The Effect of Altered Redox Homeostasis on Vascular Wall Elasticity in Patients with Chronic Kidney Disease

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Reactive oxygen species (ROS) and reactive nitrogen species (RNS) play a physiological role as secondary messengers in a variety of cellular metabolic pathways. However, in certain pathological states the disequilibrium between oxidants and antioxidant systems can alter normal cell function and even induce deleterious phenotypical changes which further aggravate the underlying pathology. This has been especially evident in the case of vascular smooth muscle cells (VSMC) which are exposed to increased amounts of ROS, in various disease processes (i.e. atherosclerosis, diabetes). The purpose of this review is to examine the current knowledge regarding the effect of oxidative stress on vascular structure in general, and in chronic kidney disease, in particular.

Keywords: reactive oxygen species, reactive nitrogen species, oxidative stress, vascular structure, chronic kidney disease

Since the publication of *Gerschman*'s free radical theory of oxygen toxicity in 1954, considerable efforts have been made to study the effects of oxidative stress in a variety of pathologies. Free radicals are molecules or molecular fragments that contain at least one unpaired electron [1]. This unpaired electron gives the free radical considerable reactivity and enables it to react with various types of molecules (i.e. proteins, lipids, DNA molecules) [2].

Thus, redox disequilibrium has been implicated in a wide spectrum of diseases ranging from cardiovascular disease (i.e. atherosclerosis, ischemic heart disease, hypertension etc) to rheumatoid arthritis, ischemic stroke and Alzheimer's disease [2-6].

The effects of ROS can be both beneficial (occurring at low or moderate concentrations, as part of cellular signaling or defense mechanisms against infectious agents) or harmful (as they occur, for example, in states of chronic inflammation, diabetes, chronic kidney disease etc) [7]. Redox homeostasis is defined as a state of equilibrium between oxidant and antioxidant elements and due to excess of oxidants or deficit of antioxidants, any disruption of this equilibrium is termed oxidative stress [4,8].

Types of free radicals

Reactive oxygen species (ROS) are the most common types of free radicals found in many living systems with aerobic metabolism. ROS of interest include singlet oxygen ($^1O_2$), hydroxyl radical (HO^-), superoxide (O_2^-), peroxyl radical and hydrogen peroxide [9].

Molecular oxygen (O_2) is itself a free radical, having two unpaired electrons, however it does not have a great oxidizing capacity since it can only react with non-radicals that have electrons with the same spin number [1].

Singlet oxygen ($^1O_2$), the electronically excited form of oxygen is known to be a major cytotoxic species being implicated in carcinogenesis by photosensitization and metabolic activation of various carcinogens [9]. It is more reactive than oxygen in its steady state because the energy input required to generate singlet oxygen re-arranges the electrons and removes the spin restriction [1,9]. In biological systems singlet oxygen can be obtained through photosensitization or chemical reactions. Porphyrins, cytochromes as well as various drugs (i.e. tetracycline, thiazides) absorb energy and produce singlet oxygen species. Chemical reactions that produce singlet oxygen are catalyzed by a variety of enzymes such as myeloperoxidases, cytochromes and lipoxygenases. It has been shown that singlet oxygen is implicated in numerous physiological functions such as the metabolism of arachidonic acid, lipid peroxidation and the so-called respiratory burst generated by neutrophils, as part of the normal immune response to the presence of bacteria or other noxiae [9,10].

Superoxide anion radical (O_2^-) is generated, under physiological conditions, by the mitochondrial respiratory chain and significantly contributes to overall redox mitochondrial homeostasis [11]. Superoxide usually reacts close to the site of its formation, making available Fe and Cu for Fenton reactions (1) which produce highly oxidizing hydroxyl radical. It can also affect cellular compartments other than the mitochondria, but in most cases the inner mitochondrial membrane is the most exposed to the full oxidizing activity of this ROS [2,11].

The Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + OH^- \quad (1)$$

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Hydroxyl radical ($HO^{\cdot}$) has a high reactivity with a very short half-life of approximately 10^{-9} s [12]. The oxidizing effects of hydroxyl radical are usually limited to the site of its generation which is currently not very well defined [11]. Looking at the mentioned Fenton reactions it is understandable how hydroxyl radical generation largely depends on H_2O_2 production. The oxidation of amines which is carried out by monoamine oxidase on the outer aspect of the mitochondrial membrane yields H_2O_2 as a by-product. H_2O_2 reacts with superoxide to generate hydroxyl radical – the Haber-Weiss reaction (2) [2,13]. Other sources of H_2O_2 are peroxisomes, which, when damaged release their contents into the cytosol significantly contributing to oxidative stress [2].

The Haber-Weiss reaction:

$$O_2^{\cdot} + H_2O_2 \rightarrow O_2 + {}^{\cdot}OH + OH^- \quad (2)$$

Peroxyl radicals ($ROO^{\cdot}$), the simplest of which is hydroperoxyl radical ($HOO^{\cdot}$), the protonated form of superoxide is responsible for lipid peroxidation. It reacts selectively with organic molecules and determines fatty acid peroxidation [2,14].

Oxidative stress defense mechanisms

Antioxidants are molecules that scavenge and reduce the overall ROS burden at a cellular level and can be largely classified as enzymatic or non-enzymatic oxidative stress defense mechanisms. Non-enzymatic antioxidants are ascorbic acid, alpha-tocopherol, glutathione, carotenoids, flavonoids etc. Enzymatic antioxidants are represented by superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) [2].

Enzymatic antioxidants

Superoxide dismutase (SOD) dismutates superoxide anion to H_2O_2. Its three isoforms, SOD1 (Cu/Zn SOD), SOD2 (MnSOD) and SOD 3 (ecSOD) have various locations but they all catalyze the same reaction (3) [15].

Dismutation of superoxide anion:

$$O_2^- \xrightarrow{SOD} O_2 + 2H^+ \xrightarrow{SOD} H_2O_2 \quad (3)$$

*Catalase (CAT) f*acilitates the conversion of hydrogen peroxide to oxygen and water (4).

Hydrogen peroxide reduction by catalase:

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2 \quad (4)$$

Glutathione peroxidase (GPx) is a Se-dependent enzyme which helps reduce lipid and hydrogen peroxide. There are four isoenzymes (GPx1 found the cytosol of virtually all cells, GPx2 found primarily in the cells of the gastrointestinal tract, GPx3 is located in the plasma and GPx4 occurs in the mitochondria and interacts with lipoproteins damaged by free radicals (5) [16].

GPx catalyzed lipid peroxid reduction:

$$H_2O_2 + \underset{\text{lipid peroxide}}{ROOH} \xrightarrow{GPx} H_2O + \underset{\text{lipid alcohol}}{ROH} \quad (5)$$

Non-enzymatic antioxidants

Vitamin C is a potent antioxidant believed to exert its effects by scavenging ROS which result from normal cellular metabolism. *Tamari Y et al* have shown that ascorbic acid reduces superoxide levels in SOD1 and SOD2 depleted cells [17].

Glutathione is an endogenously synthesized antioxidant essential in the removal of oxidants and countering lipid peroxidation. It is found in the endoplasmic reticulum, nucleus and mitochondria. It serves as a cofactor for GPx and also scavenges, directly, hydroxyl radical and singlet oxygen [2].

α-Tocopherol is a lipophilic scavanger of free radicals. It prevents the oxidation of cellular membranes by reacting with lipid radicals which result from the lipid peroxidation chain reaction [18].

The effect of ROS on vascular smooth muscle cells

Reactive oxygen species as part of cell signaling systems

Signal transduction (or cell signaling) systems are means by which cells interact with the surrounding environment and enable them to communicate and coordinate with other cells. Reactive oxygen species have only recently come to be recognized also as secondary messengers, not just as unfortunate by-products of aerobic metabolism.

Several studies have revealed that ROS are used as secondary messengers in Angiotensin-II modulated vascular hypertrophy and that exposure to increased amounts of hydrogen peroxide stimulate vascular smooth muscle cell (VSMC) proliferation in different pathologies including chronic kidney disease (CKD) [19]. *Rao and Berk* demonstrated by using catalase and superoxide dismutase (SOD) scavenging systems that H_2O_2 generated by xanthine/xanthine oxidase was capable of eliciting a mitogenic response in VSMCs by increasing accumulation of early growth response genes c-myc and c-fos mRNA. In addition, they have shown that H_2O_2 had mitogenic effects only on VSMCs and not on endothelial cells or fibroblasts [20].

Furthermore, *Griendling et al* have shown that ROS are used as secondary messengers in the Angiotensin II signal transduction cascade with potential mitogenic effects. Stimulation with Angiotensin II causes activation of phospholipase D which, among other things, produces phosphatidic acid (PA). PA, in turn, stimulates NADPH oxidase which produces large amounts of superoxide. By using oxidase inhibitors they have discovered that reduction of superoxide amounts attenuates VSMC hypertrophy [21].

Byon et al have shown that exposure to 0.1-0.4 mM hydrogen peroxide induces phenotypical changes that promote calcification of VSMCs. VSMCs exhibited lower concentrations of specific smooth muscle markers (α-SMA and SM22-α) and increased expression of bone markers such as alkaline phosphatase (ALP), type I collagen (Col1) and osteocalcin (OC). It is apparent that the transcription factor Runx 2 (Runt-related transcription factor 2), associated with osteoblast differentiation is implicated in the phenotypical switch of the VSMCs, however it cannot be specified whether expression of Runx 2 is a consequence of vascular calcification or the cause for it [22,23]. Furthermore, VSMCs under oxidative stress by exogenous hydrogen peroxide produce themselves ROS through a NAD(P)H-oxidase feed-forward mechanisms, which could promote the further degradation of overall vascular health [24].

Hydrogen peroxide also induces VSMC inflammation. As it has been established, atherosclerosis is associated with a proinflammatory status. Macrophages and monocytes infiltrate the vascular wall and induce ROS production. The presence of ROS in the vascular wall further amplify the inflammatory response as evidenced by increased expression of VCAM-1 (vascular cell adhesion molecule 1), monocyte chemoattractant protein-1 (MCP 1), fractalkine and osteopontin [22,25].

The contribution of VSMCs to vascular wall stiffness

Vascular wall elasticity can be regarded as the result of complex interactions between the three layers and their respective components, interactions which take place at a molecular level (between the cellular and non-cellular elements) and dictate the physical properties of the entire system. Thus, it is easily understandable why any changes that arise in pathological states (atherosclerosis, diabetes, dialysis etc) in the intima or media, predominantly, affect the mechanics and the performance of the vascular wall [26,27].

Impressive efforts have been made to analyze the impact of atherosclerosis on vascular wall stiffness and the number of studies on this subject underlines the overall burden atherosclerosis has on cardiovascular disease epidemiology and outcomes. However, not enough attention has been given to the consequences of the altered metabolism and general function of VSMCs that has been so well documented in various disease processes. Furthermore, it is increasingly apparent that a better understanding of vascular wall stiffness is needed to more efficiently counteract the deleterious effects that rigid arteries pose to overall health.

There are many studies that have focused on describing the role of the extracellular matrix (ECM) in the pathogenesis of arterial wall stiffness, with further insight into the mechanical properties of collagen and elastin fibers. Several authors have reported that increased amounts of collagen and decreased amounts of elastin are responsible, among other factors, for the stiffening of the vascular wall [28]. However, other authors have demonstrated that increase in collagen/elastin ratio is not invariably associated with vascular stiffness seen in hypertension or normal aging [29]. This has lead researchers to believe there are more complex phenomena involved and that further studies are needed to properly identify the factors involved in vascular wall mechanics.

Recently, focus has shifted to studying the contribution of VSMCs to arterial elasticity. VSMCs have a critical role in regulating vascular tone under normal conditions. In pathological states it has been shown that VSMCs undergo hypertrophy and can even modify their behavior by dedifferentiating to an osteogenic phenotype, thereby contributing to vascular calcification [30-33]. Furthermore, *Sehgel et al* reported that VSMCs themselves become stiff due to alterations in cytoskeleton structure associated, mostly with aging and independent of arterial pressure [30].

Additionally, VSMCs' interaction with ECM proteins through integrins and fibronectin and with each other through cadherines contribute to the overall elasticity of the system. These interactions also respond to various vasoactive agents that induce cytoskeletal changes associated with cell stiffness. Exposure to Angiotensin II increased contractile fiber thickness and orientation while adenosine decreased fiber thickness, consistent with a process of depolymerization [34]. Moreover, AngII increased adhesion probability to type I collagen fibers, observation which suggested that vasoconstrictors influence how VSMCs attach themselves to ECM [34].

Another factor which apparently contributes to the increase in vascular stiffness is chronic inflammation, as illustrated by a number of studies that have linked inflammatory disorders (i.e. rheumatoid arthritis, inflammatory bowel disease etc) to increased cardiovascular mortality. The mechanisms by which chronic inflammation affects vascular wall structure are currently not well understood, however a possible link could reside in the oxidative stress generated by the sustained activation of the non-specific immune response [30,35].

Relevant biomarkers of oxidative stress in chronic kidney disease

The study of ROS has proven relatively difficult because they are highly reactive and have short half-lives and because assessment methods vary greatly. It is therefore more efficient to determine the by-products that result from the interaction of ROS with various molecules, however these measurements have also proven quite unreliabile in establishing cause-effect relationships since they are not tissue-specific and are influenced by coexisting pathologies which are recognised as major influence factors of the oxidant/antioxidant equilibrium and are frequently associated with CKD (cardiovascular diseases, anemia, mineral and bone disorder, diabetes mellitus etc) [36-38]; furthermore, there are studies showing that hemodialysis has an negative impact on miocardium contractility (even in the absence of changes in angiography or troponin levels), probably due to enhaced oxidative stress (table 1) [39-42].

Table 1
RELEVANT BIOMARKERS OF OXIDATIVE STRESS IN CKD

NO.	STUDIES	BIOMARKER	SOURCE	CHARACTERISTICS	REFERENCES
1.	Atamer et al Yilmaz et al Rutkowski et al Kuo et al	Malondialdehyde *(MDA)*	Result of PUFA peroxidation	Positively correlates with uremic toxins Inversely correlates with GFR	[43-46]
2.	Locatelli et al Del Rio et al Nelson et al Bober et al	Thiobarbituric acid-reactive substances *(TBARS)*	Measure lipid peroxidation. Used to quantify MDA	Low specificity React with unrelated aldehydes, prostaglandins etc Positively correlate with creatinine Negatively correlate with antioxidant levels	[47-50]
3.	Roberts and Morrow Milne et al Dounousi et al Ikizler et al	F2-Isoprostanes	Generated from arachidonic acid in COX-independent pathways after oxidative injury	Can be determined in plasma and urine Correlate with CKD stage and inflammatory markers Inherent chemical reactivity	[51-56]
4.	Girotti, Annuk et al Chatterjee et al Kamat et al	Lipid hydroperoxides *(LOOHs)*	Derived from phospholipids, glycolipids and cholesterol	Trigger apoptosis Correlate with CRP, TBARS, MDA and obesity Negatively correlate with GFR and do not decrease with dialysis	[55-58]

5.	Sydow and Munzel Gocmen et al Nakamura et al Nanayakkara et al Aslam et al	Asymmetric dimethylarginine *(ADMA)*	Predictor of superoxide generation Competitive inhibitor of eNOS	Correlates with TBARS, MDA, and CKD stage Negatively correlates with CrCl Decreased amounts after antihypertensive therapy	[59-63]
6.	Sebekova et al Apeland et al Fragedaki et al Atamer et al Scholze et al Alvares Delfino et al	Homocysteine	Results from cysteine metabolism	Correlates with MDA, AOPP, Isoprostanes, CRP Increased in CKD Negatively correlates with paraoxonase-1 (PON1) Diminished by dialysis, NAC and folic acid	[43,64-68]
7.	Witko-Sarsat and Descamps-Latscha Antolini et al Kuchta et al Lentini et al	Advanced oxidation protein products *(AOPP)*	Oxidation-altered albumin, fibrinogen, lipoproteins	Markers of chronic inflammation in CKD High levels in AKI Correlate with MDA, AGE, CrCl, uremic toxins	[69-72]

§Legend: ADMA = asymmetric dimethylarginine; AGE = advanced glycation end products; AKI = acute kidney injury; AOPP = advanced oxidation protein products; CKD = chronic kidney disease; COX = cyclooxygenase; CrCl = creatinine clearance; CRP = C-reactive protein; eNOS = endothelial nitric oxide synthase; GFR = glomerular filtration rate; LOOHs = lipid hydroperoxides; MDA = malondialdehyde; NAC = N-acetylcysteine; PON1 = paraoxonase-1; PUFA = polyunsaturated fatty acid; TBARS = thiobarbituric acid-reactive substances.

§Modified after: Tucker PS, Dalbo VJ, Han T, Kingsley MI. Clinical and research markers of oxidative stress in chronic kidney disease. Biomarkers. 2013; 18(2):103-115.

The use of AOPP in evaluating patients with CKD

In terms of studying oxidative stress in patients with chronic kidney disease, advanced oxidation protein produts (AOPP) have proven useful in quantifying the amount of oxidative damage in this particular class of patients. Plasma levels of AOPP correlate with other markers of oxidative stress (dityrosine and pentosidine), are highest in patients undergoing hemodialysis, and substantially increased in patients with advanced CKD compared to healthy controls [73]; this proinflammatory state has been also documented in peritoneal dialysis patients, regardless the presence of peritonitis, with negative impact on long-term prognosis [74-76].

Unlike other markers of oxidative stress, AOPP seem to be implicated directly in the pathogenesis of several glomerular disorders. In IgA nephropathy elevated AOPP levels are a strong predictor of poor outcome [77]. In addition, AOPP are associated with proteinuria. Increased exposure to AOPP causes podocyte apoptosis and activation of the renin-angiotensin system [77].

In patiens suffering from CKD, AOPP may induce endothelial dysfunction and are independently correlated with flow mediated dilation and aggravate the atherogenic pehnotype by inhibiting plasma clearance of HDL-cholesterol and by decreasing cholesterol efflux from macrophages, thus promoting foam cell formation.

Implications of oxidative stress on vascular wall elasticity in chronic kidney disease

The association between chronic uraemia and oxidative stress has been well demonstrated by several researchers [73,78]; however, the mechanisms by which ROS affect the outcome of patients with chronic kidney disease have proven ellusive. Uremic toxins themselves are a source of oxidative stress, uric acid synthesized by the xanthine oxidoreductase complex enhances progression of CKD [78]. Furthermore, uremic toxins exert a proinflammatory effect by stimulating the innate immune response, thus increasing the generation of ROS [78-80].

More importantly, because of the negative impact of oxidative stress on the vascular wall, the influence of chronically increased ROS on the arterio-venous fistula (AVF) outcome should be considered in end-stage renal disease patients requiring vascular access creation for hemodialysis initiation. AVF surgery success and adequate maturation, still an important chalange for the physicians, require sufficiently dilated and elastic blood vessels; in this manner, an adequate blood flow will be provided for an efficient dialysis therapy [81]. However, chronic oxidative stress conditions induce stiffness of VSMCs and consequently improper vessel dilation. A very important requirement of AVF maturation is nitric oxide (NO) dependent vasodilation induced by increased flow through the newly created vascular segment. The increased shear stress stimulates endohelial cells to synthesize NO, a capacity that is diminished in states of chronic inflammation and oxidative stress, such as CKD [82,83].

While endothelial dysfunction may be a common denominator in patients experiencing AVF failure which can be attributed to systemic factors (diabetes, advanced atherosclerosis) it does not provide the sole explanation to why some forms of vascular access succeed and why others do not. Eventually all AVFs suffer from neointimal hyperplasia (NH) but this process has variable consequences depending on the preexisting conditions of the patient. For example, diabetic patients have larger vein diameters to begin with than non-diabetic patients, a characteristic which would help prevent access failure [84]. Unfortunately this has not been the case since this particular subpopulation of ESRD patient experience higher failure rates than others. It has now come to light that outward vascular remodelling is just as important as inward remodelling [85].

Further studies are required for the proper understanding of AVF failure mechanisms but it seems the understanding of oxidative stress may open new therapeutic avenues which could help prolong vascular access patency and thus improve the efficiency of hemodialysis treatment.

Conclusions

It is becoming increasingly apparent that the mechanisms which contribute to vascular wall stiffening are extremely complex and do not solely rely on endothelial dysfunction and atheromatous plaque formation, and that a closer look is required on identifying the contribution of

altered vascular smooth muscle cell metabolism and function. In addition, the key to properly explain how VSMCs are involved in the pathogenesis of arterial wall stiffness might reside in correctly understanding the effect that exposure to reactive oxygen species has on overall vascular structure and mechanical properties.

While AOPP may not be directly linked to the stiffening of the vascular wall, they may prove to be a useful prognosis marker for AVF maturation in the CKD subpopulation.

References

1.HALLIWELL, B., Plant. Physiol., **141**, nr. 2, 2006, p. 312
2.VALKO, M., LEIBFRITZ, D., MONCOL, J., CRONIN, M.T., MAZUR, M., TELSER, J., Int. J. Biochem. Cell Biol., **39**, nr. 1, 2007, p. 44
3.GERSCHMAN, R., GILBERT, D.L., NYE, S.W., DWYER, P., FENN, W.O., Science, **119**, nr. 3097, 1954, p. 623
4.MANDA, G., CHECHERITA, A.I., COMANESCU, M.V., HINESCU, M.E., Mediat. Inflamm., nr. 604208, 2015
5.CEULEMANS, A.G., ZGAVC, T., KOOIJMAN, R., HACHIMI-IDRISSI, S., SARRE, S., MICHOTTE, Y., J. Neuroinflammation, **7**, 2010, p. 74
6.PIRICI, D., ION, D.A., MOGOANTA, L., MARGARITESCU, O., PIRICI, I., FOARFA, C., TUDORICA, V., PANDURU, N.M., COCONU, M., CHECHERITA, I.A., Rom. J. Morphol. Embryol., **52**, nr. 2, 2011, p. 699
7.CHECHERITA, I.A., MANDA, G., HINESCU, M.E., PERIDE, I., NICULAE, A., BILHA, ª., GRAMATICU, A., VORONEANU, L., COVIC, A., Int. Urol. Nephrol., **48**, nr. 3, 2016, p. 373
8.DROGE, W., Physiol. Rev., **82**, nr. 1, 2002, p. 47
9.DEVASAGAYAM, T.P., KAMAT, J.P., Indian J. Exp. Biol., **40**, nr. 6, 2002, p. 680
10.CADENAS, E., SIES, H., Methods Enzymol., **319**, 2000, p. 67
11.CADENAS, E., DAVIES, K.J., Free Radic. Biol. Med., **29**, nr. 3-4, 2000, p. 222
12.PASTOR, N., WEINSTEIN, H., JAMISON, E., BRENOWITZ, M., J. Mol. Biol., **304**, nr. 1, 2000, p. 55
13.LIOCHEV, S.I., FRIDOVICH, I., Free Radic. Biol. Med., **16**, nr. 1, 1994, p. 29
14.AIKENS, J., DIX, T.A., J. Biol. Chem., **266**, nr. 23, 1991, p. 15091
15.FUKAI, T., USHIO-FUKAI, M., Antioxid. Redox Signal., **16**, nr. 6, 2011, p. 1583
16.ESPINOZA, S.E., GUO, H., FEDARKO, N., DEZERN, A., FRIED, L.P., XUE, Q.L., LENG, S., BEAMER, B., WALSTON, J.D., J. Gerontol. A. Biol. Sci. Med. Sci., **63**, nr. 5, 2008, p. 505
17.TAMARI, Y., NAWATA, H., INOUE, E., YOSHIMURA, A., YOSHII, H., KASHINO, G., SEKI, M., ENOMOTO, T., WATANABE, M., TANO, K., Free Radic. Res., **47**, nr. 1, 2013, p. 1
18.TRABER, M.G., ATKINSON, J., Free Radic. Biol. Med., **43**, nr. 1, 2007, p. 4
19.CHECHERITA, I.A., DAVID, C., STOICA, L., POPESCU, P., CIOCALTEU, A., LASCAR, I., Rom. J. Morphol. Embryol., **52**, nr. 2, 2011, p. 533
20.RAO, G.N., BERK, B.C., Circ. Res., **70**, nr. 3, 1992, p. 593
21.GRIENDLING, K.K., MINIERI, C.A., OLLERENSHAW, J.D., ALEXANDER, R.W., Circ. Res., **74**, nr. 6, 1994, p. 1141
22.BYON, C.H., HEATH, J.M., CHEN, Y., Redox Biol., **9**, 2016, p. 244
23.BYON, C.H., JAVED, A., DAI, Q., KAPPES, J.C., CLEMENS, T.L., DARLEY-USMAR, V.M., MCDONALD, J.M., CHEN, Y., J. Biol. Chem., **283**, nr. 22, 2008, p. 15319
24.LI, C., XU, Q., Cell. Signal., **19**, nr. 5, 2007, p. 881
25.HU, T., LUAN, R., ZHANG, H., LAU, W.B., WANG, Q., ZHANG, Y., WANG, H.C., TAO, L., Clin. Exp. Pharmacol. Physiol., **36**, nr. 7, 2009, p. 626
26.ROMAN-GARCIA, P., RODRIGUEZ-GARCIA, M., CABEZAS-RODRIGUEZ, I., LOPEZ-ONGIL, S., DIAZ-LOPEZ, B., CANNATA-ANDIA, J.B., Med. Princ. Pract., **20**, nr. 3, 2011, p. 203
27.DAVID, C., BOVER, J., VOICULET, C., PERIDE, I., PETCU, L.C., NICULAE, A., COVIC, A., CHECHERITA, I.A., Int. Urol. Nephrol., **49**, nr. 4, 2017, p. 689
28.MOURLON-LE GRAND, M.C., POITEVIN, P., BENESSIANO, J., DURIEZ, M., MICHEL, J.B., LEVY, B.I., Arterioscler. Thromb., **13**, nr. 5, 1993, p. 640
29.SEHGEL, N.L., SUN, Z., HONG, Z., HUNTER, W.C., HILL, M.A., VATNER, D.E., VATNER, S.F., MEININGER, G.A., Hypertension, **65**, nr. 2, 2015, p. 370
30.SEHGEL, N.L., VATNER, S.F., MEININGER, G.A., Front. Physiol., **6**, 2015, p. 335
31.LI, C., XU, Q., Cell. Signal., **19**, nr. 5, 2007, p. 881
32.GHEORGHIU, M.L., GALOIU, S., VINTILA, M., PURICE, M., HORTOPAN, D., DUMITRASCU, A., COCULESCU, M, POIANA, C., Hormones (Athens), **15**, nr. 2, 2016, p. 224
33.PIKILIDOU, M., YAVROPOULOU, M., ANTONIOU, M., YOVOS, J., J. Vasc. Res., **52**, nr. 1, 2015, p. 32
34.HONG, Z., SUN, Z., LI, M., LI, Z., BUNYAK, F., ERSOY, I., TRZECIAKOWSKI, J.P., STAICULESCU, M.C., JIN, M., MARTINEZ-LEMUS, L., HILL, M.A., PALANIAPPAN, K., MEININGER, G.A., J. Physiol., **592**, nr. 6, 2014, p. 1249
35.KLOCKE, R., COCKCROFT, J.R., TAYLOR, G.J., HALL, I.R., BLAKE, D.R., Ann. Rheum. Dis., **62**, nr. 5, 2003, p. 414
36.TUCKER, P.S., DALBO, V.J., HAN, T., KINGSLEY, M.I., Biomarkers, **18**, nr. 2, 2013, p. 103
37.NICULAE, A., DAVID, C., DRAGOMIRESCU, R.F.I., PERIDE, I., TURCU, F.L., PETCU, L.C., COVIC, A., CHECHERITA, I.A., Rev. Chim.(Bucharest), **68**, nr. 2, 2017, p. 354
38.CAPATINA, C., CARAGHEORGHEOPOL, A., BERTEANU, M., POIANA, C., Exp. Clin. Endocrinol. Diabetes., **124**, nr. 8, 2016, p. 461
39.MCINTYRE, C.W., BURTON, J.O., SELBY, N.M., LECCISOTTI, L., KORSHEED, S., BAKER, C.S., CAMICI, P.G., Clin. J. Am. Soc. Nephrol., **3**, nr. 1, 2008, p. 19
40.BURTON, J.O., JEFFERIES, H.J., SELBY, N.M., MCINTYRE, C.W., Clin. J. Am. Soc. Nephrol., **4**, nr. 5, 2009, p. 914
41. FARCAS, A., GLIGOR, F., BUCSA, C., MOGOSAN, C., BOJITA, M., DUMITRASCU, D., Farmacia, **63**, nr. 3, 2015, p. 325
42.NECHITA, A.M., PITURU, S., RADULESCU, D., PERIDE, I., NEGREANU, L., NICULAE, A., FERECHIDE, D., CHECHERITA, I.A., SINESCU, R.D., Farmacia, **64**, nr. 3, 2016, p. 348
43.ATAMER, A., KOCYIGIT, Y., ECDER, S.A., SELEK, S., ILHAN, N., ECDER, T., ATAMER, Y., J. Nephrol., **21**, nr. 6, 2008, p. 924
44.YILMAZ, M.I., SAGLAM, M., CAGLAR, K., CAKIR, E., SONMEZ, A., OZGURTAS, T., AYDIN, A., EYILETEN, T., OZCAN, O., ACIKEL, C., TASAR, M., GENCTOY, G., ERBIL, K., VURAL, A., ZOCCALI, C., Am. J. Kidney Dis., **47**, nr. 1, 2006, p. 42
45.RUTKOWSKI, P., SLOMINSKA, E.M., SZOLKIEWICZ, M., ALEKSANDROWICZ, E., SMOLENSKI, R.T., WOLYNIEC, W., RENKE, M., WISTEROWICZ, K., SWIERCZYNSKI, J., RUTKOWSKI, B., Scand. J. Urol. Nephrol., **41**, nr. 3, 2007, p. 243
46.KUO, H.T., KUO, M.C., CHIU, Y.W., CHANG, J.M., GUH, J.Y., CHEN, H.C., Eur. J. Clin. Invest., **35**, nr. 4, 2005, p. 245
47.LOCATELLI, F., CANAUD, B., ECKARDT, K.U., STENVINKEL, P., WANNER, C., ZOCCALI, C., Nephrol. Dial. Transplant., **18**, nr. 7, 2003, p. 1272
48.DEL RIO, D., STEWART, A.J., PELLEGRINI, N., Nutr. Metab. Cardiovasc. Dis., **15**, nr. 4, 2005, p. 316
49.NELSON, S.K., BOSE, S.K., GRUNWALD, G.K., MYHILL, P., MCCORD, J.M., Free Radic. Biol. Med., **40**, nr. 2, 2006, p. 341
50.BOBER, J., KEDZIERSKA, K., KWIATKOWSKA, E., STACHOWSKA, E., GO³EMBIEWSKA, E., MAZUR, O., STANIEWICZ, Z., CIECHANOWSKI, K., CHLUBEK, D., Ann. Acad. Med. Stetin., **56**, nr. 3, 2010, p. 5
51.ROBERTS, L.J., MORROW, J.D., Free Radic. Biol. Med., **28**, nr. 4, 2000, p. 505
52.MILNE, G.L., SANCHEZ, S.C., MUSIEK, E.S., MORROW, J.D., Nat. Protoc., **2**, nr. 1, 2007, p. 221
53.DOUNOUSI, E., PAPAVASILIOU, E., MAKEDOU, A., IOANNOU, K., KATOPODIS, K.P., TSELEPIS, A., SIAMOPOULOS, K.C., TSAKIRIS, D., Am. J. Kidney Dis., **48**, nr. 5, 2006, p. 752

54.IKIZLER, T.A., MORROW, J.D., ROBERTS, L.J., EVANSON, J.A., BECKER, B., HAKIM, R.M., SHYR, Y., HIMMELFARB, J., Clin. Nephrol., **58**, nr. 3, 2002, p. 190
55.GIROTTI, A.W., J. Lipid. Res., **39**, nr. 8, 1998, p. 1529
56.ANNUK, M., SOVERI, I., ZILMER, M., LIND, L., HULTHE, J., FELLSTROM, B., J. Nephrol., **18**, nr. 6, 2005, p. 721
57.CHATTERJEE, S.R., SRIVASTAVA, T.S., KAMAT, J.P., DEVASAGAYAM, T.P., Mol. Cell. Biochem., **166**, nr. 1-2, 1997, p. 25
58.KAMAT, J.P., SARMA, H.D., DEVASAGAYAM, T.P., NESARETNAM, K., BASIRON, Y., KAMAT, J.P., SARMA, H.D., DEVASAGAYAM, T.P., NESARETNAM, K., BASIRON, Y., Mol. Cell. Biochem., **170**, nr. 1-2, 1997, p. 131
59.SYDOW, K., MUNZEL, T., Atheroscler. Suppl., **4**, nr. 4, 2003, p. 41
60.GÖCMEN, A.Y., SAHIN, E., KOCAK, H., TUNCER, M., GÜMÜºLÜ, S., Clin. Biochem., **41**, nr. 10-11, 2008, p. 836
61.NAKAMURA, T., SATO, E., FUJIWARA, N., KAWAGOE, Y., UEDA, Y., SUZUKI, T., UEDA, S., ADACHI, H., OKUDA, S., YAMAGISHI, S., Pharmacol. Res., **60**, nr. 6, 2009, p. 525
62.NANAYAKKARA, P.W., TEERLINK, T., STEHOUWER, C.D., ALLAJAR, D., SPIJKERMAN, A., SCHALKWIJK, C., TER WEE, P.M., VAN GULDENER, C., Kidney Int., **68**, nr. 5, 2005, p. 2230
63.ASLAM, S., SANTHA, T., LEONE, A., WILCOX, C., Kidney Int., **70**, nr. 12, 2006, p. 2109
64.SEBEKOVA, K., GAZDIKOVA, K., SYROVA, D., BLAZICEK, P., SCHINZEL, R., HEIDLAND, A., SPUSTOVÁ, V., DZÚRIK, R., J. Hum. Hypertens., **17**, nr. 4, 2003, p. 265
65.APELAND, T., MANSOOR, M.A., SELJEFLOT, I., BRØNSTAD, I., GORANSSON, L., STRANDJORD, R.E., J. Intern. Med., **252**, nr. 5, 2002, p. 456
66.FRAGEDAKI, E., NEBEL, M., SCHUPP, N., SEBEKOVA, K., VOLKEL, W., KLASSEN, A., PISCHETSRIEDER, M., FRISCHMANN, M., NIWA, T., VIENKEN, J., HEIDLAND, A., STOPPER, H., Nephrol. Dial. Transplant., **20**, nr. 9, 2005, p. 1936
67.SCHOLZE, A., RINDER, C., BEIGE, J., RIEZLER, R., ZIDEK, W., TEPEL, M., Circulation, **109**, nr. 3, 2004, p. 369
68.ALVARES DELFINO, V.D., DE ANDRADE VIANNA, A.C., MOCELIN, A.J., BARBOSA, D.S., MISE, R.A., MATSUO, T., Nutrition, **23**, nr. 3, 2007, p. 242
69.WITKO-SARSAT, V., DESCAMPS-LATSCHA, B., Nephrol. Dial. Transplant., **12**, nr. 7, 1997, p. 1310
70.ANTOLINI, F., VALENTE, F., RICCIARDI, D., BARONI, M., FAGUGLI, R.M., Clin. Chim. Acta., **358**, nr. 1-2, 2005, p. 87
71.KUCHTA, A., PACANIS, A., KORTAS-STEMPAK, B., CWIKLINSKA, A., ZIETKIEWICZ, M., RENKE, M., RUTKOWSKI, B., Kidney Blood Press. Res., **34**, nr. 1, 2011, p. 12
72.LENTINI, P., DE CAL, M., CRUZ, D., CHRONOPOULOS, A., SONI, S., NALESSO, F., ZANELLA, M., GARZOTTO, F., BRENDOLAN, A., PICCINNI, P., RONCO, C., J. Crit. Care., **25**, nr. 4, 2010, p. 605
73.WITKO-SARSAT, V., FRIEDLANDER, M., CAPEILLERE-BLANDIN, C., NGUYEN-KHOA, T., NGUYEN, A.T., ZINGRAFF, J., JUNGERS, P., DESCAMPS-LATSCHA, B., Kidney Int., **49**, nr. 5, 1996, p. 1304
74.ISVORANU, I., RADULESCU, D., PERIDE, I., NICULAE, A., SINESCU, R.D., CHECHERITA, I.A., Rev. Chim.(Bucharest), **66**, no. 8, 2015, p. 1239
75.MITOIU, D., DAVID, C., PERIDE, I., NICULAE, A., MURESAN, A., CIOCALTEU, A., GEAVLETE, B.F., CHECHERITA, I.A., Rom. J. Morphol. Embryol., **55**, nr. 4, 2014, p. 1409
76.ISVORANU, I., PERIDE, I., RADULESCU, D., NICULAE, A., SINESCU R.D., CHECHERITA, I.A., Rev. Chim.(Bucharest)., **66**, no. 9, 2015, p. 1316
77.CAO, W., HOU, F.F., NIE, J., Kidney Int. Suppl. (2011), **4**, nr. 1, 2014, p. 102
78.SMALL, D.M., COOMBES, J.S., BENNETT, N., JOHNSON, D.W., GOBE, G.C., Nephrology (Carlton), **17**, nr. 4, 2012, p. 311
79.MARTINON, F., PETRILLI, V., MAYOR, A., TARDIVEL, A., TSCHOPP, J., Nature, **440**, nr. 7081, 2006, p. 237
80.SANCHEZ-LOZADA, L.G., SOTO, V., TAPIA, E., AVILA-CASADO, C., SAUTIN, Y.Y., NAKAGAWA, T., FRANCO, M., RODRÍGUEZ-ITURBE, B., JOHNSON, R.J., Am. J. Physiol. Renal Physiol., **295**, nr. 4, 2008, p. F1134
81.CHECHERITA, I.A., TUTA, L.A., DAVID, C., PERIDE, I., NICULAE, A., GEAVLETE, B.F., PRICOP, C., ION, D.A., Rom. J. Morphol. Embryol., **56**, nr. 1, 2015, p. 27
82.MEZZANO, D., PAIS, E.O., ARANDA, E., PANES, O., DOWNEY, P., ORTIZ, M., TAGLE, R., GONZALEZ, F., QUIROGA, T., CACERES, M.S., LEIGHTON, F., PEREIRA, J., Kidney Int., **60**, nr. 5, 2001, p. 1844
83.GEENEN, I.L., KOLK, F.F., MOLIN, D.G., WAGENAAR, A., COMPEER, M.G., TORDOIR, J.H., SCHURINK, G.W., DE MEY, J.G., POST, M.J., PLoS One, **11**, nr. 1, 2016, p. e0146212.
84.LAZICH, I., CHANG, A., WATSON, S., DHAR, P., MADHURAPANTULA, R.S., HAMMES, M., Hemodial. Int., **19**, nr. 4, 2015, p. 490
85.HAMMES, M., Biomed. Res. Int., **2015**, 2015, p. 171674.

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