Oxidative Stress & Hypertension: A Balancing Act

Inaugural Lecture of Prof. Carina Mels

10 December 2021

NWU® | Hypertension in Africa Research Team

1. Introduction

What is balance? As a noun balance is "*an even distribution of weight enabling someone or something to remain upright and steady*" or "*a situation in which different elements are equal or in the correct proportions*." That sounds easy enough, but why do we keep on getting it wrong? In our everyday life we fight constantly to maintain balance between work, our lives, our families, and our health. While we are struggling on the outside to maintain what is known as work-life balance, the human body is facing its own internal challenges to maintain homeostasis.

In this regard the human body is in constant chemical communication with the external environment. When we eat, nutrients are absorbed through the lining of the digestive tract. When we breath gases such as oxygen and carbon dioxide move across the epithelium of the alveoli of the lungs, and waste products are being excreted on a continuous basis. Even though these processes occur at various specialised sites in the human body, they affect every organ, tissue and cell instantaneously due to the fact that all parts of the human body are linked to the cardiovascular system [1].

2. The cardiac cycle

To gain a complete understanding of the cardiovascular system, you must start at the heart of it all – the cardiac cycle. The cardiac cycle can be defined as the period between two consecutive heart beats and include periods of contraction and relaxation. At the start of the cardiac cycle known as *ventricular diastole*, the ventricles are filled with blood. At the end of *ventricular diastole*, the atria will contract to complete ventricular filling, followed by *ventricular systole* when the ventricles will start to contract and the atrioventricular valves will close. The increase in pressure inside the ventricles will lead to the opening of the pulmonary and aortic valves and when these valves open, blood is forced out of the ventricles into the aorta and pulmonary arteries from where the blood will be distributed to all parts of the human body. Thereafter the ventricles will start to relax, the pressure inside the ventricles will drop and the aortic and pulmonary valves will shut until a new cardiac cycle commence [1].

During the cardiac cycle pressure in the large and small arteries will fluctuate, increasing during ventricular systole and decreasing during ventricular diastole. Systolic blood pressure is the highest blood pressure measured during systole, and diastolic blood pressure the lowest blood pressure at the end of diastole. In the reporting of blood pressure measurements this will be reported as two separate values such as 120/80 mmHg [1].

In addition to the contribution of the cardiac cycle (or cardiac output) to blood pressure, peripheral arterial resistance and vascular compliance (elastic properties) also play important roles. To maintain blood flow, the pressure in the circulation must be great enough to

overcome the resistance to flow provided by the entire cardiovascular system – known as total peripheral resistance. Total peripheral resistance is a function of vascular resistance, the viscosity of blood and turbulence. Viscosity is determined by the number of cells such as red blood cells; white blood cells and platelets present in the blood and an increase in viscosity will lead to increased resistance to flow. While turbulence is caused by high flow rates or irregular surfaces due to injury of the blood vessel. The most important determinant of vascular resistance is friction between blood and the vessel wall, where the amount of friction will depend on the length and the diameter of the vessel. Since the length of a blood vessel can't be changed, vascular resistance is determined by changes in the diameter of blood vessels via contraction and relaxation of the smooth muscle cells in the vessel walls [1].

3. Blood pressure regulation

Given the very important role of the cardiovascular system, it is no surprise that blood pressure is tightly regulated. When the balance or homeostasis in the human body is disturbed, the cardiovascular system must adapt very quickly to ensure adequate blood flow to the tissue or organ where the change occurred. The cardiovascular system therefore engages in resource allocation. For example, when you exercise the skeletal muscles, the heart and the lungs will have a higher demand for blood flow but following a meal the demand for blood flow will shift towards the digestive system. Various mechanisms are therefore employed to regulate blood pressure, including autoregulation, neural and endocrine mechanisms [1].

Autoregulation is the main mode of blood pressure regulation under normal resting conditions when cardiac output remains stable and entail adjusting of peripheral resistance. Upon alterations in the local environment such as changes in oxygen or carbon dioxide levels, the smooth muscle cells will respond automatically to these changes in the local environment via the release of either a vasodilator such as nitric oxide or a vasoconstrictor such as endothelin-1 [1].

When autoregulation becomes ineffective a neural response will be triggered where the nervous system will act via two receptor reflexes namely the baroreceptor and the chemoreceptor reflexes. The baroreceptors are responsible to detect changes in pressure and the chemoreceptors changes in oxygen, carbon dioxide and pH levels. Stimulation of these reflexes with result in the adjustment of cardiac output and peripheral resistance to maintain adequate blood flow [1].

The endocrine mechanism relies on the release of hormones such as epinephrine and nor-epinephrine which will result in short-term changes in cardiac output and peripheral resistance. Whereas, long-term regulation relies on various other hormones (such as antidiuretic hormone (ADH), the renin-angiotensin-aldosterone system (RAAS), erythropoietin (EPO) and atrial

natriuretic peptide (ANP)) to control blood volume, sodium levels and red blood cell production [1].

4. Ethnic disparities in blood pressure regulation

Although mechanisms of blood pressure regulation are universal to mankind some ethnic disparities - most likely related to external factors such as rapid urbanization are evident. In this regard it was shown in a study done in China, that cardiovascular disease events such as heart attacks and stroke were projected to double in number in urban areas. It was further indicated that rural to urban migration may lead to rapid changes in cardiovascular risk factors such as increases in blood pressure as demonstrated by a higher systolic blood pressure of 6-7 mmHg in urban migrants when compared to their rural counterparts [2].

Bringing this back to sub-Saharan Africa, it is interesting to note that less than 100 years ago, it was nearly impossible to find a single patient with hypertension in a hospital in Kenya [3]. However, with the recent demographic transition on the African continent, adverse health behaviours are expected to escalate with strong trends already evident. Africa also experience some of the world's greatest economic, educational and health inequalities, which may also contribute significantly to this problem [4,5].

In a recent study by Gafane-Matemane et al., we provided a detailed profile of the RAAS, electrolytes, volume loading, blood pressure and total peripheral resistance in healthy young black and white adults. We found that plasma renin activity in the healthy black adults was almost half of what we observed in their white counterparts. Longitudinal associations between aortic blood pressure and the RAAS revealed that an increase in aortic blood pressure over ≈4.5 years was associated with elevated aldosterone levels – only in black adults [6]. In fact, higher 24-hour, daytime, and nighttime blood pressure were previously reported in both black men and women compared to their white counterparts and may also be associated with a phenomenon known as early vascular ageing [7].

Early vascular ageing reflects increased arterial stiffness at younger chronological ages and in a recent review article, ethnic disparities in routine blood pressure measures, hypertension and cardiovascular outcomes were further investigated in the context of early vascular ageing. In many studies comparing arterial stiffness between ethnic groups over the life-course (including children as young as 6 years of age) ethnic disparities were found, where populations from African descent presented with increased aortic stiffness from young ages [8].

Other mechanisms such as the balance between factors associated with autoregulation of blood pressure may further explain the observed ethnic disparities and that was the focus of my research till date.

5. The balancing act between vasodilation and vasoconstriction

The concept of changes in the diameter of blood vessels via the contraction and relaxation of smooth muscle cells in the vessel walls are closely related to endothelial function. Nitric oxide and endothelin-1 are natural counterparts in this equation and tipping the balance between vasodilation and vasoconstriction may lead to endothelial dysfunction and vascular remodelling.

Under normal physiological conditions the bioavailability of the vasodilator – nitric oxide will be high, which will lead to the inhibition of endothelin-1 synthesis and reduced binding of endothelin-1 to its receptors and consequently all downstream activities including vasoconstriction. However, with a reduction in nitric oxide bioavailability, endothelin-1 activities such as increased vasoconstriction and vascular remodelling will be unmitigated [9].

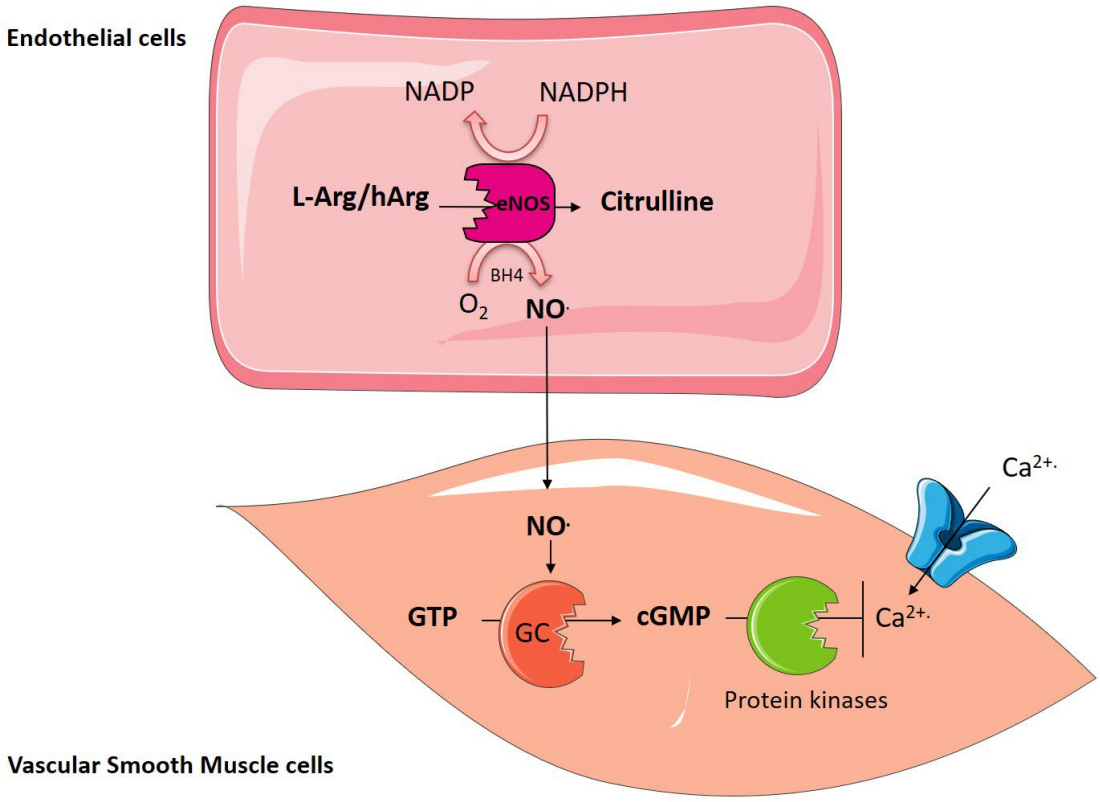

Figure 1: Nitric oxide synthesis and its role in vasodilation

L-Arg, L-Arginine, hArg, Homoarginine; eNOS, endothelial Nitric oxide synthase; NADP, Nicotinamide adenine dinucleotide phosphate; NADPH, Reduced Nicotinamide adenine dinucleotide phosphate; O_2, Oxygen; BH4, Tetrahydrobiopterin; $NO^{\cdot}$, Nitric oxide; GTP, Guanosine triphosphate; GC, Guanylyl Cyclase; cGMP, cyclic Guanosine monophosphate; $Ca^{2+\cdot}$, Calcium.

The bioavailability of nitric oxide is determined by the balance between the rate of its synthesis and the rate of its breakdown or inactivation. In the vasculature (Figure 1), nitric oxide is

produced form the amino acid L-arginine (or its homologue homoarginine) and oxygen in a reaction catalysed by the enzyme endothelial nitric oxide synthase (eNOS). This enzymatic conversion of arginine to nitric oxide also requires the presence of reduced cofactors including tetrahydrobiopterin (BH4) and nicotinamide adenine dinucleotide phosphate (NADPH). After synthesis, nitic oxide diffuses into vascular smooth muscle cells where it activates soluble guanylyl cyclase (sGC). This will lead to the production of cyclic guanosine monophosphate (cGMP), which will reduce calcium release and calcium influx due to the action of protein kinases to elicit vasorelaxation [10,11].

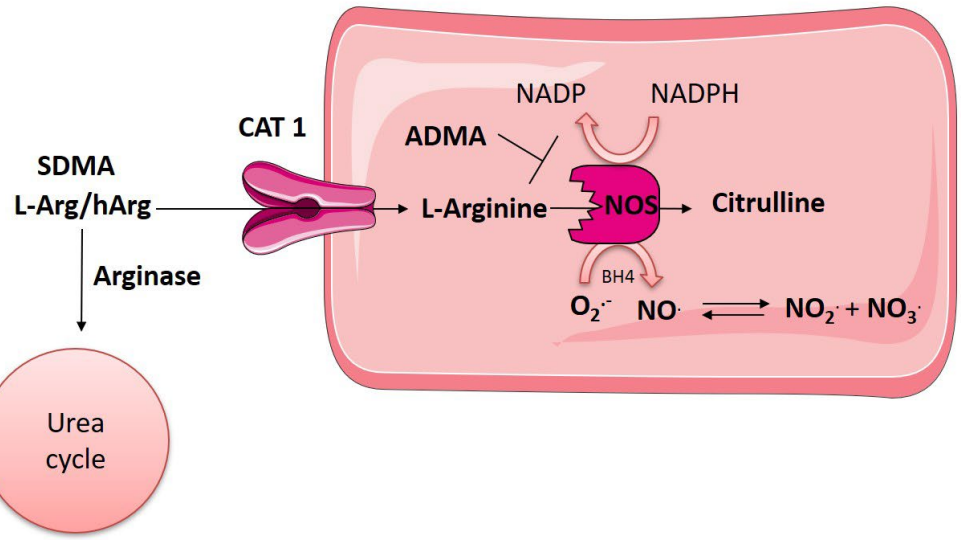

Figure 2: Factors influencing nitric oxide bioavailability

SDMA, Symmetric dimethylarginine; L-Arg, L-Arginine; hArg, Homoarginine; CAT1, Cationic amino acid transporter 1; ADMA, Asymmetric dimethylarginine NADP, Nicotinamide adenine dinucleotide phosphate; NADPH, Reduced nicotinamide adenine dinucleotide phosphate; NOS, Nitric oxide synthase; O_2, Oxygen; BH4, Tetrahydrobiopterin; $NO^{\cdot}$, Nitric oxide; $NO_2^{\cdot}$, Nitrite; $NO_3^{\cdot}$, Nitrate.

Factors influencing nitric oxide bioavailability include the availability and transport of its main substrate(s) namely L-arginine and homoarginine, endogenous inhibitors of the NOS enzyme and nitric oxide inactivation (Figure 2). L-arginine is a semi-essential amino acid which can be

synthesized from essential amino acids in cases of dietary insufficiency. Although L-arginine deficiency is rare, it may occur with increased L-arginine metabolism via the enzyme arginase in the urea cycle. Transport of L-arginine into endothelial cells occur via the cationic amino acid transporter (CAT1). However, symmetric dimethyl arginine (SDMA) may lower L-arginine uptake by competing with L-arginine for transport, which may indirectly affect nitric oxide synthesis. Asymmetric dimethyl arginine (ADMA) may also impact on nitric oxide synthesis as it is a competitive inhibitor of the eNOS enzyme. Alternatively, since nitric oxide has a high affinity for superoxide, binding of these two molecules will lead to the formation of peroxinitrite leading to the inactivation of nitric oxide. Nitric oxide can also undergo metabolism to nitrite and nitrate, which may serve as a nitric oxide store as it can be reduced back to release nitric oxide via several pathways [12,13].

6. Atherosclerosis and arterial stiffness

Apart from its role in vasodilation, nitric oxide also plays an important role in several inhibitory actions including the inhibition of platelet aggregation, monocyte adhesion, smooth muscle cell proliferation, oxidative stress, and oxidative modification of low-density lipoprotein (LDL) cholesterol the so called “bad” cholesterol. In turn, all these inhibitory actions of nitric oxide maintain healthy endothelial function and thus prevent the onset of atherosclerosis and arterial stiffness [14].

Atherosclerosis is the term used for the process of plaque (or fatty deposit) build up in the inner lining of an artery. With the build-up of plaque, the wall of the blood vessel will thicken and consequently this will narrow the blood vessel and thereby reduce blood flow. This is a slow, lifelong process that may start in childhood and get worse as you age. One of the important initiating factors in the development of atherosclerosis is the transport of oxidized LDL cholesterol into the artery wall at sites where the endothelium is damaged. When endothelial cells are damaged adhesion molecules (such as P-selectin), and chemotactic factors (such as monocyte chemoattractant protein-1) are expressed. These adhesion molecules and chemotactic factors will lead to the attachment of leukocytes such as monocytes and T-lymphocytes to the endothelial cells. The endothelial cells, leukocytes and smooth muscle cells will secrete growth factors and chemo-attractants with consequent migration of the monocytes and leukocytes into the subendothelial space. The uptake of lipoproteins by the monocytes will transform these cells into macrophages and eventually into foam cells. When foam cells combine with leukocytes fatty streaks will form and smooth muscle cells will start to migrate into the intima of the vasculature where they will start to proliferate. More advanced lesions will start to form, and a fibrous plaque may bulge into the arterial lumen. Calcification and continued fibrosis may lead to the formation of a fibrous cap, which may ultimately rupture leading the formation of a thrombus (blood clot). This whole

process can be measured by means of ultrasound where we measure the thickness of the intima media layer of the carotid arterial wall and an indication of plague formation [15].

Several mechanisms involved in the development of atherosclerosis are also associated with changes in the extracellular matrix of the vessel wall and these processes may also lead to the stiffening of arteries. With contraction of the left ventricle a pulse wave is generated which travels down the arterial tree. When you are young, your aorta is still elastic, and the pulse wave takes longer to travel through the arterial tree, but with ageing, the aorta and other arteries in the body lose their elastic properties and the pulse wave will travel much faster through the arterial tree. This will cause the reflected wave to return earlier to the heart and will increase the pressure in the aorta. Over time the increased aortic pressure will impact negatively on your heart and other organs, known as target organ damage. The time it takes for this pulse wave to travel between two sites in the arterial tree also known as pulse wave velocity can be measured non-invasively to give an indication of arterial stiffness. The measurement of pulse wave velocity in the carotid to femoral segment is considered as the "golden standard" measurement for arterial stiffness [16].

7. The mosaic theory of hypertension

When keeping the various blood pressure regulation mechanisms in mind, it is no surprise that hypertension (chronically elevated blood pressure) is a complex, multifactorial, and multisystem disorder. The multifactorial nature of hypertension was described for the first time in the 1940's by Dr. Irvine Page when he formulated the mosaic theory of hypertension. This theory proposed that the etiology of hypertension is multifactorial, and that genetic, environmental, anatomic, adaptive, neural, endocrine, humoral and hemodynamic forces all contribute to the development of hypertension [17]. Since then, vast progress was made in terms of discoveries explaining the molecular and cellular basis underlying hypertension and the work of three Nobel Prize winning scientists in 1998 for their discovery of nitric oxide made a huge contribution in this regard [18]. Later, in 2013, upon revisiting this theory of Dr. Irvine Page it became evident that all the components of the mosaic theory also link with oxidative stress [19]. Recently, Dr. Rhian Touyz published a review paper in which oxidative stress is portrayed as a unifying paradigm in hypertension [20] (Figure 3).

8. Oxidative stress

If oxidative stress is at the centre of hypertension development, it is important to know what is oxidative stress? According to the textbook definition oxidative stress is "*an imbalance between oxidants and antioxidant in favor of the oxidants that leads to a disruption of oxidation-reduction (redox) signaling and control and molecular damage*" [21].

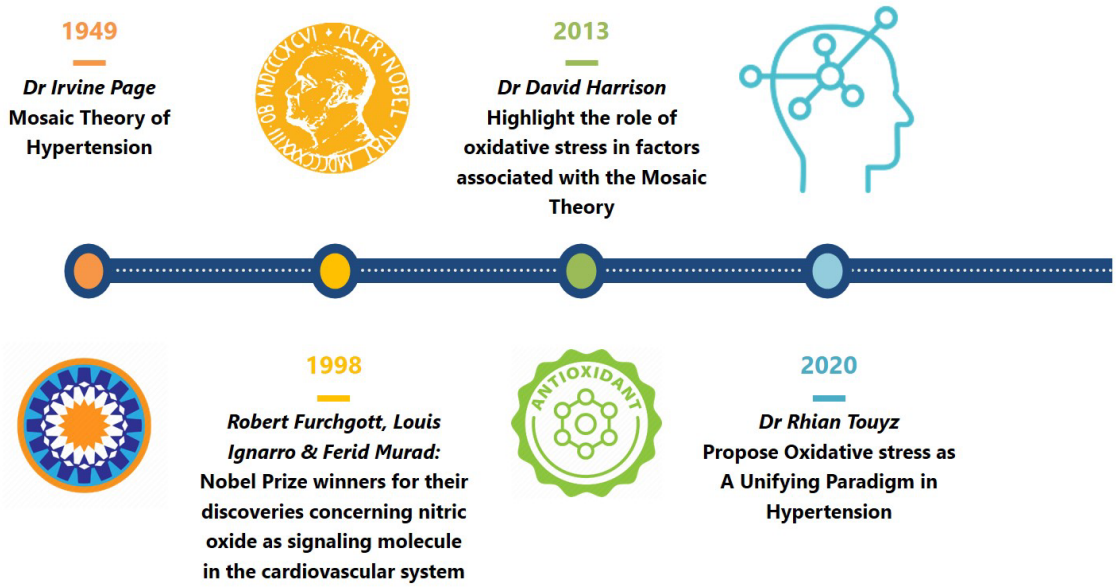

Figure 3: Evolution of the mosaic theory of hypertension

But what is oxidants and antioxidants? All atoms contain electrons in spaces known as orbitals and normally these electrons are present in pairs. However, some molecules known as free radicals have an unpaired electron rendering these molecules unstable (and unhappy). To regain stability, free radicals have the habit of stealing electrons from other molecules. Antioxidants on the other hand are very generous molecules willing to donate their spare electrons.

Various normal physiological processes are involved in the production of free radicals known as reactive oxygen species (ROS), including NADPH oxidases – the major source of ROS production in the cardiovascular system, mitochondrial oxidases, xanthine oxidase, endoplasmic reticular oxidases, and uncoupled nitric oxide synthase [20].

Fortunately, the production of ROS in the human body is balanced by its own endogenous antioxidant enzyme system with support from the dietary intake of various antioxidants in healthy food. The enzymatic antioxidant system consists firstly of the enzyme superoxide dismutase responsible for the binding of superoxide anions with an electron leading to the formation of hydrogen peroxide. Hydrogen peroxide can be transformed into hydroxyl radicals in the Fenton's reaction in the presence of transition metals. Alternatively, hydrogen peroxide can also be reduced to water and oxygen via the action of either catalase or glutathione peroxidase. In the reaction catalysed by glutathione peroxidase, glutathione (GSH) will be oxidized (GSSG). Here the enzyme glutathione reductase plays an important role to transform glutathione disulphide (GSSG) back to its reduced form [22-24].

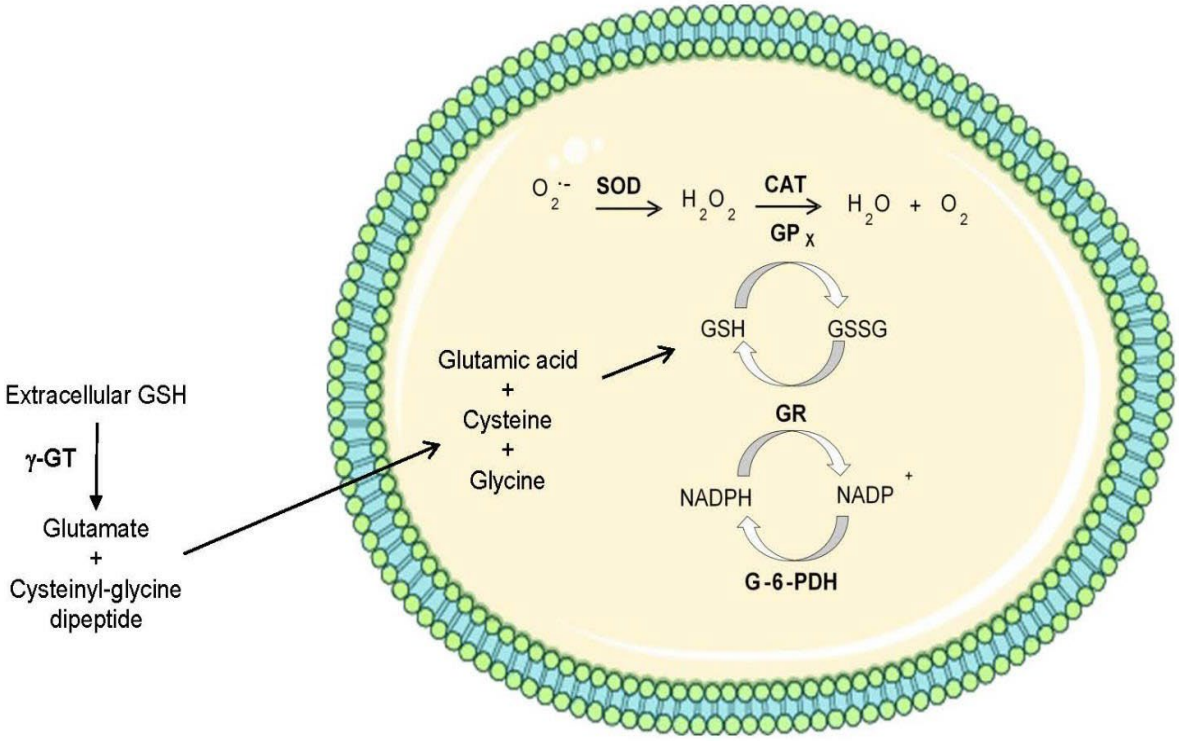

Figure 4: Antioxidant enzyme system

γ-GT, γ-glutamyl transferase; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; GR, glutathione reductase; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NADP+, oxidized nicotinamide adenine dinucleotide phosphate; G-6-PDH, glucose-6-phosphate dehydrogenase [22-25].

Importantly, at physiological levels the production of ROS plays an important role to induce protective effects via redox signaling pathways such as an increase in the expression of antioxidant enzymes to improve antioxidant capacity. However, when the production of ROS outweighs the antioxidant capacity, the high ROS levels may lead to cell damage and endothelial dysfunction. In the context of cardiovascular disease, it is important to note that different cardiovascular disease risk factors such as dyslipidemia, diabetes, obesity, hypertension, and ageing, are also associated with increased oxidative stress and may lead to vascular dysfunction and eventually the development of atherosclerosis and arterial stiffness [26].

9. A “Balancing Act” over the life-course

To assess the “Balancing Act” between oxidative stress and hypertension it is important not to include only elderly and diseased populations, but to rather investigate the interplay between these factors over the life-course. Therefore, findings from data in children (6-8 years of age), young adults (20-30 years) and older adults will be discussed.

9.1. Arterial Stiffness in Offspring Study

The Arterial Stiffness in Offspring Study (ASOS) is a cross-sectional, observational study aimed to investigate tendencies of high blood pressure, arterial stiffness and body composition in 6–8-year-old black and white school children [27].

9.2. African Prospective Study on the Early Detection and Identification of Cardiovascular Disease and Hypertension (African-PREDICT)

The African-PREDICT study is a longitudinal study with the central aim to follow young (aged 20-30 years), apparently healthy black and white adults over a 10-year period to identify novel markers related to early cardiovascular disease development [28].

9.3. Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study

The SABPA study is a psychophysiological prospective cohort study conducted in 409 black and white schoolteachers, aimed to investigate the brain-heart link and neural response pathways to describe plausible mechanisms for cardiometabolic morbidity [29].

9.4. Prospective Urban and Rural Epidemiology study

The South African leg of the international PURE study, which was performed in the North West Province of South Africa aimed to track changes in lifestyle and cardiovascular disease risk factors over a period of 10 years in black participants.

10. Ethnic disparities in nitric oxide bioavailability

Maintaining endothelial function is dependent on the balance between nitric oxide synthesis and inactivation, where oxidative stress plays an important role. Low nitric oxide bioavailability in early life may be a predisposition for early development of cardiovascular disease and explain (at least in part) some of the ethnic disparities previously noted. In black children (6-8 years of age) and young adults (20-30 years), we noted lower nitric oxide metabolites (nitrate and nitrate-to-nitrite ratio (UNOxR) (all $p \leq 0.003$) [30] but higher L-arginine (NOS substrate) and ADMA (NOS inhibitor) levels [31], suggesting an unfavourable nitric oxide bioavailability profile. Upon further investigations into how this profile link with blood pressure, arterial stiffness, carotid intima media thickness (cIMT) and the urinary albumin-to-creatinine ratio (uACR), a surrogate marker for renal endothelial function, we found inverse associations of nitrate, $U_{NOx}R$, and homoarginine (NOS substrate) with blood pressure, cIMT and uACR in young black children and adults [30-32].

In the SABPA cohort with older adults and some having blood pressure levels in the hypertensive ranges we found an overall more favourable nitric oxide synthesis capacity

profile as evidenced by the higher substrate (L-arginine and homoarginine) and lower nitric oxide inhibitor (ADMA and SDMA) levels in the black compared to the white group [33]. However, in the context of the oxidative stress profile (higher glutathione reductase and glutathione reductase:glutathione peroxidase ratio (GR:GPx ratio)) the seemingly more favorable nitric oxide synthesis capacity may be counteracted by increased nitric oxide inactivation. In fact, we indicated that the nitric oxide inhibitor, ADMA, was inversely related to glutathione peroxidase amongst other oxidative stress markers [33]. We also explored change in cIMT and change in ADMA and SDMA levels over time and how these factors relate to each other. No change in the levels of cIMT and ADMA were observed, and SDMA even decreased over time, but change in cIMT was positively associated with change in ADMA and SDMA levels. These findings suggest that ADMA and SDMA lowering strategies may delay carotid wall thickening [34]. Since modifiable cardiovascular disease risk factors such as physical inactivity may affect the vasculature via mechanisms associated with nitric oxide bioavailability, we further investigated the link between these factors in physically active and inactive groups. Despite only reaching moderate physical activity levels we found higher Windkessel compliance and homoarginine levels in the physically active group. In the physically inactive group, 24-hour blood pressure was positively associated with SDMA levels. Our findings suggest that even moderate physical activity levels may have a beneficial effect on the nitric oxide profile, which may mitigate the development of cardiovascular disease in this population [35].

In the PURE study we had the opportunity to investigate whether blood pressure after 10 years associates with the baseline nitric oxide synthesis profile in participants who remained normotensive or developed hypertension over this time. Despite having similar homoarginine levels at baseline ($p = 0.39$) and follow-up ($p = 0.93$), we found a positive association between various blood pressure measures (after 10 years) and baseline homoarginine levels in the group that remained normotensive. No significant associations were found in the group that developed hypertension after 10 years. This may suggest a protective role for homoarginine to maintain normal blood pressure, up until a certain threshold. However once hypertension develops other factors may surpass the protective effects of homoarginine [36]. When we investigated the nitric oxide synthesis profile in relation with carotid wall thickening, we found inverse associations between cIMT and cross-sectional wall area after 5 years with homoarginine levels at baseline. Again, suggesting that homoarginine may play a protective role against vascular injury [37]. The potential protective effects of homoarginine were further supported when we investigated whether homoarginine levels are associated with 10-year risk for all-cause and cardiovascular mortality. We found that survivors had higher homoarginine

levels when compared to non-survivors and that higher homoarginine levels are associated with a reduced risk for 10 year cardiovascular and all-cause mortality [38].

11. Cardiovascular disease risk factors and oxidative stress

When reflecting on the mosaic theory, it remains striking that cardiovascular disease risk factors such as smoking, hypercholesterolemia, family history, ageing, hypertension, kidney disease, hyperglycaemia and obesity are all associated with oxidative stress. Since familial cardiovascular and lifestyle risk may impact on the development of cardiovascular disease, we investigated the link between oxidative stress and vascular function in boys stratified by maternal cardiovascular risk. Only in children with maternal risk we found positive associations between blood pressure and arterial stiffness with thiobarbituric acid-reactive substances (TBARS), a marker of lipid peroxidation. In the same group, blood pressure and uACR were positively associated with 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), a marker of DNA damage. These findings support that the link between family history and the early onset of cardiovascular disease may be modulated by oxidative stress [39].

Similarly, we also investigated the link between these two markers of lipid and DNA damage respectively in relation with blood pressure, total peripheral resistance, and arterial compliance in an adult population. Surprisingly, we found lower 8-oxodG levels ($p<0.001$) and inverse associations of 24-hour blood pressure with 8-oxodG in the black compared to the white group [40]. In contrast, higher TBARS and along with a positive association with total peripheral resistance and an inverse association with arterial compliance were found in the black group [41]. Collectively these findings may suggest that oxidative damage to lipids may play a role in early vascular changes, but that oxidative stress levels may not have reached a level where DNA damage is relevant possibly due to the upregulation of DNA repair mechanisms to maintain DNA integrity.

Another important cardiovascular disease risk factor – obesity is associated with oxidative stress and inflammation. In this regard we investigated the link between uric acid and bilirubin, two endogenous molecules with pro- and antioxidant properties, with blood pressure in young lean vs. overweight/obese men and women. In women with a BMI greater than 25 kg/m^2, higher 24-hour blood pressure and uric acid levels were found when compared to their lean counterparts. This was accompanied by an adverse association between 24-hour blood pressure and uric acid only in young women with increased adiposity. These findings may suggest that the pro-oxidative effects of uric acid may mediate this relationship, where increased uric acid levels may translate to more pronounced vascular damage over time [42].

Apart from the link between cardiovascular risk factors and oxidative stress markers, we also investigated the link between oxidative stress and other mechanisms associated with

peripheral resistance such as haemostasis (the blood clotting profile) and endothelin-1 levels (responsible for vasoconstriction). Previous work indicated an altered haemostatic, inflammatory and oxidative stress profile, but it was unclear whether these components function independently or whether they are related. We found that in the context of lower glutathione activity, von Willebrand Factor (a marker of endothelial function), and clot lysis time were inversely associated with glutathione peroxidase activity [43]. Regarding endothelin-1, the levels were found to be similar when comparing black and white groups, but unique associations were found between endothelin-1 and markers of oxidative stress. In black men endothelin-1 was positively related with glutathione activity and the GR:GPx ratio, while in black women endothelin-1 levels correlated negatively with total glutathione [44]. These findings suggest an interrelationship between oxidative stress and different mechanisms associated with peripheral vascular resistance, where decreased antioxidants may alter the haemostatic profile, while ET-1 levels may contribute to the upregulation of antioxidant enzymes.

In one of the very first oxidative stress related papers by the Hypertension in Africa Research Team, the link between glutathione, an important antioxidant molecule, and cIMT was investigated in hypertensive black men. It was shown that cIMT was inversely associated with glutathione, suggesting a possible contributory role for attenuated glutathione levels in the early developmental phases of atherosclerosis [45]. Since glutathione levels are regulated by various factors, including the action of the glutathione peroxidase and reductase enzymes, we investigated a broader profile of antioxidant enzymes. In black compared to white groups we found higher serum peroxide levels, higher catalase but lower glutathione peroxidase activity, higher glutathione reductase and higher total glutathione levels and higher GR:GPx ratio, a surrogate measurement for glutathione turnover in the glutathione cycle. These findings clearly suggest that the balance is shifted more towards an oxidative state. In black women, 24-hour blood pressure measures were inversely associated with glutathione peroxidase activity and in black men cIMT was positively associated with glutathione reductase activity. These results suggest that lower glutathione peroxidase may play a role in the development of hypertension while upregulation of higher glutathione reductase may be a compensatory response to prevent arterial remodelling. Interestingly, no associations were found in the white men or women [46].

With limited data relating changes in oxidative stress with deterioration in the vascular and renal system over time, we investigated whether changes in oxidative stress over 3 years are associated with target organ damage. Remarkably, over 3 years black men exhibited decreased serum peroxide levels, superoxide dismutase and glutathione reductase activities. Additionally, positive associations of cross-sectional wall area with change in serum peroxide

and change in superoxide dismutase activity were noted, suggesting than an improvement in the functioning of the antioxidant system may delay target organ damage [47].

With the above-mentioned findings limited to the SABPA cohort (older individuals, some with blood pressure levels in the hypertensive ranges) it remained unclear whether these findings were confounded by increased age and blood pressure. We therefore aimed to profile oxidative stress markers in the young black and white adults of the African-PREDICT study. We found higher total glutathione but lower glutathione peroxidase activity and total antioxidant status in the black compared to white adults. A negative association between 24-hour pulse pressure and glutathione peroxidase were found in the black men, suggesting that oxidative stress may be associated with early vascular changes [48].

From the previous findings it became evident that the lower glutathione peroxidase activity may be a “weak link” in the enzymatic antioxidant enzyme system and we therefore investigated the availability of selenium, an important co-factor for this enzyme. We found that one in ten black men and one in five black women were selenium deficient (<8µg/100ml). No associations between 24-hour blood pressure or arterial stiffness and either selenium or glutathione peroxidase activity was evident in the black men and women. Interestingly, in white men, inverse associations between 24-hour blood pressure and selenium and between arterial stiffness and glutathione peroxidase activity were found. These findings suggest that lower serum selenium levels in black populations from the same geographical region as their white counterparts may impact on the loss of the vasculoprotective effects of selenium and selenoproteins such as glutathione peroxidase [49].

Again, it was unclear whether the findings in the SABPA cohort was confounded by age or blood pressure values in the hypertensive ranges. To better understand the involvement of selenium and glutathione peroxidase in the early development of cardiovascular disease, we investigated in young, healthy black and white men and women whether measures of the micro- and macrovasculature are related to selenium and glutathione peroxidase activity. Only 2% of the total African-PREDICT cohort was selenium deficient. We also found that both markers of the micro- and macro vasculature were associated with serum selenium and glutathione peroxidase, with the nature of these associations pointing to the vascular protective role for selenium and glutathione peroxidase in these vascular beds [50].

In the PURE study we had the opportunity to investigate the long-term associations between selenium and atherosclerosis, arterial stiffness, and hypertension. In normal and selenium-deficient groups we investigated if large artery structure and function after 10 years are associated with baseline serum selenium levels. In the normal selenium group, we found a long-term vascular protective association of selenium on arterial stiffness and blood pressure,

supporting the notion that selenium has vascular protective effects. In the highest quartile of serum selenium, a potential detrimental association between selenium and carotid wall thickness was identified. These findings highlight the importance of balancing selenium levels [51].

12. Metabolomics and oxidative stress

The constant "balancing act" to maintain a healthy redox state and hence a healthy cardiovascular system also became evident in the application of novel omics technologies. Omics can be defined as the "*analysis of large amounts of data representing an entire set of some kind, especially the entire set of molecules, such as proteins, lipid or metabolites, in a cell, organ or organism*". Different types of omics will thus provide a holistic view, for example genomics provide an overview of the complete set of genetic instructions provided by the DNA, while transcriptomics investigate gene expression patterns. In proteomics dynamic protein products and their interactions are studied, while the interaction between genetic make-up and environmental factors can be studied with metabolomics.

We used metabolomics to assess the early molecular determinants of arterial stiffness and hypertrophy of the left ventricular wall. In young black and white adults with similar arterial stiffness profiles, central systolic blood pressure was higher in the black compared to the white group. We also found lower dietary protein intake. In metabolomics analyses we found more abundant levels of several non-essential amino acids including 4-hydroxyproline, alanine, glutamine, glycine, histidine and serine in the black compared to the white group. It was further found that central blood pressure was inversely associated with these amino acids, again only in the black group [52]. Since increased central blood pressure may impact on the heart and lead to hypertrophy of the left ventricular wall, we also investigated the link between the metabolomics profile and left ventricular mass. We found that three metabolites including hydroxyproline, glycine and trimethylamine were more abundant and inversely associated with left ventricular mass index in the black group when compared to their white counterparts [53]. Since these amino acids play a pivotal role in maintaining collagen biosynthesis – a process with oxidative stress at the center - these ethnic-specific finding may suggest that biosynthesis of nonessential amino acids may be upregulated to protect the cardiovascular system against early deterioration.

To increase our understanding of the mechanistic pathways associated with CVD risk, we use a similar metabolomics approach to profile cardiovascular disease risk factors (obesity, physical activity, smoking, excessive alcohol intake, masked hypertension, hyperglycemia, dyslipidemia, and socio-economic status) and cardiovascular disease risk clusters in comparison with a control group without any risk factors. When we compared the

metabolomics profile between the CVD risk groups, a hyperglycemic state showed the most differences when compared to the control group, followed by a low socio-economic status and obesity. In these risk groups and, also in the risk clusters we identified three affected metabolic pathways, including branched chain amino acid metabolism, pathways associated with energy metabolism such as β-oxidation, glycolysis, creatine metabolism and oxidative stress and in particular - glutathione metabolism.

13. Summary and future prospects

To summarise our findings related to ethnic disparities in nitric oxide bioavailability (Figure 5), in black children and young adults, lower nitric oxide bioavailability may contribute to the development of hypertension in later life via mechanisms associated with endothelial dysfunction, atherosclerosis and arterial stiffness. Findings in an older population indicated that maintaining optimal homoarginine levels may protect against the development of hypertension, atherosclerosis and mortality. However even with a favorable nitric oxide synthesis capacity profile, the availability of nitric oxide may be counteracted by the presence of increased oxidative stress, underpinning the importance of lifestyle modifications such as engaging in moderate physical activity to maintain balance between these role-players.

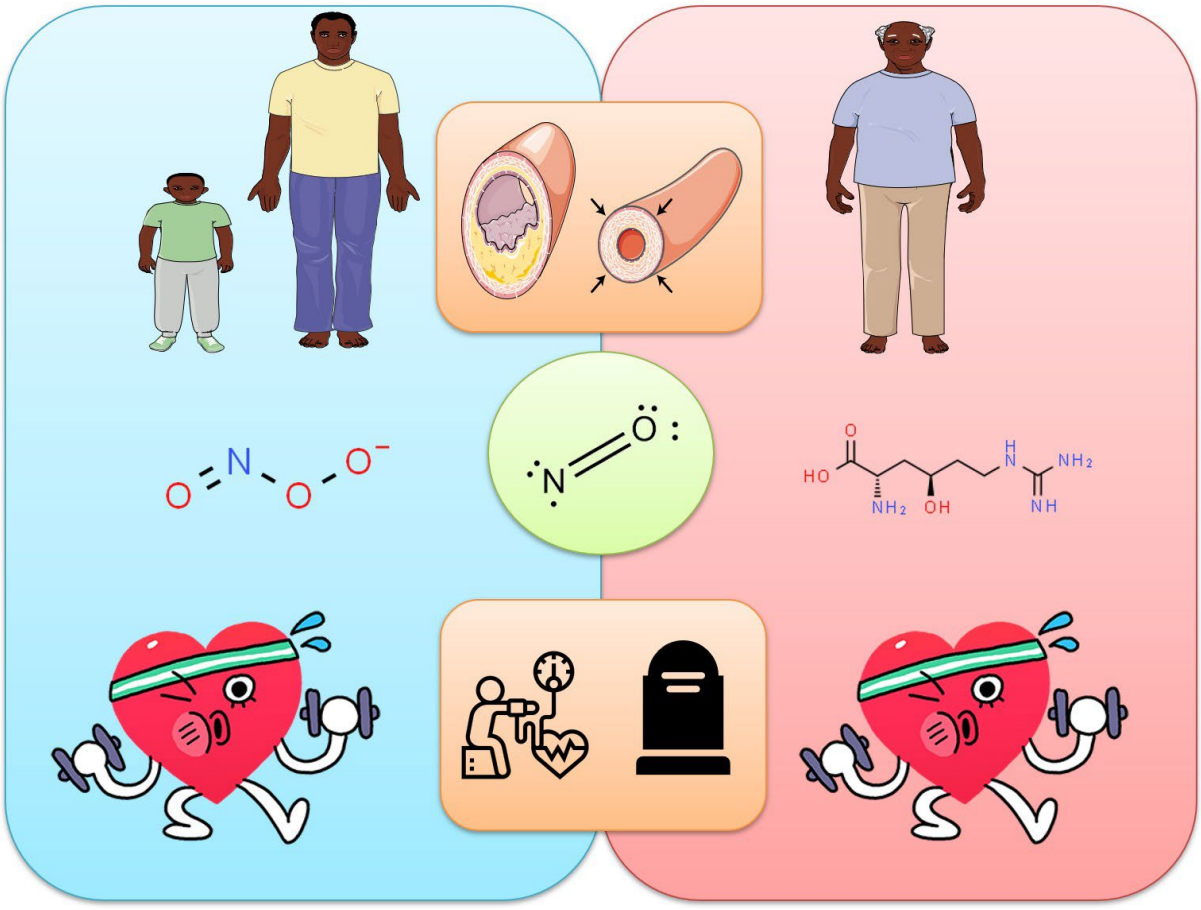

Figure 5: Graphical abstract on ethnic disparities in nitric oxide bioavailability

Our findings related to cardiovascular disease risk factors and oxidative stress clearly support the link of oxidative stress with factors of the mosaic theory (Figure 6). We demonstrated a link between maternal risk and the early onset of cardiovascular disease and that this may be modulated by oxidative stress. Environmental factors such as diet and balanced levels of the micronutrient – selenium, an important role player in the optimal functioning of the antioxidant system were linked with vascular protection. Regarding anatomical, adaptive and hemodynamic factors, we linked oxidative stress to blood pressure, arterial compliance, and peripheral resistance, arterial stiffness, carotid intima media thickness, cross-sectional wall area and microvascular calibers. We also indicated a link between oxidative stress markers and factors determining peripheral resistance such as nitric oxide, endothelin-1 and haemostatic markers. Even when following a holistic approach such as metabolomics when investigating the factors associated with the early phases of cardiovascular development, the involvement of oxidative stress was identified as a prominent feature related to cardiovascular disease risk. Several of the factors included in the Mosaic Theory remains to be investigated in these bi-ethnic cohorts and should be the focus of future studies.

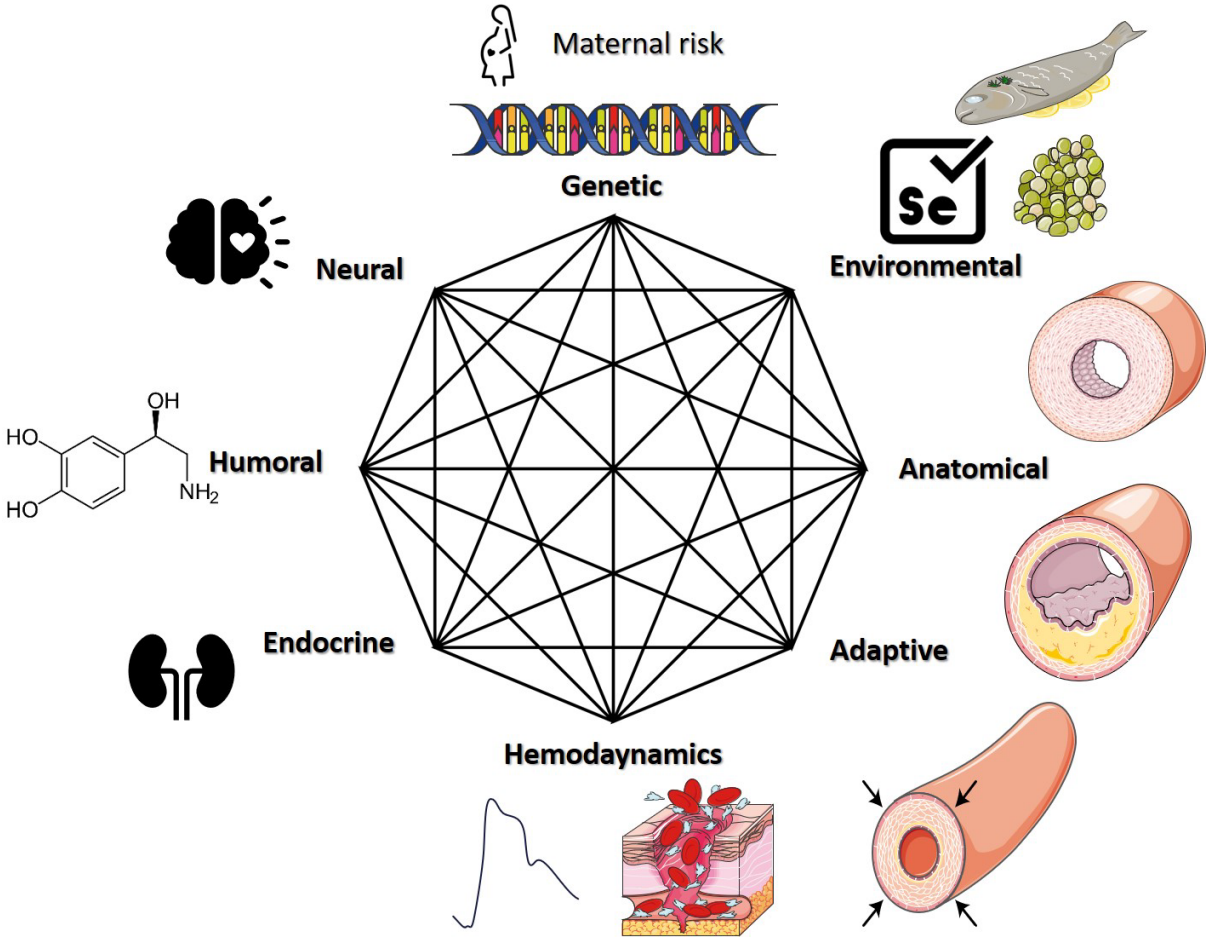

Figure 6: Summary of findings in support of the role of oxidative stress in the Mosaic Theory

With such a prominent role in the development of hypertension, antioxidant therapy has been the focus of multiple studies. In a recent review article, it was highlighted that modulation of oxidant systems in animal models provided encouraging findings, but translation to human disease is rarely successful, probably due to the multifactorial nature of hypertension [54]. Limited success in clinical trials may be the result of various limitations (i) the short half-life of antioxidants; and (ii) crosstalk with other substances may reduce the anti-hypertensive effects of the antioxidants tested. A further limitation may include the inadequate homogeneity of patients included in these trials [54], suggesting that an individualised rather than a one-size-fits-all approach may be more effective. However, in the context of sub-Saharan Africa with economic, educational and health inequalities, the focus should perhaps rather be directed at health education.

14. Acknowledgements

I would like to acknowledge the principal investigators of the following studies: i) Prof Ruan Kruger (ASOS); Prof Alta Schutte (African-PREDICT); Prof Leone Malan (SABPA study) and the late Prof Annemarie Kruger, Prof Minrie Greeff and Prof Lanthé Kruger (PURE). The ASOS study was supported by grants from NRF South Africa and the South African Sugar Association (SASA). The African-PREDICT study was funded as part of an ongoing research project financially supported by the South African Medical Research Council (SAMRC) with funds from National Treasury under its Economic Competitiveness and Support Package; the South African Research Chairs Initiative (SARChI) of the Department of Science and Technology and National Research Foundation (NRF) of South Africa (GUN 86,895); SAMRC with funds received from the South African National Department of Health, GlaxoSmithKline R&D (Africa Non-Communicable Disease Open Lab grant), the UK Medical Research Council and with funds from the UK Government's Newton Fund; as well as corporate social investment grants from Pfizer (South Africa), Boehringer-Ingelheim (South Africa), Novartis (South Africa), the Medi Clinic Hospital Group (South Africa) and in kind contributions of Roche Diagnostics (South Africa). The SABPA study was supported by the National Research Foundation; the National Research Foundation Thuthuka (80643) the North West Education Department; the Medical Research Council of South Africa; the North-West University; Roche Products (Pty) Ltd., South Africa; and the Metabolic Syndrome Institute, France. I would also like to acknowledge all the participants that took part in these studies as well as the students, support staff and researchers at the Hypertension Research and Training Clinic of the Hypertension in Africa Research Team. I would like to acknowledge the technical support of Mrs Tina Scholtz and Sr Chrissie Lessing for their contributions to the SABPA study and the support of the Africa Unit for Transdisciplinary Health Research (AUTHeR) and Dr S Yusuf

(PURE-International) and the PURE project staff at the PHRI, Hamilton Health Sciences and McMaster University, ON, Canada for their contributions to the PURE study.

15. References

1. Martini F, Bartholomew E (eds). Essentials of Anatomy and Physiology, Fifth Edition. Benjamin Cummings; 2006.

2. Chan F, Adamo S, Coxson P, Goldman L, Gu D, Zhao D, et al. Projected impact of urbanization on cardiovascular disease in China. Int J Public Health 2012; 57:849-854.

3. Donnison C. Blood Pressure in the African Native. Its Bearing upon the Aetiology of Hyperpiesia and Arterio-Sclerosis. Lancet 1929.

4. Canning D, Raja S, Yazbeck A. Africa's demographic transition: dividend or disaster? : World Bank Publications; 2015.

5. Gordon T, Booysen F, Mbonigaba J. Socio-economic inequalities in the multiple dimensions of access to healthcare: the case of South Africa. BMC Public Health 2020; 20:289.

6. Gafane-Matemane LF, Kruger R, Smith W, Mels CMC, Van Rooyen JM, Mokwatsi GG, et al. Characterization of the Renin-Angiotensin-Aldosterone System in Young Healthy Black Adults: The African Prospective Study on the Early Detection and Identification of Hypertension and Cardiovascular Disease (African-PREDICT Study). Hypertension 2021; 78:400-410.

7. Schutte AE, Botha S, Fourie CMT, Gafane-Matemane LF, Kruger R, Lammertyn L, et al. Recent advances in understanding hypertension development in sub-Saharan Africa. J Hum Hypertens 2017; 31:491-500.

8. Schutte AE, Kruger R, Gafane-Matemane LF, Breet Y, Strauss-Kruger M, Cruickshank JK. Ethnicity and Arterial Stiffness. Arterioscler Thromb Vasc Biol 2020; 40:1044-1054.

9. Bourque S, Davidge S, Adams M. The interaction between endothelin-1 and nitric oxide in the vasculature: new perspectives. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 2011; 300:R1288-R1295.

10. Ahmad A, Dempsey SK, Daneva Z, Azam M, Li N, Li PL, et al. Role of Nitric Oxide in the Cardiovascular and Renal Systems. Int J Mol Sci 2018; 19:2605.

11. Schlossmann J, Feil R, Hofmann F. Signaling through NO and cGMP-dependent protein kinases. Ann Med 2003; 35:21-27.

12. Levine AB, Punihaole D, Levine TB. Characterization of the role of nitric oxide and its clinical applications. Cardiology 2012; 122:55-68.

13. Gamil S, Erdmann J, Schwedhelm E, Bakheit KH, Abdalrahman IBB, Mohamed AO. Increased Serum Levels of Asymmetric Dimethylarginine and Symmetric Dimethylarginine and Decreased Levels of Arginine in Sudanese Patients with Essential Hypertension. Kidney Blood Press Res 2020; 45:727-736.

14. Kumar S, Bogle R, Banerjee D. Why do young people with chronic kidney disease die early? World J Nephrol 2014; 3:143.
15. Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. Arterioscler Thromb Vasc Biol 2005; 25:29-38.
16. Jeroncic A, Gunjaca G, Mrsic D, Mudnic I, Brizic I, Polasek O, et al. Normative equations for central augmentation index: assessment of inter-population applicability and how it could be improved. Sci Rep 2016; 6:1-9.
17. Page IH. The mosaic theory of arterial hypertension--its interpretation. Perspect Biol Med 1967; 10:325-333.
18. Furchgott R, Ignarro L, Murad F. Discover concerning nitric oxide as a signaling molecule in the cardiovascular system. Nobel Prize in Medicine and Physiology 1998.
19. Harrison DG. The mosaic theory revisited: common molecular mechanisms coordinating diverse organ and cellular events in hypertension. J Am Soc Hypertens 2013; 7:68-74.
20. Touyz RM, Rios FJ, Alves-Lopes R, Neves KB, Camargo LL, Montezano AC. Oxidative Stress: A Unifying Paradigm in Hypertension. Can J Cardiol 2020; 36:659-670.
21. Jones DW, Hall JE. Racial and ethnic differences in blood pressure: biology and sociology. Am Heart Assoc; 2006.
22. Li H, Horke S, Förstermann U. Oxidative stress in vascular disease and its pharmacological prevention. Trends Pharmacol Sci 2013; 34:313-319.
23. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet 2008; 371:64-74.
24. Mironczuk-Chodakowska I, Witkowska AM, Zujko ME. Endogenous non-enzymatic antioxidants in the human body. Adv Med Sci 2018; 63:68-78.
25. Myburgh C. Exploring the link between oxidative stress and the vasculature in a bi-ethnic population. North-West University; 2019.
26. Munzel T, Camici GG, Maack C, Bonetti NR, Fuster V, Kovacic JC. Impact of Oxidative Stress on the Heart and Vasculature: Part 2 of a 3-Part Series. J Am Coll Cardiol 2017; 70:212-229.
27. Mokwatsi GG, Schutte AE, Kruger R. Ethnic differences regarding arterial stiffness of 6-8-year-old black and white boys. J Hypertens 2017; 35:960-967.
28. Schutte AE, Gona PN, Delles C, Uys AS, Burger A, Mels CM, et al. The African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT): Design, recruitment and initial examination. Eur J Prev Cardiol 2019; 26:458-470.
29. Malan L, Hamer M, Frasure-Smith N, Steyn HS, Malan NT. Cohort Profile: Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) prospective cohort study. Int J Epidemiol 2015; 44:1814-1822.

30. Craig A, Mels CMC, Schutte AE, Tsikas D, Kruger R. Nitric oxide-related markers link inversely to blood pressure in black boys and men: the ASOS and African-PREDICT studies. Amino Acids 2020.
31. Craig A, C. MCM, Tsikas D, Boeger RH, Schwedhelm E, Schutte AE, et al. Central systolic blood pressure relates inversely to nitric oxide synthesis in young black adults: the African-PREDICT study. J Hum Hypertens 2021; 35:985-993.
32. Craig A, Mels C, Schutte A, Bollenbach A, Tsikas D, Schwedhelm E, et al. Urinary albumin-to-creatinine ratio is inversely related to nitric oxide synthesis in young black adults: the African-PREDICT study. Hypertens Res 2021; 44:71-79.
33. Mels CMC, Huisman HW, Smith W, Schutte R, Schwedhelm E, Atzler D, et al. The relationship of nitric oxide synthesis capacity, oxidative stress, and albumin-to-creatinine ratio in black and white men: the SABPA study. Age 2016; 38:9.
34. Mels CMC, Schutte AE, Huisman HW, Smith W, Kruger R, van Rooyen JM, et al. Asymmetric dimethylarginine and symmetric dimethylarginine prospectively relates to carotid wall thickening in black men: the SABPA study. Amino Acids 2017; 49:1843-1853.
35. van Niekerk E, Botha Le Roux S, Atzler D, Schwedhelm E, Boger RH, van Rooyen JM, et al. Blood pressure and nitric oxide synthesis capacity in physically active and inactive groups: the SABPA study. J Hum Hypertens 2021; 35:325-333.
36. Mokhaneli MC, Botha-Le Roux S, Fourie CMT, Boger R, Schwedhelm E, Mels CMC. Homoarginine and blood pressure: a 10-year prospective relationship in normotensives. J Hum Hypertens 2021:1-9.
37. Mokhaneli MC, Fourie CMT, Botha-Le Roux S, Boger RH, Schwedhelm E, Mels CMC. Asymmetric dimethylarginine and L-homoarginine prospectively relate to carotid wall thickness in a South African cohort. Amino Acids 2020; 52:965-973.
38. Mokhaneli MC, Botha-Le Roux S, Fourie CMT, Böger R, Schwedhelm E, Mels CMC. L-homoarginine is associated with decreased cardiovascular-and all-cause mortality. Eur J Clin Invest 2021; 51:e13472.
39. Craig A, Mels CMC, Kruger R. Thiobarbituric acid-reactive substances relate to arterial stiffness and blood pressure in 6 to 8-year-old boys stratified by maternal risk. Free Radic Res 2018; 52:180-187.
40. Mels CMC, Schutte AE, Schutte R, Pretorius PJ, Smith W, Huisman HW, et al. 8-Oxo-7, 8-dihydro-2'-deoxyguanosine, reactive oxygen species and ambulatory blood pressure in African and Caucasian men: the SABPA study. Free Radic Res 2014; 48:1291-1299.
41. Mokhaneli MC, Fourie CMT, Botha S, Mels CMC. The association of oxidative stress with arterial compliance and vascular resistance in a bi-ethnic population: the SABPA study. Free Radic Res 2016; 50:920-928.

42. Du Toit WL, Schutte AE, Mels CMC. The relationship of blood pressure with uric acid and bilirubin in young lean and overweight/obese men and women: the African-PREDICT study. J Hum Hypertens 2020; 34:648-656.
43. Lammertyn L, Mels C, Pieters M, Schutte A, Schutte R. Ethnic-specific relationships between haemostatic and oxidative stress markers in black and white South Africans: The SABPA study. Clin Exp Hypertens 2015; 37:511-517.
44. du Plooy CS, Mels CMC, Huisman HW, Kruger R. The association of endothelin-1 with markers of oxidative stress in a biethnic South African cohort: the SABPA study. Hypertens Res 2017; 40:189-195.
45. Schutte R, Schutte AE, Huisman HW, van Rooyen JM, Malan NT, Peter S, et al. Blood glutathione and subclinical atherosclerosis in African men: the SABPA Study. Am J Hypertens 2009; 22:1154-1159.
46. van Zyl C, Huisman HW, Mels CMC. Antioxidant enzyme activity is associated with blood pressure and carotid intima media thickness in black men and women: The SABPA study. Atherosclerosis 2016; 248:91-96.
47. Myburgh C, Huisman H, Mels C. Three-year change in oxidative stress markers is linked to target organ damage in black and white men: the SABPA study. Hypertens Res 2019; 42:1961-1970.
48. Myburgh C, Huisman HW, Mels CMC. The relation of blood pressure and carotid intima-media thickness with the glutathione cycle in a young bi-ethnic population: the African-PREDICT study. J Hum Hypertens 2018; 32:268-277.
49. Swart R, Schutte AE, van Rooyen JM, Mels CMC. Serum selenium levels, the selenoprotein glutathione peroxidase and vascular protection: The SABPA study. Food Res Int 2018; 104:69-76.
50. Swart R, Schutte AE, van Rooyen JM, Smith W, Mels CMC. The Association of Measures of the Micro- and Macro-Vasculature with Selenium and GPx Activity in a Young Bi-Ethnic Population: The African-PREDICT Study. J Am Coll Nutr 2019; 38:614-622.
51. Swart R, Schutte AE, van Rooyen JM, Mels CMC. Selenium and large artery structure and function: a 10-year prospective study. Eur J Nutr 2019; 58:3313-3323.
52. Mels CM, Delles C, Louw R, Schutte AE. Central systolic pressure and a nonessential amino acid metabolomics profile: the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension. J Hypertens 2019; 37:1157-1166.
53. De Beer D, Mels CM, Schutte AE, Louw R, Delles C, Kruger R. Left ventricular mass and urinary metabolomics in young black and white adults: The African-PREDICT study. Nutr Metab Cardiovasc Dis 2020; 30:2051-2062.
54. Sorriento D, De Luca N, Trimarco B, Iaccarino G. The Antioxidant Therapy: New Insights in the Treatment of Hypertension. Front Physiol 2018; 9:258.