology

Estrogen therapy has an opposite effect on vascular lumen as ovariectomy and AngII treatment, which produces eutrophic remodeling. Estrogen may therefore be considered to counterbalance the adverse changes seen in the vascular wall of intraluminal coronaries in the early stages of chronic hypertension. In the absence of estrogen NO-mediated dilation becomes vulnerable in the face of early chronic hypertension, opening the gate to further remodeling and vascular damage. Estradiol therapy leads to an elevation of spontaneous tone, allowing for greater range for vasomotion, meaning a greater capacity for dilation.

Conclusions regarding the effects of an increase in venous pressure and decrease of venous flow on the geometrical and biomechanical characteristics of the saphenous vein main branch in chronic main branch stricture model in rats

Involution of the wall and lumen was observed. Blood flow decreased and pressure elevated under high pressure low-flow conditions in the saphenous vein model. Contrary to expectations, the main branch did not dilate morphologically, instead involution with morphologically reduced lumen, reduced wall thickness and reduced wall mass was observed. High-pressure low-flow condition induced morphological

shrinking of the lumen in veins may override pressure-induced morphological distension.

Conclusions regarding evidence of collateral network formation following partial clipping of the main branch of the saphenous vein of the rat

The high-pressure low-flow environment created by partiel occlusion of the saphenous vein led to the development of a rich bypassing collateral network on the clipped side, morphologically reminiscent of early stage varicose disease, with brush-like conglomerates.

Conclusions regarding evidence of macrophage invasion and new cell formation at the clipping site in chronic main branch stricture model in rats

As a result of low-flow induced remodeling (i.e-morphological shrinkage) may override the passive distention induced by chronically elevated venous pressures. Loosening of the force-bearing elements during flow-induced wall remodeling may be an important pathological component in varicosity. Elastic modulus is dependent on the presence and amount of fragmented elastic elements in the media, but outward from the inner elastic membrane. The scattering of contractile elements leads to a substantial loss of contractile force.

In case of both elastic and contractile elements, not only the amount of tissue, but also their distribution in the different layers of the media has a decisive mechanical role. Such observations, we believe, should be taken into consideration when explaining the pathomechanism of varicose disease.

DOI:10.14753/SE.2020.2305

VIII. Summary

The aim of this thesis was to gain a more thorough understanding some of the hemodynamic and endocrine factors that play a role in the remodeling of the vascular wall of coronaries in hypertension, and the network of the saphenous vein in varicosity.

Differences between the sexes in terms of vascular remodeling in the coronary resistance arteries of the rat following early stages of angiotensin II induced hypertension was studied in a chronic Angiotensin II infusion model via in vitro pressure-angiometry. Different adaptation mechanisms were observes in males and females. Although we observed inward eutrophic remodeling in females, an increase in wall stress and elastic modulus dominated in males.

The effects of ovariectomy and hormone replacement on the remodeling of intramural coronaries were studied in the same chronic angiotensin II model, and via in vitro pressure angiometry. In hypertension, intramural small coronaries show inward eutrophic remodeling after ovariectomy comparing with hypertensive controls. Estrogen therapy had an opposite effect on vessel diameter. Hormone therapy led to an increase in spontaneous tone, allowing for greater dilatative capacity. Estrogen may therefore be considered to counterbalance some of the adverse changes seen in the wall of intramural coronaries in the early stages of chronic hypertension.

The effects of an increase in venous pressure and decrease of venous flow on the saphenous vein in chronic main branch low-flow high pressure stricture model in rats was studied via a partial chronic occlusion model. In vitro pressure-angiometry, intravital videomicroscopy, hemodynamic measurements, histology and immune-histology of the main branch and preparation of Batson 17 plastic casts were performed. Contrary to expectations, the main branch did not dilate morphologically, instead involution with morphologically reduced lumen, reduced wall thickness and reduced wall mass was observed. A rich collateral system appeared on the clipped side. Loosening of the force-bearing elements during flow-induced wall remodeling may be an important pathological component in varicosity.

DOI:10.14753/SE.2020.2305

IX. Összefoglaló

Jelen tanulmány célja a hipertóniában, illetve a vena saphena hálózatában kialakuló varicositásbetegségben kialakuló érfalátépülés hemodinamikai illetve endokrin hátterének feltérképezése.

Krónikus angiotenzin II infúzió adagolása mellett vizsgáltuk a nemek közötti különbségeket az intramurális koronáriák érfalátépülésében a következményesen kialakult korai hypertoniában; az érszakaszokat nyomás-angiometriai vizsgálatnak vetettük alá. A hímek illetve a nőstények különböző módon reagáltak a nyomásemelkedésre. A nőstények jelentősebb relaxációt mutattak bradykinin adása esetében. A nőstények eutróf érfalátépüléssel, míg a hímek magasabb falstressz értékekkel, illetve emelkedettebb elasztikus modulussal reagáltak a nyomásterhelésre.

Ugyanezt az angiotenzin II indukálta magasvérnyomás állatmodellt alkalmaztuk az ovariektómia és a hormonpótlás tanulmányozása céljából. Az érszakaszokat ebben az esetben is nyomás-angiometriával vizsgáltuk. Hipertóniában ovariektomiát követően az intramurális koronáriákon eutróf érátépülést tapasztaltunk a hipertenzív csoporthoz képest. Az ösztogénkezelés hatása ezzel ellentétes volt az érátmérő tekintetében. A hormonpótlás fokozta a vizsgált érszakaszok spontán tónusát, így egyben növelték a dilatációs tartalákot. Tekinthetjük tehát úgy, hogy az ösztrogén ellensúlyozhatja a korai hipertóniában tapasztalt érfalelváltozások egy részét az intramurális koronáriákban.

A vena sephena beömléséhez felhelyezett részleges okklúzió segítségével kialakított állatmodellen vizsgálatuk a nyomásterhelés és áramláscsökkenés hatását a vena saphena ágrendszerére. In vitro nyomás-angiometria mellett intravitális videomikroszkópos vizsgálatokat, hemodinamikai méréseket, szövettni és immunhisztológiai vizsgálatokat végeztünk, valamint Batson 17 mintákat készítettünk. A várakozásokkal ellentétben a vena saphena főága nem tágult ki, hanem involúciót tapasztaltunk, morfológiailag csökkent lumen, csökkent falvastagság illetve faltömeg mellett. Az klippelt oldalon gazdag kollaterálisrendszer kialakulását tapasztaltuk. A varikózitásbetegség kialakulásában a struktúrális elemek fellazulása fontos pathológiai tényező lehet.

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DOI:10.14753/SE.2020.2305

XI. Publications by the Author in this field

1. Máté Mátrai; Judit R Hetthéssy; György L. Nádasy; Emil Monos; Béla Székács; Szabolcs Várbíró; **2012** *Sex Differences in the Biomechanics and Contractility of Intramural Coronary Arteries in Angiotensin II–Induced Hypertension*
   Gender Medicine
   Vol. 9, No 6, pp. 548-56 IF: 1,69
2. Máte Mátrai; Judit R Hetthéssy; György L. Nádasy; Béla Székács;Metin Mericil;Nándor Ács; Emil Monos; Nissim Arbib; Szabolcs Varbiro*;* **2016**
   Estrogen therapy may counterbalance eutrophic remodeling of coronary arteries and increase bradykinin relaxation in a rat model of menopausal hypertension
   Menopause The Journal of The North American Menopause Society
   Vol. 23, No. 7, pp. 778-783 IF:3,172
3. Judit R Hetthéssy; Anna-Mária Tőkés; Sándor Kérész; Petra Balla; Gabriella Dörnyei; Emil Monos; György L. Nádasy*;* **2017**
   High pressure–low flow remodeling of the rat saphenous vein wall
   Phlebology 2018 Mar;33(2):128-137. doi: 10.1177/0268355516688984. Epub 2017 Jan 17. IF: 1,413

DOI:10.14753/SE.2020.2305

XII. Acknowledgements

I would like to express my very great appreciation to Dr. György László Nádasy, MD, PhD, Dr.habil., for his tireless patience, unwavering support, and continued faith in our work.

I would like to offer my special thanks to Professor Emil Monos for his professional guidance and valuable contributions during the planning and development of this research work.

I would like to express my deep gratitude to Dr. Máté Mátrai and Dr. Szabolcs Várbíró for contributing their skills, talent and many work hours to this research, and for their faith, support and friendship throughout the years.

I would like to express my very great appreciation to all of my co-authors for their many hours of labor and all of their contributions to this project.

Special thanks should be given to Professor Miklós Szendrői for his encouragement support and faith in this research.

I am grateful for the valuable support of Professor György Szőke.

I thank the expert technical assistance of Ms. Ildikó Oravecz.

I would also like to thank Dr. Zoltán Várady, Venenklinik, Frankfurt am Main, Germany for his valuable advice and continuous support.

I thank the Hungarian Hypertension Society for their support.

I would like te express my gratitude to my family and friends for their encouragement and support.

I would like to dedicate this work to my father for the life that made this work possible, and to my husband whose love knows no bounds.

Aunapuu, & Arend. (2005). Histopathological changes and expression of adhesion molecules and laminin in varicose veins. *Vasa, 34*(3), 170-175.