EFFECTS OF MODERATE TREADMILL ACTIVITY ON CARDIOVASCULAR FACTORS IN SPONTANEOUSLY HYPERTENSIVE RATS

By

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DECLARATION

I, **Kibwe Mwewa** do hereby declare that all information provided in this thesis entitled:

"Effects of moderate treadmill activity on cardiovascular factors in spontaneously hypertensive rats"

Is the result of my own investigation and all the information taken from research papers is properly referenced. I understand that copying or plagiarism could lead to my degree being disqualified.

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LIST OF ABBREVIATIONS

µl	Microlitre
ATP	Adenosine triphosphate
BH4	Tetrahydrobiopterin
BHT	Butylated hydroxytoluene
BP	Blood pressure
CAT	Catalase
cDNA	Complimentary Deoxyribonucleic
ClHO4	Perchloric Acid
CP	Crossing point
CRP	C-reactive protein
Ct	Threshold cycle
CT-1	Cardiotrophin-1
Cu	Copper
Cu-ZnSOD	Copper-zinc superoxide
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
DNA	Deoxyribonucleic acid
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic Acid
ELISA	enzyme linked immunosorbent assays
eNOS	Endothelial nitric oxide synthase
EPO	Eosinophil peroxidase
Fe	Iron
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GPX	Glutathione peroxidase
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase family of enzymes
GSSG	Glutathione disulfide
H&E	Heamatoxylin and Eosin
H_2O	Water
H_2O_2	Hydrogen peroxide
HAE	Hereditary Angioedema
HCl	Hydrochloric acid
HNO_2	Nitrous acid
HO-1	Heme oxygenase
HOBr	Hypobromous acid
HOCL	Hypochlorous acid
hs-CRP	high-sensitivity c-reactive protein
ICAM-1	intercellular adhesion molecule type 1

ICAM-1	Intercellular Adhesion Molecule 1
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-6	Interleukin-6
Kg	Kilogram
LC	Light cycler
LDL	Low-density lipoprotein
MDA	Malondialdehyde
MF	Myofibres
mg	Milligram
min	Minute
mL	millilitre
mmHg	millimetre of mercury
Mn	Manganese
NAD^+	Nicotinamide adenine dinucleotide reduced
NADH	Nicotinamide adenine dinucleotide oxidized
NADPH	Nicotinamide adenine dinucleotide phosphate-oxidase
NaOH	Sodium Hydroxide
NaOH	Sodium hydroxide
NHCL	Hydrochloric Acid solution
NIBP	Non-invasive blood pressure
nm	Nanometer
NO	Nitric oxide
NO_2^-	Nitrite ion
NO_3^-	Nitrates
NOS	Nitric oxide synthase
O_2	Oxygen
O_3	Ozone
OD	Optical density
OD	Optimum density
OH	Alcohol
ONOO-	Peroxinitrite
OXPHOS	Oxydative phosphorylation
PCT	Proximal convoluted tube
PHGPx	Phospholipid hydroperoxide glutathione peroxidase
RAS	Renin angiotensin system
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROH	Alcohol
ROOH	Hydroperoxide
ROS	Reactive oxygen species

RT-PCR	Real-time polymerase chain reaction
SBP	Systolic blood pressure
Se	Selenium
SEM	Standard error of means
SHR	Spontaneously hypertensive rat
SHR EX	spontaneously hypertensive rat exercised
SHR NEX	Spontaneously hypertensive rat non-exercised
SOD	Superoxide dismutase
STAT-3	transducer and activator of transcription-3
TAC	Total antioxidant capacity
TAS	Total antioxidants status
TBA	Thiobarbituric acid
Tm	Melting temperature
UV	Ultra violet
VVG	Verhoeff-Van Gienson
WHO	World Health Organization
WISTAR	Wistar Non- exercise
WISTAR EX	Wistar exercised
WKY	Wistar-Kyoto
WKY	Wistar Kyoto
XO	Xanthine oxidase
Zn	Zinc

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ABSTRACT

Hypertension is a major health problem throughout the world because of its high prevalence and its association with increased risk of cardiovascular disease. Oxidative stress due to either increase of reactive oxygen species (ROS) or a compromised antioxidant status has also been positively correlated with cardiovascular diseases. The beneficial effect of physical activity has been well documented in the literature but studies have shown that in the SHR physical activity leads to oxidative stress. The SHR is an excellent model of essential hypertension and hence the present study was designed to investigate the effect of moderate treadmill activity on various cardiovascular factors in SHR. Sixteen male SHR and male Wistar rats (n=16) weighing between 70 and 90 g were used and they were randomly divided into four groups: The SHR exercised group (n=8), Wistar exercised group (n=8), SHR group (n=8) and Wistar (n=8). All the rats in exercised group were subjected to a weekly increase in the rate of activity on the treadmill. Blood pressure, blood glucose and body mass were recorded weekly. At the end of the 8 week experimental protocol, animals were fasted for 12 hours, anaesthetized with halothane and blood and tissue samples harvested. The C-reactive protein (CRP) and antioxidant-associated trace elements such as copper (Cu), iron (Fe), manganese (Mn), selenium (Se), and zinc (Zn) were measured in the blood, brain and skeletal muscle. Cardiotrophin-1 (CT-1) was determined in the plasma, total antioxidant capacity (TAC) and malonyldialdehyde (MDA) were determined in blood and skeletal muscle, plasma TAC levels was also measured. The superoxide dismutase (SOD), IkB and nuclear factor-kappa (NF-kB) gene expression were also measured in skeletal muscle and liver. Kidney sections were stained with Haematoxylin-Eosin (H&E) and sections of aorta were stained with Verhoeff-van Gieson (VVG). The results show that physical activity did not significantly change both the systolic and diastolic blood pressures in SHR. Plasma levels of CRP and NF-kB mRNA expression were increased in both SHR and Wistar exercised groups. An increase in oxidative stress due to physical activity was evident by an increase in TAC and MDA levels in the skeletal muscle. A significant decrease in blood TAC and SOD mRNA expression was also evident in the SHR exercise group. Physical activity also resulted in significant shifts in trace elements that are associated with a compromised antioxidant system.

***Key words**: Antioxidants, oxidative stress, exercise and hypertension*

INTRODUCTION

Free radicals are continuously produced in the various mammalian organ systems, as a result of normal metabolic processes. These free radicals are quenched by antioxidants under normal conditions (Uttara et al., 2009). If there is high production of free radicals in the body systems or a compromised antioxidant that overwhelms antioxidants, that imbalance leads to the situation called oxidative stress. Oxidative stress is a term generally used to describe the state in which potential damage could occur due to the free radical. (Lobo et al., 2010).

Research has shown that reactive oxygen species (ROS) play an important role in the development of cardiovascular diseases, including hypertension. This is in large part due to the ROS production such as superoxide (O_2^-) , hydroxyl radical and hydrogen peroxide (H_2O_2) levels and the decreased nitric oxide bioavailability in the vasculature (Paravicini and Touyz, 2008).

There is a potential consequence of oxidative stress to tissue in a variety of diseases such as atherosclerosis, diabetes, cancer and neurodegenerative diseases. The mechanism of organ damage which explains the development of essential hypertension is still under investigation. Free radicals have been implicated in playing a major role in the pathogenesis of hypertension, and its associated organ damage (Rubattu et al., 2015).

Physical activity is recognised worldwide to have a beneficial effect on a number of physiological and psychosocial problems such as heart disease, diabetes, depression and hypertension (Buttar et al., 2005). Paradoxically, physical activity can potentially cause oxidative stress in the body by increasing free radical production that may overwhelm antioxidants (Powers and Jackson, 2008a). The aetiology of the oxidative that is associated with physical activity is not entirely conclusive and is hence is still under investigation. This study will expand our knowledge on the effect of physical activity on the antioxidants status in this model of essential hypertension.

Previous studies have shown that trace elements form part of an integral of metalloproteins and antioxidant enzyme systems which play a major catalytic role in antioxidant reactions (Rahman, 2007a). Trace elements have a role to play in the regulation of blood pressure and imbalances or deficiencies they can lead to the development of high blood pressure (Loyke, 2002b).

The spontaneously hypertensive rat (SHR) has been established and commonly used as a model of essential human hypertension. The development of hypertension in the SHR, as in humans, is multifactorial and polygenic in origin (Dornas and Silva, 2011).

The broad aim of this study was therefore to investigate the effects of moderate treadmill activity on the pathogenesis of hypertension using a well-established animal model of genetic hypertension viz the SHR rat.

The objectives are listed as follows:

1. To determine the effects of 8 week moderate physical activity on a treadmill and the effects on the progression of hypertension and associated parameters in young male SHR rats.

2. To determine the effects of exercise on the antioxidant status of the animals by assessing the total antioxidant capacity (TAC) in the blood and in the skeletal muscle. Additionally, tissue oxidative stress will be assessed indirectly by determining lipid peroxidation in skeletal muscle via the malondialdehyde (MDA) assay.

3. To assess effects of physical activity on the expression of SOD mRNA in the skeletal muscle and liver tissue as an indicator of the adaptive response of the antioxidant system to the moderate physical activity.

4. To determine the effects of exercise on vascular remodelling in the cardiovascular system. This will include a histological study of myocardium, aorta and the kidney. Plasma cardiotrophin-1 levels, an indicator of cardiac and vascular remodelling, will determined in the myocardium and aorta. C-reactive protein, nuclear factor-kappa B and IkB mRNA expression will also be determined to assess inflammation and associated gene activation.

5. Trace elements such as Cu, Fe, Mn, Se and Zn play a catalytic role in the antioxidant system and play a role in blood pressure regulation. These will be measured in the blood and tissue to assess any shift or imbalance that may contribute to oxidative stress or hypertension.

The outcome of this study will hence be useful to elucidate the potential therapeutic role of antioxidant therapy and physical activity in hypertension.

LITERATURE REVIEW

2.1 Hypertension

High blood pressure is defined as elevated systolic blood pressure (SBP) above 140 mmHg and a diastolic blood pressure above 90 mmHg. Its consequences are one of the major causes of mortality and morbidity globally (Chalmers, 2011). It has been associated with increased risk of cardiovascular complications such as coronary heart disease, stroke, heart failure and oxidative stress which most developed and developing countries are facing (Kashyap et al., 2005, Pal et al., 2013). Vascular oxidative stress has been shown to play a large role in cardiovascular pathogenesis due to the presence of ROS as well as reactive nitrogen species (RNS) (Rodrigo et al., 2007)

The World Health Organisation (WHO) has developed a set of guidelines which classify the disorder into categories for the purposes of clinical management (Table 1).

Table 1. Systemic classification of blood pressure values for adults.

Parameters	Systolic (mmHg)	Diastolic (mmHg)
NORMAL	Less than 130 mmHg	Less than 85 mmHg
HIGH-NORMAL	130-139 mmHg	85–89 mmHg
HYPERTENSION	140 mmHg or greater	90 mmHg or greater
Stage 1 (Mild)	140–159 mmHg	90–99 mmHg
Stage 2 (Moderate)	160–179 mmHg	100–109 mmHg
Stage 3 (Severe)	180–209 mmHg	110–119 mmHg
Stage 4 (Very Severe)	210 mmHg or greater	140 Hg or greater

Source: (Tran and Giang, 2014)

2.2 High blood pressure in sub-Saharan Africa

Sub-Saharan Africa is a region of considerable socio-economic and demographic diversity. Epidemiological studies reveal that hypertension is now a major public health concern in this region, particularly in urban areas. Research conducted in Tanzania, Nigeria, Namibia, Kenya and South Africa, which differ considerably in terms of socio-economic conditions, living environment and geographical location indicates that high blood pressure is the most commonly observed risk factor for cardiovascular disease (Addo et al., 2007, Hendriks et al., 2012).

Thus, hypertension constitutes a burden in the lives of a large proportion of the population in sub-Saharan Africa, and the risk of cardiovascular disease is linearly related to blood pressure (Kuller, 2007). The evidence shows that lifestyle changes in sub-Saharan Africa contribute to the increased prevalence of cardiovascular disease including hypertension (Opie and Seedat, 2005).

2.3 Pathogenesis of high blood pressure

Hypertension can be put into two broad categories, namely essential and secondary hypertension, and 90–95% of hypertension diagnoses fit into the category of essential hypertension (Calhoun et al., 2008). The origin of essential hypertension is multifactorial and several hypotheses have been put forward in this regard, including genetic factors. The pathogenesis of hypertension includes factors such as increased sympathetic nervous system activity that plays a role in raising of blood pressure and contributes to the progress and maintenance of hypertension through stimulation of the heart, peripheral vasculature, and kidneys, which causes increased cardiac output, increased vascular resistance and fluid retention. In addition, autonomic imbalance (increased sympathetic tone accompanied by reduced parasympathetic tone) is associated with many metabolic and hemodynamic abnormalities that result in increased cardiovascular mortality (Mark, 1996).

The increased sympathetic nervous system mechanisms active in hypertension are multifactorial and involve changes in baroreflex and chemoreflex pathways at both peripheral and central levels. There is a central resetting of the aortic baroreflex in hypertensive patients, resulting in suppression of sympathetic inhibition after activation of aortic baroreceptor nerves (Joyner et al., 2008). This baroreflex resetting seems to be mediated by a central action of angiotensin II. Angiotensin II increases the response to sympathetic stimulation by means of a presynaptic (peripheral) mechanism, which facilitates the modulation of norepinephrine release (Guyenet, 2006). Furthermore, small-molecule mediators such as ROS and endothelin suppress baroreceptor activity and contribute to an increased sympathetic activity which may lead to hypertension (Converse et al., 1992). Research has shown that increased ROS production in the system during exercise has an impact on the vascular wall and can potentially lead to oxidative stress. For example, antioxidant status can be overwhelmed by the production of free radicals through aerobic metabolism and later may cause deactivation of vasodilators such as nitric oxide on vascular endothelium and results in high blood pressure. The remaining 5–10% of cases are secondary hypertension resulting from known causes such as kidney disease, endocrine disorders and complications in some drug treatments.

2.4 Free radicals

Free radicals are defined as molecules that contain one or more unpaired electrons in atomic or molecular orbitals (Valko et al., 2006). The unpaired electrons usually give a considerable degree of reactivity to the free radical. These electrons are unstable and make these molecules unstable. Aerobic metabolism normally produces oxygen-centred radicals which are also called ROS (Halliwell, 2006).

Numerous free radicals can be found in living systems even if most molecules *in vivo* are non-radicals (Scott and Malcolm, 2008).

Any free radical containing oxygen is referred to as ROS and includes superoxide anion radical (O_2^-) and hydroxyl radical (.HO) (Lin et al., 2013). Superoxide is formed when oxygen receives one electron which triggers a quick flow of events that create other free radicals, terminating eventually in the formation of H_2O. Since superoxide is the most commonly produced free radical, it can be found in the phagocytic cells such as macrophages and neutrophils which are the prominent sources of superoxide (Szabo et al., 2007). During inflammation free radicals are produced by cells such as neutrophils in order to attack invading pathogens such as bacteria (Madamanchi et al., 2005).

Hydrogen peroxide (H_2O_2) is formed when the oxygen molecule attracts two hydrogen molecules. Technically, hydrogen peroxide cannot be considered an oxygen-free radical but as a member of the ROS family. and may play a role in the generation of free radicals (Dröge, 2002b). Most of the H_2O_2 is broken down to oxygen and water by the cellular enzyme catalase. The hydroxyl radical is the most reactive of free radical molecules, and can actually damage cell membranes and lipoproteins by lipid peroxidation. In atherosclerosis, lipid peroxidation causes damage to lipids in low-density lipoprotein (LDL) and plays an important role in the formation of the foam cell within the vessels (Dröge, 2002b, Steinberg, 2009). Superoxide anion (O_2^-) is the precursor of most ROS and plays a large role as mediator in oxidative chain reactions. The reactions are shown below:

$O_2 + e^- \rightarrow O_2^-$- Superoxide radical*(1)*

$O_2 + H_2O \rightarrow HO_2\cdot + OH^-$- Hydroperoxyl radical*(2)*

$HO_2 + e^- + H \rightarrow H_2O_2$- Hydrogen peroxide*(3)*

Superoxide (O_2^-) and hydroxyl (OH^-) are among the examples of reactive oxygen. The term ROS can refer to oxygen-derived non-radicals such as hydrogen peroxide (H_2O_2), ozone (O_3), hypochlorus (HOCl) and singlet oxygen ($^1O_2$).

There are also nitrogen radicals such as nitric oxide ($NO^{-\bullet}$) and nitrogen dioxide (NO_2^-), but the term ROS also includes certain non-radicals such as nitrous acid (HNO_2) and Peroxynitrite ($ONOO^-$) (Hancock et al., 2001).

ROS play an important role in various physiological functions including cell signalling, inflammation and immune defence. Research has shown that elevation in the production of free radicals can lead to increased cellular stress and may contribute to the variations in molecular pathways that contribute to the pathogenesis of many diseases in humans, including heart disease, neurological disease and in the physiological ageing process (Dröge, 2002b).

Table 2. Reactive Oxygen Species

Species	Common Name	Half-Life (37^0C)
HO	Hydroxyl radical	10^{-9} second
HO_2	Hydroperoxyl radical	1 nanosecond
O_2	Superoxide anion radical	unstable
$^1O_2$	Singlet oxygen	enzymatic
RO	Alkoxyl radical	1 microsecond
ROO	Peroxyl radical	1 microsecond
NO	Nitric oxide radical	7 seconds
H_2O_2	Hydrogen peroxide	1-10 seconds
HOCl	Hypochlorus acid	stable

Source: (Palmieri and Sblendorio, 2007)

The stated molecules are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, leading to cellular damage. The generation of ROS is done by different mechanisms (Lobo et al., 2010). Below are some of the sources of ROS that arise from normal metabolic processes:

2.4.1 Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase

Superoxide is a one of the free radicals that originates from the NADPH oxidase enzyme complex. NADPH does not act on the vasculature only; its action can be found in other organs, for example the kidney (Korge et al., 2008). In phagocytic cells NADPH plays a major role in the production of ROS in response to foreign pathogens (Elahi et al., 2009).

Different parameters are involved in the regulation of NADH/NADPH activity. Some of them include hormones, local metabolic changes and haemodynamic forces. Angiotensin II contributes to the increase of NADH and NADPH through superoxide production that is localised in aortic adventitial fibroblast and vascular smooth muscle cells (VSMC) (Touyz, 2004). The oxidase activation can be mediated by intracellular second messengers.

2.4.2 Xanthine oxidoreductase system

Xanthine oxidase and xanthine dehydrogenase are forms of the same enzyme, known as xanthine oxidoreductase. Both forms of xanthine oxidoreductase catalyse the conversion of hypoxanthine to xanthine and xanthine to uric acid; however, only the oxidase form generates O_2^- and H_2O_2 (Battelli et al., 2014). The enzyme typically exists in the dehydrogenase form, but under certain stressful conditions, such as hypoxia, the oxidase isoform predominates. Studies show that xanthine oxidase is implicated as a source of ROS after reperfusion of ischaemic tissue in several organs (Halliwell, 2006).

Further, xanthine oxidase may act as NO_3^- and NO_2^- reductase to generate NO under hypoxic conditions. In addition, xanthine oxidase expression and O_2^- production are up-regulated by NADPH oxidase, which is an indication that factors regulating NADPH oxidase may also have an influence on xanthine oxidase. (Szabo et al., 2007)

As noted above, there are two forms of xanthine oxidoreductase, namely xanthine dehydrogenase and xanthine oxidase. NAD^+ is reduced in the first form while the second reacts with molecular oxygen, leading to the production of superoxide anion and hydrogen peroxide. Xanthine acido-reductase catalyses oxidative hydroxylation of hypoxanthine to xanthine in the purine catabolism, and then from xanthine to uric acid, which is a strong antioxidant and free radical scavenger (Nishikawa et al., 2000). Thus, Xanthine oxidase can play a dual role as regulator of the cellular redox state.

Xanthine oxidoreductase is an important source of oxidative stress under physiological stress conditions. It generates ROS through the metabolism pathway and contributes to endothelial dysfunction in patients with cardiovascular disease such as contractile dysfunction in heart failure (Wu and Cederbaum, 2003).

2.4.3 Mitochondrial respiration

In the process of ATP production, mitochondrial respiration involves transport of electrons from NADH or flavoprotein-linked dehydrogenases which finally result in reduction of oxygen to water. This transport chain involves oxidative phosphorylation (OxPHOS) of complexes that are both nuclear and mitochondrial DNA encoded. Through aberrant O_2 reaction mitochondria produce significant amounts of cellular ROS (Campbell and Reece, 2005). About 2–5% of electrons escape to react with O_2, resulting in the production of ROS, which mainly occurs at complexes I and III. In physiological conditions this process is controlled with the majority of ROS produced remaining inside intact mitochondria (Valko et al., 2007).

Furthermore, some elements in the outer membrane such as monoamine oxidases produce NO or H_2O_2 which result in the increase of free radical stress. Mitochondrial respiration rate and ROS formation are mostly influenced by the couple reaction of the mitochondria in which electrons are

transferred is coupled with the ATP production and this turn by factors such as internal and external Ca^{2+} levels and antioxidant activity (Turrens, 2003).

2.4.4 Uncoupling of nitric oxide synthase (NOS)

Uncoupling of NO synthase plays a role in the generation of ROS and results in vascular endothelial dysfunction (Landmesser et al., 2003). Endothelial NOS (eNOS) is a cytochrome P450 reductase-like enzyme that catalyses flavin-mediated electron transport from the electron donor NADPH to a prosthetic heme group. In addition, eNOS is able to produce both NOS via its oxygenase function and superoxide through its reductase function, the latter dependent on NADPH. This enzyme requires tetrahydrobiopterin (BH-4) bound near this heme group to transfer electrons to guanodino nitrogen of L-arginine to form NO (Apel and Hirt, 2004). eNOS uncoupled plays a role in the production of ROS when there is a deficiency of L-arginine or BH4. The lack of L-arginine or BH-4, eNOS can produce O_2^- and H_2O_2 The outcome of the reaction between NO and O_2^- can oxidise BH_4 and this may result in the uncoupling of eNOS (Jin and Loscalzo, 2010).

ROS may also affect NO responses by oxidising sites on the protein that reacts with NO or which would otherwise influence NO binding (Powers and Jackson, 2008c). Recently, it was revealed that under certain pathological circumstances, eNOS becomes dysfunctional and produces superoxide rather than NO. The pathophysiological role of dysfunctional eNOS has attracted attention in vascular disorders, including atherosclerosis, hypertension, and diabetes mellitus (Yokoyama and Hirata, 2007)

2.5 Reactive oxygen species and diseases

Free radicals are constantly produced from various normal physiological and biochemical processes in all living organisms (Valko et al., 2007). These radicals could potentially damage cellular biomolecules such as proteins, enzymes, nucleic acids, lipids and carbohydrates leading to many disease conditions such as neurodegerative diseases, heart disease, cancers etc. Therefore, the balance between the production of free radicals and the antioxidant defences is important from a health point of view (Jenner, 2003).

Studies show that overproduction of free radicals due to overexposure to environmental factors such as smoking, ultraviolet radiation, and pollutants lead to tissue damage. This is a result of an imbalance in oxidant-generating versus oxidant-catalysing systems, leading to an increased ROS in the vasculature, cardiac and renal tissues. Superoxide anion is an endogenously important ROS, primarily in ROS metabolic pathways, which by itself decreases NO bioavailability (Touyz and Schiffrin, 2004) and attenuates vasodilatory mechanisms, thereby affecting the elevation of blood pressure (Radomski and Moncada, 1993).

2.6 Free radical production during physical activity

Many tissues that may potentially produce ROS during physical activity such as heart, lungs, white blood cells and skeletal muscle have been studied. Skeletal muscle has been the most studied at the subcellular level, as sources of free radicals during physical activity. Evidence has shown that during aerobic activity mitochondria increase oxygen consumption and would lead to an increase in $O_2^{\cdot -}$ formation from complexes I and III. The latter has been confirmed in recent studies that in complex I, the main site of electron leakage to oxygen appears to be the iron-sulphur clusters, and in complex III, it appears to be the Q_{10} semiquinone which releases superoxide from both sides of the inner mitochondrial membrane (Powers and Jackson, 2008b). Nevertheless, recent studies demonstrate that mitochondria may not be the dominant source of ROS during physical activity. Intense physical activity, especially eccentric contractions, may generate ROS through NADPH (NAD(P)H) oxidase from neutrophils and sarcolemma and, secondarily, through myeloperoxidase (Gomez-Cabrera et al., 2013).

It has been demonstrated that these enzymes produce superoxide anions which regulate contractile function via calcium release in the cardiac muscle. An important enzyme involved in the metabolism of membrane polyunsaturated fatty acid during inflammation, has been identified as a modulator of cytosolic oxidant production in skeletal muscle. Other studies have shown that phospholipase A_2 is an enzyme that cleaves membrane phospholipids to release arachidonic acid which is a substrate for ROS-generating enzyme systems. It was shown that activation of PLA_2 stimulates ROS generation in muscle mitochondria and cytosol and releases ROS into the extracellular space (Powers and Jackson, 2008b).

Mechanisms were identified in which increases in Ca^{++} concentration during physical activity may activate the enzyme phospholipase $A_{2,}$ which releases the arachidonic acid from the phospholipids (Schneider and Oliveira, 2004). The cyclooxygenase reacts with the arachidonic acid to form the hydroxyl radical. Its function is essential for the rise in intracellular ROS that occurs during repetitive, fatiguing contractions. Cyclooxygenase and lipoxygenase are involved in ROS production with phospholipase A_2. The role of xanthine oxidase (XO) in oxidant generation during high-intensity intermittent exercise has already been identified (Nethery et al., 1999).

In addition, research has shown that within the skeletal muscle there is a continuous production of NO by NO synthase with an important function to regulate vascular smooth muscle tone (Gomes et al., 2012). Therefore, increased muscle contraction can increase NO production via activation of eNOS or iNOS which may have a negative effect due to the highly reactive peroxynitrite which is considered as a danger. Figure 1 shows different sites of free radical production in the skeletal muscle.

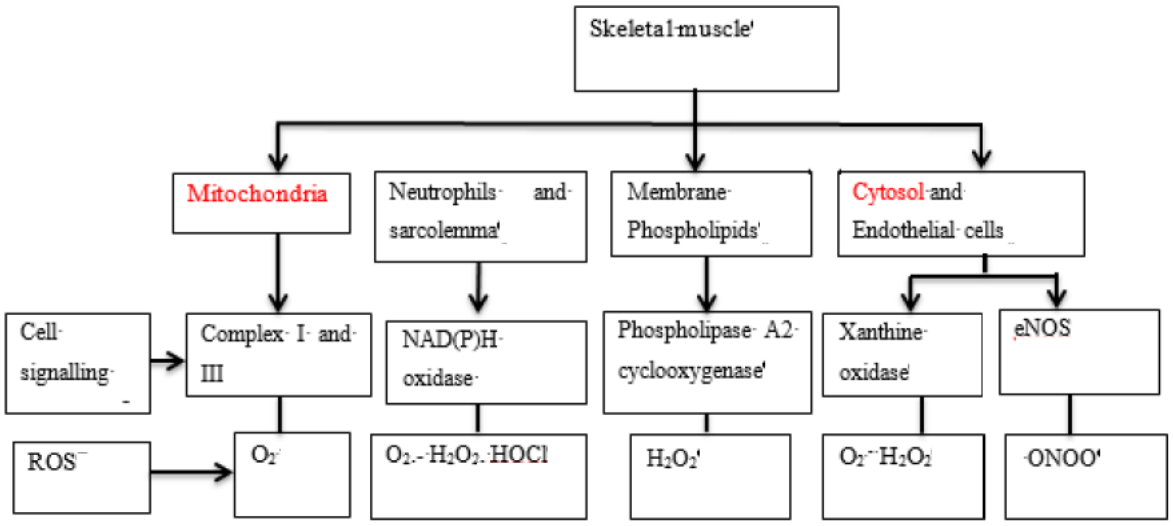


Figure 1. Reactive Oxygen Species
(Palmieri and Sblendorio, 2007)

There are several sources of free radicals in skeletal muscle. They are located in mitochondria, cytosol, sarcolemma, and endothelial cells. Abbreviations: ROS=reactive oxygen species; NOS= nitric oxide synthase; NAD(P)H = NADPH; ONOO = peroxynitrite.

Some of the mechanisms of the generation of ROS have been established during physical activity. Research has shown that temporary interruptions of the calcium-dependent (Ca^{++}) ATP pumps lead to increases of the calcium intracellular concentrations, which may activate the path of the XO during physical activity (Schneider and Oliveira, 2004). Increased concentrations of intramuscular calcium during periods of progressive intensity physical activity may activate the calcium-dependent proteases, which convert xanthine dehydrogenase into XO. The XO uses the molecular oxygen instead of NAD^+ as an electron acceptor, thus generating the superoxide radical (Meneshian and Bulkley, 2002). While looking at the white blood cell during physical activity, the leukocytes activation were shown to stimulate the production of free radicals to improve the host defence mechanism in response to the muscular damage induced by physical activity. Especially, the neutrophils may reduce the molecular oxygen into superoxide radical through NADPH oxidase, which is inactive in cells in rest. The same results were observed in monocytes and eosinophils (Schneider and Oliveira, 2004).

2.7 Cellular antioxidant defences

Antioxidants are the substances that present naturally in plants and animals and protect the cell from harmful effects (Devasagayam et al., 2004) and they can be referred to any molecule capable of stabilising or deactivating free radicals before they cause damage to the cells. There are enzymatic and non-enzymatic antioxidants in the human body and these work in combination to protect the cells and systems of the body against free radicals (Halliwell, 1994). The antioxidants can be endogenous or obtained exogenously, for example as a part of the diet or as dietary supplements (Rahman, 2007b).

Studies also reveal that antioxidants are substances that directly or indirectly protect cells against adverse effects of xenobiotics, drugs, carcinogens and toxic radical reactions (Halliwell, 2006). However, antioxidant properties can also be found in other compounds including vitamin C (ascorbic acid), vitamin E (α-tocopherol), vitamin A, b-carotene, metallothionein, polyamines, melatonin, NADPH, adenosine, coenzyme Q-10, urate, ubiquinol, polyphenols, flavonoids, phytoestrogens, cysteine, homocysteine, taurine, methionine, s-adenosyl-L-methionine, s-adenosyl-L-methionine, resveratrol, nitroxides, GSH, glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), thioredoxin reductase, NOS, heme oxygenase-1 (HO-1) and eosinophil peroxidase (EPO) (Matés et al., 2000).

2.7.1 Superoxide dismutase

This is one of the most effective intracellular enzymatic antioxidants and it catalyses the conversion of superoxide anions to dioxide and hydrogen peroxide (Petrulea et al., 2012). Subsequently H_2O_2 is reduced to H_2O and O_2 by peroxidases such as glutathione peroxidase or catalase. SOD is found in in the cytoplasm as well as on the endothelial cell surface with either Cu or Zn (CuSOD, ZnSOD) and in the mitochondria with Mn (MnSOD). This reaction is generally considered to be the body's primary antioxidant defence because it prevents further generation of free radicals (Liochev and Fridovich, 2000). The highest levels of SOD are found in the liver, adrenal gland, kidney, and spleen (Kuo et al., 2005).

2.7.2 Glutathione peroxidase

GPX is an enzyme responsible for reducing H_2O_2 or organic hydroperoxides in the presence of reduced glutathione (GSH) to H_2O and alcohol respectively. GSH is used by this enzyme as the electron donor and requires Se as a cofactor (Flora, 2009a). GPX is highly specific for its electron donor (GSH), but this enzyme has low specificity for substrates: i.e. GPX will reduce a wide range of hydroperoxides, from H_2O_2 to complex organic hydroperoxides, (Powers, 1999). This characteristic makes GPX an important cellular protectant against ROS-mediated damage to membrane lipids and other molecules sensitive to oxidation (Cabrera and Chihuailaf, 2011).

Glutathione peroxidases are widely distributed in animal tissues. Reduced glutathione (GSH) plays a major role in the regulation of the intracellular redox state of vascular cells by providing reducing equivalents for many biochemical pathways (Ay et al., 2013).

Dietary selenium actively participates in the catalytic reaction, and this is often the basis for antioxidant protection offered by supplemental selenium. Selenium-containing peroxidases comprise a family of enzymes of at least four types. The "classic" glutathione peroxidase (cGPx) acts on H_2O_2 and hydroperoxides of fatty acids and cholesterol, but not esterified lipids such as those present in

lipoproteins. Phospholipid hydroperoxide glutathione peroxidase (PHGPx) is the only enzyme known to reduce complex lipid hydroperoxides in lipoproteins (Stocker and Keaney Jr, 2004).

In the absence of GPX activity or inadequate glutathione levels, hydrogen peroxide and lipid peroxides are not detoxified and may be converted to hydroxyl radicals and lipid peroxyl radicals, respectively, by transition metals (e.g., Fe^{2+}). The GPx/glutathione system is thought to be a major defence in low-level oxidative stress (Shree et al., 2013).

Reduced glutathione (GSH) is recognised as one of the most important non-enzymatic oxidant defences within the body. It exists in very large quantities (mM levels) within cells where it acts to detoxify peroxides as well as maintain other physiologically important antioxidants in their reduced form (Agarwal et al., 2012). Studies have postulated that, "the balance of GSH and GSSG provides a dynamic indicator of oxidative stress *in vivo*". Therefore glutathione levels *in vivo* are an important indicator of oxidative stress (Jones, 2002).

2.7.3 Catalase (CAT)

As an antioxidant enzyme, CAT catalyses the breakdown of H_2O_2 to form water and O_2 (Dröge, 2002b). Catalase requires Fe^{3+} as a cofactor in order to maintain its catalytic activity (Powers, 1999). Catalase works to detoxify oxygen-reactive radicals by catalysing the formation of H_2O_2 derived from superoxide. High concentrations of catalase are found in both peroxisomes and mitochondria, although it is widely distributed in the cell (Victor et al., 2005).

There are also non-enzyme antioxidants which exist in cells. The most important non-enzyme defences include vitamin E, vitamin C, GSH, carotenoids, bilirubin and ubiquinone. Under normal conditions, there is a balance between both the activities and the intracellular levels of these antioxidants and this balance is essential for the survival of organisms (Powers, 1999).

Table 3. Cellular location and antioxidant properties of primary antioxidant enzymes in cells

Enzymatic antioxidants	Cellular locations	Antioxidant properties
Mn superoxide dismutase	Mitochondria	Dismutates superoxide radicals
Cu-Zn superoxide dismutase	Cytosol	Dismutates superoxide radicals
GSH peroxidase	Cytosol and mitochondria	Removes H_2O_2 and organic hydroperoxides
Catalase	Cytosol and mitochondria	Removes H_2O_2

Table 4. Cellular location and antioxidant properties of non-enzymic antioxidants in cells

Non-enzymic antioxidants	Cellular locations	Antioxidant properties
Vitamin E	Lipid-soluble phenolic compound; located in membranes	Major lipid peroxidation chain-breaking antioxidant
Vitamin C (ascorbic acid)	Water-soluble; located in cytosol	Quenches a wide variety of aqueous-phase ROS; regenerates vitamin E from it's oxidised product
GSH	Non-protein thiol in cells; located in both cytosol and mitochondria	
Lipoic acid	Endogenous thiol; located in both lipid and aqueous phase of cell	Effective as an antioxidant and in recycling vitamin C; may also be an effective GSH substitute
Ubiquinones	Lipid-soluble quinone derivatives; located in membranes	Reduced forms are efficient antioxidants
Carotenoids	Lipid-soluble; located primarily in membranes of tissue	Antioxidant; reduces lipid peroxidation

2.8 Hypertension and antioxidant enzymes

The cardiovascular system may be compromised during hypertension. Studies in hypertensive animals confirmed that the level of oxidative stress in these animals was increased and the hypertensive myocardium is more susceptible to post-ischaemic damage (Dröge, 2002b). However, changes in antioxidant enzymes during hypertension are observed in different tissues, including myocardium, vascular endothelium, skeletal muscle, liver, kidney and erythrocytes. Changes observed in these tissues may allow an increased level of superoxide anion in the affected tissues (Dröge, 2002a).

The number of abnormalities observed in hypertensive patients has been attributed due to increased superoxide levels including effects on Ca^{2+} signalling processes and blood pressure. In the latter case, angiotensin II-induced hypertension is mediated by superoxide anion elevations, and angiotensin II-decreased antioxidant enzyme expression in the renal cortex. Hypertension effects of oxidative stress are most due to endothelial dysfunction from disturbances of vasodilator systems, particularly degradation of NO by oxygen-free radicals (Kachhawa et al., 2014). Antioxidant therapy has been shown to play a role in hypertension in order to create a balance against free radicals. Thus superoxide

dismutase mimetic causes up-regulation of NO synthase with either a concomitant decrease in hypertension or an inhibition of vascular remodelling in stroke-prone SHRs (Rehman et al., 2012).

2.9 Physical activity and antioxidant enzymes

Physical activity is now recognised worldwide to have a beneficial effect on a number of physiological and psychosocial problems such as heart disease, hypertension, diabetes and depression. The benefit of regular physical activity has been shown to improve cardiovascular functions in patients with chronic heart failure and other cardiovascular diseases (Buttar et al., 2005). Reactive oxygen and nitrogen species play an important role in the regulation of cardiovascular functions, inflammatory responses and hypertension. However, animal studies have shown that physical activity can potentially induce significant oxidative stress in animal systems because increased oxygen consumption for respiration may generate free radical production. Considering the mechanism such as the XO-catalysed reaction being part of oxidative stress associated with physical activity (Gielen et al., 2010), such increase in free radical production is reflected in increases in the circulatory levels of thiobarbituric acid reactive substances (TBARS) and oxidised glutathione and this may be responsible for increased levels of oxidative damage (Flora, 2009b).

Skeletal muscle is recognised as a major source of free radical production, because of its large mass in relation to body weight and because of its aerobic capacity. Therefore, studies have confirmed that contractile activity directly induces oxidative stress by the increased production and release of superoxide anions from exercising skeletal muscle *in vivo* (Gosker et al., 2000).

It is known that changes in antioxidant enzymes during physical activity do not only occur in skeletal muscle, but also in other tissues. In erythrocytes, aerobic training in humans resulted in increased superoxide dismutase and glutathione peroxidase activities. Thus, the type of physical activity can also influence the results in the level of antioxidants (Mairbaurl, 2013).

2.10 Oxidative stress

The concept of oxidative stress started to be used frequently in the late 1970s, but its origin can be traced from the 1950s where more research was done on the toxic effects of ion radiation, free radicals and the potential role of these molecules in many disease states. (Buettner and Jurkiewicz, 1996). At the beginning the implication of free radicals in the disease state was controversial due to many theories at the time. The implication of free radicals production causing disruption in the biological systems was acknowledged during 1968 (Hybertson et al., 2011).

Free radicals are continuously being produced in the cells and ROS as part of the metabolic processes. During normal conditions the increased production of free radicals in the system is quenched by the antioxidant defence mechanism (Belviranlı and Gökbel, 2006). However, an imbalance between the production of oxidants and the defence systems of an organism results in oxidative stress, which is the

pathologic result of ROS increase that overwhelms the antioxidant defence mechanism (Halliwell, 2006).

2.10.1 Implications of oxidative stress

Production of vascular oxygen species in different tissues such as endothelial, adventitial, and VSMCs comes from NAD(P)H oxidase and it is known that that the regulation of vascular NAD(P)H oxidase is done by humoral (cytokines, growth factors, and vasoactive agents) and physical factors (stretch, pulsatile strain, and shear stress) (Balaban et al., 2005, Elahi et al., 2009, Virdis et al., 2011, Drummond and Sobey, 2014).

During pathological circumstances, these molecules tend to increase, therefore causing malfunctioning of certain tissues such as endothelial cells, VSMC growth which may lead to different cascades such as increased contractility ,monocyte invasion, lipid peroxidation, inflammation, and increased deposition of extracellular matrix proteins, important factors in hypertensive vascular damage (Rodrigo et al., 2013). The decreased NO bioavailability has been linked to the impaired endothelium-mediated vasodilation in hypertension (Förstermann et al., 1994).

2.10.2 Lipid peroxidation

Lipid peroxidation is a well-established cellular mechanism that occurs in both plants and animals and it has been used a biomarker of oxidative stress in cells and tissues. Lipid peroxides are unstable and decompose to form a complex of compounds including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) upon decomposition. Measurement of MDA and HAE has been used successfully as an indicator of lipid peroxidation (Gutteridge, 1995).

The detection of lipid peroxidation in blood plasma, urine or tissue which has been used before was found to be irrelevant. Most studies over the recent years rely on the detection of thiobarbituric acid (TBA)-reactive substances such as MDA or other reactive aldehydes generated *in vivo* or *in vitro* by the decomposition of lipid peroxidation products (Tarpey et al., 2004).

Lipid peroxidation can be tested using other analytical tests which include the following techniques: fluorometry of lipofuscin-like substances in serum, spectrophotometry of conjugated dienes in lipid extracts of plasma and microsomes, gas chromatography of ethane or pentane in exhaled breath, hydroperoxide determination, fluorometry, high-performance liquid or gas-liquid chromatography, measurements of other saturated and unsaturated aldehydes and oxygen uptake during peroxidation (Wilson et al., 1997). The above-mentioned methods are used for the general determination of oxidative stress *in vivo* and *in vitro*.

Lipid peroxidation has been linked to a variety of disorders, including atherogenesis, diabetes and UV-induced carcinogenesis (Rahman, 2007b)

2.11 Free radicals and oxidative stress linked to hypertension

Studies in the past years have demonstrated that both ROS and RNS play a big role in the pathogenesis of high blood pressure (Delles et al., 2008). However, any excess of ROS production in the system or a compromised endogenous antioxidant can lead to cell and tissue damage. Since nitric oxide plays a role as a vasodilator of the vessels, NO can rapidly interact with superoxide to form peroxynitrite. The latter has the potential to deactivate NO bioavailability that leads to vasoconstriction and results in elevated blood pressure. (Fulda et al., 2010).

2.11.1 Nitric oxide (NO)

Essential hypertension in several animal models of hypertension, including SHRs, is associated with increased peripheral vascular resistance (Lee and Griendling, 2008). Due to NO as endogenous vasodilator, different hypotheses have been made available to explain why nitric oxide reduction or its bioavailability would lead to vasoconstriction and therefore increased peripheral vascular resistance (Hu et al., 2013).

Various studies suggest that the superoxide radical interacts with NO and thus limits its bioavailability. The affinity of NO for superoxide is so high that its reaction rate is limited only by diffusion. Because superoxide effectively degrades NO, the biological activity of NO may be determined by the availability of superoxide (Sharma et al., 2012).

During oxidative stress, the deactivation of NO is the result of superoxide anion reaction that leads to the peroxynitrite formation. The later can lead to a cascade of effects such as lipid peroxidation, protein nitration, DNA degradation and enhanced tubuloglomerular feedback responses (Förstermann and Sessa, 2012, Drummond and Sobey, 2014).

2.11.2 Cardiotrophin-1

Cardiotrophin-1 (CT-1) is a protein member of the IL-6 family of cytokines that signals via leukaemia inhibitory factor receptor gp130-dependent pathways and was originally characterised as a factor that induces cardiomyocyte growth and survival (Monserrat et al., 2011b). Previous studies on CT-1 stipulated that it plays an important role on the myocardial structural changes, therefore contributing to the remodelling of the left ventricle and hence leading to left ventricle failure which would result in cardiac diseases such as cardiomyopathy, coronary artery disease, aortic stenosis and hypertension (Kuwahara et al., 1999).

Although cardiotrophin-1 action has been found prominently on the heart tissue, studies have shown that it can also be found on other organs. Apart from inducing stress by releasing cytokine in the cells

against foreign stimulation, CT-1 was found to play an up-regulatory role in the cardiomyocyte and non-cardiomyocytes by different factors such as mechanical (Calabro et al., 2009), neurohumoral, and metabolic (Asai et al., 2000, Mitsumoto et al., 2001).

Clinical studies suggest that excessive levels of CT-1 in the myocardium that happen in cases of pressure overload may lead to cardiomyocyte hypertrophy that results in pathologic left ventricular hypertrophy (LVH) (Latchman, 2000, Monserrat et al., 2011a).

2.11.3 CT-1 and cardiovascular disease

Since CT-1 action is more pronounced in the heart in most cases, it has been considered as a biomarker of disease. However, it also contributes in the pathogenesis of cardiovascular diseases including hypertension, atherosclerosis and coronary diseases (González et al., 2012). Studies over the past years showed the dual role of CT-1 of being both a protective case of physiological function myocardium and a pathological condition because it can induce a malfunction in the myocardium (Ghosh et al., 2000).

Since hypertension is one of the cardiovascular diseases that is defined by the presence of hypertrophy of the left ventricle, it was also shown that increased collagen fibres would lead to myocardial fibrosis that results in ventricular remodelling during high blood pressure. But studies on rat animal models of hypertension have confirmed that interleukin-6-related cytokines would play a role in the occurring of LVH (González et al., 2012). In cases of patients with high blood pressure CT-1 plasma levels have been confirmed to have a strong relationship with inappropriate left ventricular mass. Several stress pathways are activated due to mechanical overload on the left ventricle and left ventricular mass is increased during increased systemic hypertension in particular. In this regard, cardiac secretion of CT-1 has been proposed to be induced by ventricular stretch or pressure. Moreover, CT-1 participates in the cardiac survival factor pathways and can prevent apoptosis occurring in cardiac myocytes. This happens through the signal transducer and activator of transcription 3 (STAT3) and mitogen-activated protein (MAP) kinase-dependent pathways (Thiriet, 2015).

2.11.4 Vascular changes

High blood pressure is characterised by the remodelling of the vascular wall which is manufactured by smooth muscle cells, endothelium cells and fibroblasts (Hopkins and McLoughlin, 2002). There are many changes that occur within the wall during vascularisation. The remodelling of the vasculature may be observed during cell migration, cell growth, cell death and creation of extracellular matrix (Davis and Senger, 2005). In arterial diseases such hypertension, there are vascular wall changes and lumen diameter reduction in the resistance vessels is present.

The role of ROS in the vascular wall is important in the fact that superoxide and hydrogen peroxide, for example, can contribute to the induction of apoptosis particularly in endothelial cells, and results

in the atherogenesis state. Angiotensin II also plays a big role in the vascular wall changes by inducing the production of the hydrogen peroxide anion that acts on the endothelin system by following the protein kinase C-dependent mechanism and leads to apoptosis (Gutierrez et al., 2006).

Studies on SHR have shown that angiotensin II can trigger inflammation through its stimulatory effect such as nuclear factor kB and AP-1, vascular cell adhesion molecule 1 and chemokine production (Luft, 2001a). Among the three layers of the vascular wall, the adventitia happens to be the most neglected area. However, it has been proven to generate ROS, thus contributing to the pathogenesis of the vascular wall. Increase in wall thickness results in decreased vessel compliance with a resultant increase in resistance and its pressor effect (Li and Fukagawa, 2010).

The term remodelling was proposed to be used where there is structural change in lumen diameter and this remodelling can be classified in term of inward or outward, depending on whether the process has resulted in a decrease or increase, respectively, in the diameter (Mulvany, 1999). This remodelling can result in either an increase, no change, or a decrease in the amount of material and this is sub-classified into hypertrophic inward remodelling which represents a decrease in lumen diameter associated with an increase in vessel wall material, Eutrophic inward remodelling that refers to a decrease in lumen diameter without a change in the composition or amount of vessel wall material and hypertrophic remodelling which involves cell division and enlargement (Mulvany, 1999, Renna et al., 2013) . Studies have shown that the wall to lumen ratio was increased during development of hypertension and that gives an indication that during remodelling the wall to lumen ratio is also an important factor in vascular remodelling (Mulvany, 1999) .

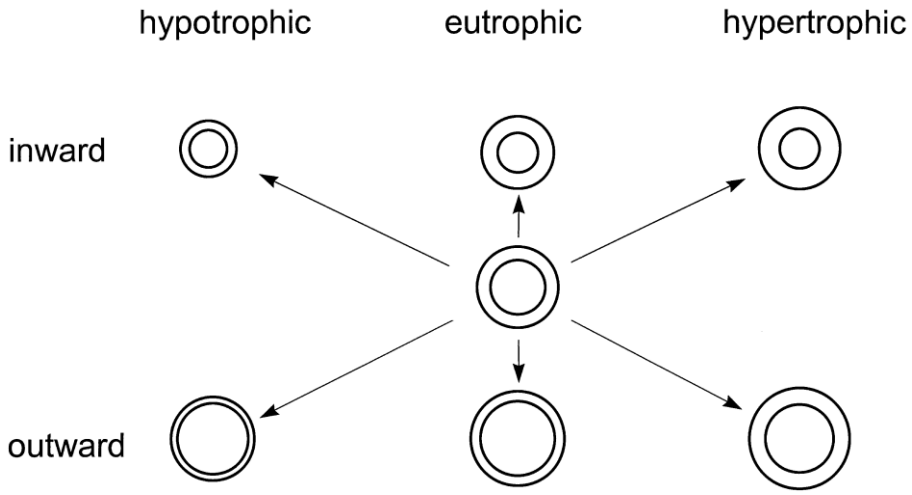


Figure 2. Different types of remodelling blood vessel (Mulvany, 1999).

2.12 Trace elements and blood pressure

All humans and other species need dietary trace elements in minor quantities for normal function of the body, as they cannot be synthesized by the biological organ system. Some of these trace element are Cu, Fe, Mn, Se and Zn. These elements are important for the regulation of blood pressure such as copper that plays a role in the elasticity of the vessels and zinc in the synthesis of collagen. Therefore, the imbalance of these elements in the blood can alter blood pressure control and participate in development of hypertension. Adequate intake and normal absorption, transport, distribution, and metabolism of trace elements are needed for optimal health (Speich et al., 2001).

Table 5. Effects of trace element imbalance

Increase BP	**Decrease BP**	**Combined**
Sodium	Potassium	Cadmium
Iron	Magnesium	Lithium
Lead	Zinc	Selenium
Arsenic	Calcium	-
Thalium	-	

Source: (Loyke, 2002a)

2.13 Antioxidant-associated trace elements in hypertension

Previous studies have shown that trace elements are bound to proteins or enzyme properties and play a catalytic role in different reactions (Tinggi, 2008). Most of the proteins in the biological system are bound to a metal and they are called metalloproteins. They play a major role in the cell in protein transport and storage, enzymes and signal transduction protein. Like ferritin an iron (III) storage and work in terms of buffering against deficiencies of iron in the biological organ system. Since trace elements have a role to play in the regulation of blood pressure, their imbalances due to dietary or metabolic factors can be triggered in the disease state where they play an important role as a mediator in the development of elevation of blood pressure. Metal-binding enzymes responsible for the defense mechanism in the tissue include copper-zinc superoxide dismutase**,** mitochondrial manganese superoxide, iron-catalase and selenium-glutathione peroxidase (Husain, 2004). However, any deficiency of these metal-binding enzymes could lead to a potential source of increased free radicals within the endothelial cells. The endothelium is the key element where free radical production can

increase and act on the arteries and eventually cause damage to the cells that results in hypertension (Singh et al., 2002).

Table 6. Trace element Components of antioxidant Systems (Loyke, 2002b)

Nutrient	**Activity**
Zinc	Constituent -cytosolic SOD
Selenium	Constituent -glutathione peroxidase
Copper	Constituent -cytosolic SOD and Ceruloplasmin
Iron	Constituent –catalase
Manganese	Constituent mitochondrial SOD

2.14 Oxidative stress and physical activity

Physical activity plays an important role in human health, preventing the risk of many chronic diseases and it has routinely been used to prevent cardiovascular diseases (Gielen et al., 2010). The WHO defines physical activity as any bodily effort which involves skeletal muscle contraction which results in increased energy production. Physical activity is a planned, structured, and repetitive body movement which aims to maintain physical fitness. Studies have also revealed that regular physical activity contributes to cardiovascular fitness and results in the reduction of blood pressure, that would benefit in cases of high blood pressure prevalence (Pal et al., 2013).

Paradoxically, studies have shown that aerobic activity increases the production of free radicals which could overwhelm the defence antioxidant mechanism and potentially may result in oxidative stress. (Higashi and Yoshizumi, 2004). There are several ways in which free radicals are generated during physical activity. The mitochondria are some of the organelles involved in oxygen production as a consequence of aerobic metabolism, and can generate free radicals. Therefore, the increase of free radicals during activity is due to oxidative phosphorylation (Belviranlı and Gökbel, 2006). Apart from the mentioned sources, many other sources of free radicals during activity are also involved, for example catecholamine, prostanoid metabolism, XO and NAD(P)H oxidase. (Urso and Clarkson, 2003, Gomes et al., 2012).

Studies have shown that athletes use antioxidant supplements to decrease the level of oxidative stress during physical activity (Felice et al., 2010). But the hypothesis that there is a need for any additional antioxidant supplement in diet still needs to be confirmed. It has been shown that a human system needs a certain level of ROS for muscle adaptation (Rahman, 2007b, Uttara et al., 2009).

2.14.1 C-reactive protein (CRP)

CRP is a pentameric protein which is mainly manufactured in the liver by specialised cells called hepatocytes. CRP has been used in many studies as an indicator of inflammation due to tissue damage, malignant neoplasia and infection (Pepys and Hirschfield, 2003, Savoia and Schiffrin, 2007). However, CRP is not only found in the liver; it can also be found in different tissues such as endothelial cells and VSMC. High levels of CRP in the blood may be a sign of inflammation and may lead to the prediction of cardiovascular disease (Savoia and Schiffrin, 2007).

Studies on women's health looking at the effect of cardiovascular risk factors in over 15 000 women have shown an increased level of CRP which is a predictor of cardiovascular risk factors, and this indication of high levels of CRP was enough to predict the risk of cardiovascular disease (Anderson, 2006).

Apart from being an indicator during inflammation, CRP acts on the endothelial cells by releasing the increased expression of cell adhesion molecules, endothelin-1 and PAI-1. Endothelin-1 is known as a vasoconstrictor factor; it can be increased by the production of superoxide which reacts with nitric oxide to form peroxynitrite. The latter is a strong free radical and can penetrate the membrane itself by deactivation of the nitric oxide bioavailability which results in an elevation in high blood pressure (Manolov et al., 2003, Montecucco and Mach, 2008).

2.15 Animal models in hypertension

Different kinds of animal models of hypertension have been developed over the past 50 years. A number of animal strains, that include several strains of rats, at least one strain of rabbit and one strain of dog readily display spontaneous hereditary hypertension. Experimental hypertensive rats are widely employed in investigative studies on the pathogenesis of human hypertension. The SHR and the Wistar-Kyoto rat (WKY) as the normotensive control have been the most employed in the studies of hypertension (Lerman et al., 2005, Sarikonda et al., 2009).

SHRs are descendants of an outbred Wistar male with spontaneous hypertension from a colony in Kyoto, Japan. The progression of hypertension starts at about 4–6 weeks of age without any induction or manipulation of pharmacological or physiological intervention. The development of hypertension in this model is similar to human essential hypertension where the blood pressure can reach up to 200 mmHg (Dornas and Silva, 2011) . The advantage of this model is that it respects the stages of hypertension which start with pre-hypertension followed by the development of hypertension, terminated by sustained high blood pressure. That allows the animal model to mimic the study of essential hypertension in humans and this is one of the reasons the strain is well accepted by most medical research centres (Lacy et al., 1998).

MATERIALS AND METHODS

3.1 Animals

Male SHR (n=16) and Male Wistar rats (n=16) weighing between 70 and 75 g bred and maintained at the Biomedical Research Unit, University of KwaZulu-Natal were used for this study. Female rats were excluded due to possible variations the estrus cycle may have on the measured parameters. Rats were randomly divided into four groups of eight animals each: exercised or sedentary SHR, and exercised or sedentary Wistar. Each group was housed in separate cages and was fed a standard rat diet and allowed access to water and food *ad libitum*.

3.2. Ethical considerations

Ethical clearance was obtained from the University of KwaZulu-Natal`s Ethics committee (reference: 027/14/Animal)

3.3 Experimental design

The training programme commenced a week after the animals were obtained from the biomedical resource unit of the University of KwaZulu-Natal, and animals in the exercised groups were trained for a period of two weeks on a treadmill in order to familiarise them with the instrument before the actual experiment commenced. This regimen used was based on a modified method employed by Somani et al. (1995) and Husain (2004) where speed, intensity and duration of exercise guidelines were considered. Metal grids of 25 volts of electricity were used to motivate the animals to run and in case the rat stepped off the belt. Animals were subjected to a graded exercise training regimen over an eight-week period using a variable speed moving-belt treadmill. Training commenced at week 1 at a treadmill belt speed of 4.0 m/min and 0° inclination. The belt speed was increased incrementally each week until a maximum of 15 m/min was reached by week 8. All animals were exercised 4 times a week.

Table 7. Training protocol

Week	Belt speed (m/min)
1	4.0
2	6.0
3	7,5
4	9.0
5	9.0
6	11.0
7	13.0
8	15.0

The inclination was at 0^0 and the time was consistent at 8 minutes

3.4 Blood pressure

Blood pressure was monitored weekly for the duration of the study on all groups and it was monitored using the non-invasive tail-cuff method. Systolic and diastolic blood pressure were recorded using the IITC Model 31 NIBP blood pressure recording equipment in the department of Physiology (IITC Instruments, USA).

3.4.1 Blood pressure training protocol

All animals were trained during the acclimatisation week. The animals were then exposed to the warming chamber at 32 degrees. SBP and diastolic blood pressure were obtained by the use of a non-invasive computerised tail-cuff system. The system comprises an automatic scanner and pump, a tail cuff with a photoelectric sensor and amplifier to measure and count the pulse rate in the animal's tail (II TC Model 31 NIBP, USA). The principle of operation is based on Riva-Rocca method used in humans.

Small, medium and large sizes of restraining devices were used. This was to compensate for the increase in mass, size and diameter of the tail of the animals during the eight-week duration of the study. The restrainers used were hollow Perspex cylinders, which fitted the rats snugly so as to minimise voluntary movement of the experimental animal, which could have an effect on the blood pressure values. The restrainers allowed for the protrusion of the tail through the tail cuff at one end and ventilation of the head at the other end. The tail cuff was attached to the restrainer by a studded end plate.

Animals were allowed to pre-heat in the restrainer for 20 minutes in a warming chamber maintained at ~31^{o}C**.** This was found to be the optimum temperature at which consistent pulses could be detected. The warming chamber consisted of a circulating heated fan and mounted, ventilated Perspex walls and cover. The warming chamber allowed for 12 animals to be pre-heated and monitored simultaneously. The optimum temperature in the warming chamber was maintained by the heated circulating fan and a temperature control.

After preheating the animals, the tail cuff was automatically inflated by the pump, which resulted in the arterial blood supply to the tail being occluded. The tail cuff was then slowly deflated and at the reappearance of a pulsation, which was detected by the photoelectric sensor, was taken as the SBP. As the pressure continued to fall, the computer automatically stored the detected high pulse point, which was accepted as the mean pressure if there was no subsequent higher pulse pressure within the next two seconds. The diastolic pressure for recording was computed using the equation Diastolic = (3mean – Systolic) / 2. This feature was part of the software used (BPMON Version 2.1., California).

The results were displayed as data plots and summary data of systolic, diastolic, mean blood pressure and heart rate on the computer screen, this information being available in printable form. The blood pressure measurement information was displayed in two forms, namely plots of analogue waveforms and digital values. The same occluding tail cuff was used for all animals, to minimise any variables in blood pressure monitoring. All results were done with the artefact filter switched on. The validation of the method and equipment was previously carried out in the same laboratory (Somova et al., 1998).

Table 8. Time line of the experiment

Time	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Procedure	Blood pressure	Blood glucose	Exercise	Exercise	Exercise	Exercise

A repeated cycle was applied for over eight weeks to achieve the goal of this project.

3.5 Sample collection and storage.

At the end of the eight-week experimental protocol, food was removed for 12 hours and the rats were anesthetised with halothane (100 mg/kg) inside an anaesthetic chamber over a period of three minutes. Before dissection, blood was taken via a cardiac puncture and then rats were dissected and samples of the abdominal aorta, brain, heart, kidney and muscles were harvested. After dissection, the brain was weighed and put into the liquid nitrogen in order to stop any metabolic process. All the samples harvested were stored at −80 °C in a bio-freezer until required for analysis. The heart was removed, washed in the saline solution and blotted on the filter paper before weighing and then was stored in the buffered neutral formalin. The kidney and abdominal aorta were placed in the buffered neutral 10% formalin for histology analyses. Blood samples were drawn in the morning after the overnight fast via cardiac puncture. Venous blood was collected into a precooled heparinised evacuated tube (approximately 7 ml) and placed on ice. One millilitre of blood was withdrawn from the heparinised tube to a normal test tube for trace element analysis. The rest of the blood was centrifuged at 3 000 rpm for 10 minutes and plasma was collected and aliquoted in 0.5 mL fractions and stored in microfuge tubes then were frozen at −80 °C until analysed.

3.6 Measurement of CRP

The CRP test was based on the principle of latex particles coated with antibody specific to rat CRP aggregate in the presence of CRP from the sample, forming immune complexes. The immune complexes cause an increase in light scattering which is proportional to the concentration of CRP in the plasma. The light scattering was measured by reading turbidity (absorbance) at 340 nm. The CRP concentration was determined from a calibration curve from CRP standards of known concentration.

The standard was done in duplicate. The kit was purchased from Randox Laboratories Limited, United Kingdom. The sample preparation was determined as follows:

Table 9. Standard preparation

	Blank, standard/sample Q.C	Test standard/sample/Q.C.
Assay Buffer	1.0 ml	1.0 ml
Standard/	100 µl	100 µl
	Tubes were vortexed and allowed to incubate for 3 minutes	
Saline	150 µl	-
Antibody	-	150 µl

3.7 Measurement of total antioxidant capacity

The total antioxidant status was determined using a commercially available kit that a 96 well plate (DTAC-100 Bioassays Systems). This assay was based on the principle in which Cu^{2+} was reduced by antioxidant to Cu^{+}. The resulting Cu^{+} specifically formed a coloured complex with a dye reagent. The colour intensity at 570 nm was proportional to TAC in the sample. The assay standards were prepared as specified in Table 10 below. Assay samples were prepared as recommended by the manufacturer.

Table 10. Standard preparation for TAC assay.

N0	1mM Trolox + H_2O	Vol (µL)	Trolox (µM)
1	100µL + 0µL	100	1000
2	60µL + 40µL	100	600
3	30µL + 70µL	100	300
4	0µ + 100µL	100	0

3.8 Preparation for trace elements for the blood, kidney and the brain

One ml of whole blood was put in a clean test tube and mixed with seven ml of 2N HCl and one ml of perchloric acid ($ClHO_4$). The kidney was washed in the deionised water, the capsule was removed, and two ml of 2N HCl was added to the test tube. The whole brain was treated similarly. Both the brain and kidney were homogenised using a graded ultra-sonicator (Nano Lab, Inc, USA). For the standard preparation see table 16. All the samples were capped and incubated in a water bath at 50 °C for digestion for approximately 24 hours. The tubes were removed, capped and centrifuged at 3 000

rpm for one hour. The supernatant was decanted into test tubes, diluted five times and filtered through 0.45 (Merck Millipore) µm syringe filter (Zeng et al., 2009). The trace element levels in blood, kidney and the brain were determined using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) at the Chemistry department at the University of KwaZulu-Natal. In ICP-OES, samples are introduced into the core of inductively coupled argon plasma (ICP), which generates a temperature of approximately 8 000 °C. At this temperature all elements emit light at their characteristic wavelengths. This light is collected by the spectrometer and the absorbance is measured. Standard curve of the absorbance vs its concentrate is constructed and the unknown elements are measured by the machine (Figure 3).

Figure 3. Inductively Coupled Plasma-Optical Emission Spectroscopy

3.9 Cardiotrophin-1

The cardiotrophin-1 was determined using a commercially available ELISA kit from Biovision (USA). This kit is based on the ELISA principle in which a microplate was coated with antibody, samples and CT-1 were added into the wells and combined antibody which with HRP labelled, became antibody-antigen-enzyme-antibody complex. After washing completely, TMB substrate solution was added, the TMB added became blue in colour at HRP enzyme-catalysed and the reaction was determined by the addition of a sulphuric acid solution and the colour change was measured using a spectrophotometer at 450 nm wavelength and the concentration of the rat CT-1 in the samples was then determined by comparing the OD of the samples to the standard curve, and all precautions were followed to prevent the edge and hook effects. The preparation of standard curve was determined as follows:

Table 11. Standard dilution

80 ng/L	5 standard	150 µ l Original Standard + 150ul Standard diluent
40ng/L	4 Standard	150 µ l 5 Standard + 150 ul Standard diluent
20ng/L	3 Standard	150 µ l 4 Standard + 150u µ l Standard diluent
10ng/L	2 Standard	150 µl 3Standard + 150 µl Standard diluent
5ng/L	1 Standard	150 µl 2 Standard + 150 µl Standard diluent

3.10 Lipid peroxidation

3.10.1 Total malondialdehyde (MDA)

MDA concentration was quantified in the skeletal muscle. The method based on that of Somova et al. (1998), was adopted for the analysis of tissue. The method is based on the principle that MDA is formed from the breakdown of polyunsaturated fatty acids, that serves as a convenient index for determining the extent of the peroxidation reaction. MDA has been identified as the product of lipid peroxidation that reacts with TBA.

Tissue for MDA analysis was stored in a bio-freezer at −80 $^{\circ}$C, to prevent extrinsic lipid peroxidation. Tissue was collected from the bio-freezer and immediately placed on ice (4 $^{\circ}$C), to thaw; the reason for using the ice was to lower the temperature gradient that the tissue was exposed to during the thawing process. The tissue was slow thawed and excess tissue was discarded, and no tissue was exposed to more than one freeze-thaw cycle.

The thawed tissue was then weighed (50 mg) and washed in deionised water to remove any excess blood on the tissue; the tissue was then blotted dry on filter paper. The method was based on that of Taulavuori et al. (2001).

The weighed tissue was transferred into a glass homogenising tube and homogenised by adding 0.2% phosphoric acid (450 µl) using a tissue homogeniser. The homogenising step was standardised for all samples, all samples were subjected to the same number of homogenising pulses (50) and the revolutions per minute (rpm) were kept at a constant 5 000 rpm. The homogenising tube was held in a beaker containing ice during the entire homogenising process, to minimise a drastic temperature increase of the suspension. The homogenate was centrifuged at 10 000 rpm for 10 minutes and the supernatant was then transferred in a glass test tube (Pyrex) and stored on ice. The supernatant was added to 500 µl of 2% phosphoric acid and vortexed and then 200 µl of 7% phosphoric acid was added and vortexed again. Finally, 400 µl BHT/TBA solution was added. The supernatant, 100 µl of

1M HCl, was then added. This reaction mixture was then lightly vortexed, and immediately transferred to a boiling water bath (100 ^{o}C) and incubated for 15 minutes.

The test tubes were then removed and immediately placed in ice slurry, for rapid cooling, to stop the reaction and inhibit further formation of the red species. To the cooled tubes 1.5 ml of butanol were added. They were then vortexed, and the top phase was transferred to microplate.

The absorbance was read at 532 nm and 600 nm and the concentration of MDA in the sample was calculated by using:

$$\text{Conc.} = \frac{\boldsymbol{A}532 - \boldsymbol{A}600}{1.56\text{nm}}$$

3.10.2 Reagents

1. BHT: 20 mM
2. NaOH: 50 mM
3. BHT/TBA solution: by adding 5 ml of 20 mM BHT to 100ml of NaOH solution, mix and then add 1 g of TBA
4. 0.2% phosphoric acid: 170 µl of 85% phosphoric acid into 100 ml of H_2O
5. 2% phosphoric acid: 1.7 ml of 85% phosphoric acid into 100 ml H_2O
6. 7% phosphoric acid: 5.95 of 85% phosphoric acid into 100 ml H_2O
7. 1M HCl
8. Butanol (99.8%)
9. Ethanol (99.8%)

3.11 Optimising gene expression

3.11.1 Introduction

Currently, tools for studying gene expression at the mRNA level can be divided into three major groups, each being an excellent tool for its specific purpose. The major groups are: hybridisation-based techniques, PCR-based techniques and sequence-based techniques (Kozian and Kirschbaum, 1999, Tseveleki et al., 2010)

Real-time PCR technology has recently reached a level of accuracy and practical ease which supports its use as a routine technique for gene expression studies. Several applications have already been implemented in various research areas, including cancer and hypertension research. Data obtained from real-time PCR can provide both researchers and clinicians with valuable information related to disease processes (Mocellin et al., 2003).

3.12 Background

3.12.1 Real-time PCR

3.12.2 The kinetics of real-time PCR (RT-PCR)

A real-time PCR reaction profile has three segments: an early background phase, an exponential growth phase (or log phase) and a plateau phase. The background phase lasts until the fluorescence signal from the PCR product is greater than the background fluorescence. The exponential growth phase begins when sufficient product has accumulated to be detected above background, and ends when the reaction slows and enters the plateau (Higuchi et al., 1993).

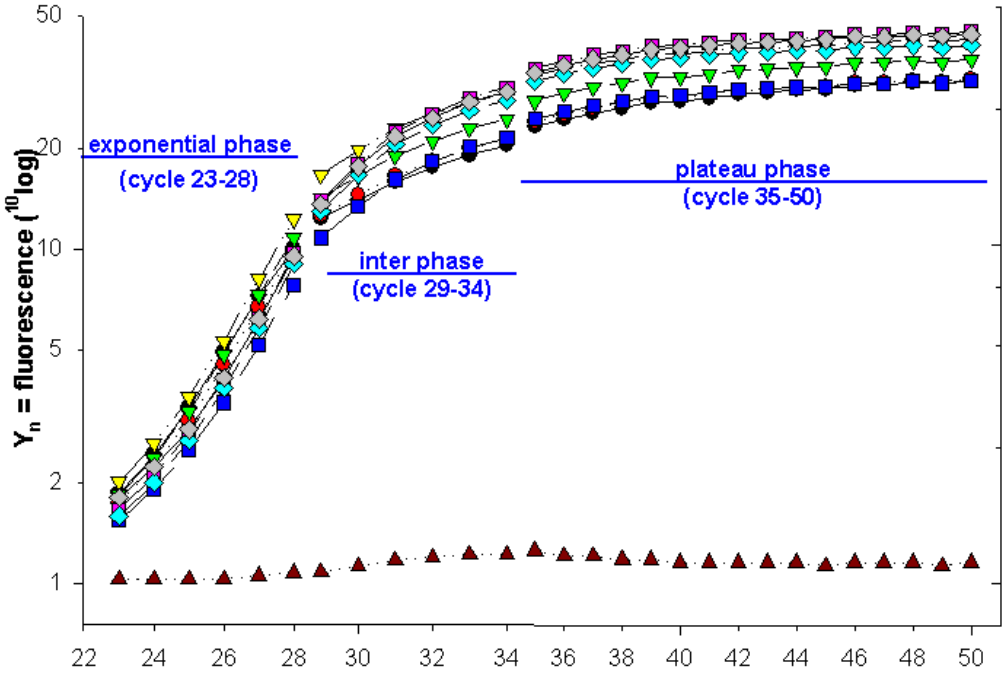


Figure 4. The amount of DNA vs number of cycles

3.12.3 Instrumentation: The Lightcycler

Real-time PCR is performed using a plate-based machine LC 480. The core facility has two Roche LightCycler 480 (LC 480) machines for quantitative PCR analysis. Each LC 480 is a plate-based machine that comes with two exchangeable thermal blocks, one with 384 wells, and the other with 96 wells. Both thermal blocks are fast-cycling, meaning that typical run times are slightly under one hour. The range of reaction volume is 20–100 µl for the 96 well block and 5–20 µl for the 384-well block with reaction volumes of 10 µl, which leads to significant savings in reagent costs. The latter uses a xenon lamp as its light source and has a number of possible excitation/emission wavelengths to choose from, making multiplexing much easier. The excitation/emission filters can be set

independently. The LC 480 can measure more than one fluorescence wavelength per well and typical run times are slightly under one hour. This means that endogenous controls can be run either as parallel reactions in separate wells or within the same well as the sample (multiplex PCR) and the range of reaction volumes is 20–100 microlitre for the 96 well blocks.

Figure 5. The Roche LightCycler 480

3.12.4 DNA binding dyes

This method involves detection of the binding of a fluorescent dye (SYBR Green) to DNA (Morrison et al., 1998). The unbound dye exhibits little fluorescence in solution, but during elongation, increasing amounts of dye bind to nascent double-stranded DNA. When monitoring in real time, this results in an increase in the fluorescence signal that can be observed during the polymerisation step and that falls off when the DNA is denatured. Consequently, fluorescence measurements at the end of the elongation step of every PCR cycle are performed to monitor the increasing amount of amplified DNA (Ririe et al., 1997).

With dsDNA-specific dyes, amplification can be stopped after an amount of product is synthesised, thus avoiding over amplification of alternative templates. The extension phase of each cycle needs to be continued only as long as fluorescence increases (Gustafson et al., 1993, Barnes and Karin, 1997).

3.13 The threshold cycle

The concept of the threshold cycle (Ct) is at the heart of accurate and reproducible quantification using fluorescence-based RT-PCR (Higuchi et al., 1993). Fluorescence values are recorded during every cycle and represent the amount of product amplified to that point in the amplification reaction. The more templates present at the beginning of the reaction, the fewer number of cycles it takes to reach a point in which the fluorescent signal is first recorded as statistically significant above background (Gibson et al., 1996). This point is defined as the Ct, and will always occur during the exponential phase of amplification (Higuchi et al., 1993, Gibson et al., 1996).

3.14 Standard curves

Fluorescence for the standard curves is easily generated because of the large dynamic range in its linear response. There are either absolute or relative quantification of mRNA transcription. In the relative quantification there is change in the steady-state transcription of a gene and it is often adequate (Bustin et al., 2005, Kosaka et al., 2010). The calibrator and sample constitutes part of a relative standard which is used to create a dilution series with arbitrary units. During the RT-PCR assay the target Ct is compared directly with the calibrator Ct and is recorded as containing either more or less mRNA (Bustin, 2002).

3.15 Methods

3.15.1 Overview

RNA was extracted from kidney tissue, cDNA synthesis was performed as described later, followed by real-time PCR for respective genes. Finally a standard curve was extrapolated and used for the quantification of gene expression of test samples.

3.15.2 RNA isolation

RNA was extracted from rat skeletal muscle and liver tissue using the modified Trizol method (Perou et al., 1999). Tissue (50–100 mg) was homogenised in 1 ml Trizol reagent, followed by incubation for 10 minutes at room temperature. The homogenate was centrifuged for 10 minutes at 4 °C at 12 000 rpm followed by addition of 300 µl of chloroform. The chloroform homogenate was shaken vigorously for 30 seconds and incubated at room temperature for 5 minutes, followed by centrifugation for 15 minutes at 4 °C at 12 000 rpm. The supernatant which contained the total RNA was transferred into a new 1.5 ml microfuge tube.

The total RNA was precipitated with the addition of 500 µl isopropanol and 1.5µl glycogen and incubated for 10 minutes at room temperature. This was followed by centrifuging for 10 minutes at 4°C at 12 000 rpm. The supernatant was discarded and the RNA pellet was washed in 1 ml 70% ethanol, followed by centrifugation for five minutes at 4 °C at 12 000 rpm. The supernatant was discarded and RNA pellet was air dried. The RNA pellet was resuspended in 25 µl (DEPC) water.

The absorbance was measured at 260 nm and the RNA was run on a 2% agarose gel. RNA was stored at −60°C for further use. RNA was treated with DNase (TURBO DNASE cat. No. 1907) as per manufacturer`s protocol. PCR was then performed on the RNA after treating with DNase. The housekeeping-gene (GAPDH) was used in this PCR to confirm if DNA contamination existed. cDNA was synthesised using a GeneAmp 9700 PCR System (Applied Biosystems, California, USA).

3.15.3 Reverse transcriptase PCR (cDNA synthesis)

cDNA synthesis was performed using the Biorad iScript cDNA synthesis kit according to the manufacturer`s protocol. The reaction mixture was constituted as follows: 4 µl of 5 × iScript reaction mix, 1 µl of iScript reverse transcriptase, 8 µl of nuclease free water and 7 µl of RNA template. The above reagents were added to a sterile PCR tube and kept on ice. The reaction protocol entailed incubating the reaction mix at 25 °C for 5 minutes, 42 °C for 30 minutes, 85 °C for 5 minutes and holding at 4 °C for 45 minutes in the GeneAmp 9700 PCR System (Applied Biosystems, California, USA).

Primers used in the experiment are listed in Table 12 and the specific mRNA sequences were amplified using the following primer pairs (from 5' to 3')

Table 12. Primers used in the experiment

Gene	Oligo-nucleotides sequence	Cycling conditions
NF-kBforward	CCTAGCTTTCTCTGAACTGCAAA	95°C for 15 s, 60°C for 15s, and 72°C for 15 s
NF-kBreverse	GGGTCAGAGGCCAATAGAGA	
IkBforward	TGGCTCATCGTAGGGAGTTT	95°C for 15 s, 60°C for 15s, and 72°C for 15 s
IkBreverse	CTCGTCCTCGACTGAGAAGC	
SODforward	CGTCATTCACTTCGAGCAGAAGG	95°C for 15 s, 60°C for 15s, and 72°C for 15 s
SODreverse	GTCTGAGACTCAGACCACATA	
GAPDH forward	GCCAAAGGGTCATCATCTCCGC	95°C for 15 s, 60°C for 15s, and 72°C for 15 s
GAPDH reverse	GGATGACCTGCCCACAGCCTTG	

The LightCycler master mix consisted of: 1 µl of water, 0.5 µl forward primer (0.5 µM), 0.5 µl reverse primer (0.5 µM) and 5µl Fast Start SYBR Green I; (Roche Diagnostics). LightCycler master mix (9 µl) was added to the plate.

Real-time PCR was performed using the LightCycler 480 (Roche Diagnostics) with the following PCR conditions: one cycle consisting of 95 °C for 10 minutes followed by 55 cycles of 95 °C for 15 sec, 60 °C for 15 seconds, 72 °C for 15 seconds and 78 °C or 83 °C for 20 seconds with a single fluorescence measurement. Melting curve analysis was done at 78–95 °C with a heating rate of 0.1 °C per second and a continuous fluorescence measurement. This was followed by a final cooling step to 40 °C.

To improve the SYBR Green quantification, high temperature fluorescence measurements at 78 °C for NF-KappaB, IkB, and SOD were carried out. In addition the fluorescence measurement for GAPDH was captured at 83 °C at the end of the fourth segment (Pfaffl, 2001). This step destroys non-specific PCR products below the chosen temperature, e.g. primer dimers. In addition, non-specific fluorescence signals are eliminated and this ensures accurate quantification of the desired NF-Kappa, IkB, SOD and GAPDH real-time products, respectively.

For the mathematical model it was necessary to determine the crossing points (CP) for each transcript. CP is defined as the point at which the fluorescence rises appreciably above the background fluorescence (Wilkening and Bader, 2004). The "Second Derivative Method" was performed with the LightCycler 480 software version 1.5.1 (Roche Diagnostics).

3.15.4 Construction of standards curves

Real-time PCR was performed on one cDNA sample from each group (i.e. NF-KappaB, IkB, SOD and GAPDH). After melt curve analysis and fragment size analysis, the absorbance was measured at 260 nm.

A 1:10 dilution was performed on the real-time PCR product and absorbance was noted at 260 nm (GeneQuant). The concentration of double-stranded DNA was also noted.

Using the following four equations the number of molecules corresponding to the initial standards was calculated and used to extrapolate standard curves for NF-KappaB, IkB, SOD and GAPDH.

1. Concentration of double-stranded DNA (GM) = (absorbance at 260nm) × (concentration µg/ml) × (dilution factor) × (volume used in gene quant)
2. Average molecular weight (MW) of a DNA base pair: 660 (Da), mm or MW of dsDNA = (number of base pairs) × (660Da)
3. Number of moles (n) = Concentration of double-stranded DNA (GM) / Average MW of dsDNA (mm)
4. Number of molecules = Number of moles (n) × (6.022×10^{23}) (Avogadro's number)

For each gene a standard curve was generated with 10-fold serial dilutions of the DNA standards (real-time PCR product) ranging from 1020 to 1011 copies per PCR reaction, seven dilutions were chosen: 10^{-3}, 10^{-4}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-10}, 10^{-12}. Quantitative analysis of the data was done using the LightCycler 480 analysis software (Version 1.5.1; Roche Diagnostics).

3.15.5 Melting curve analysis of PCR products

Melting curve analysis was used to determine the presence of non-specification products. The melting temperature (Tm) is defined as the temperature at which half of a duplex-DNA becomes single-stranded (Schalasta et al., 2000).

3.15.6 Histological study

For the light microscopic study, the specimens were fixed in 10% formaldehyde, underwent a dehydration process with series, and embedded in paraffin wax using the tissue-embedding centre (LEICA EG 1160, WETZLAR, Germany). Tissues were sectioned (3–5 sections/specimen) using a microtome 5 μm thin sections (MICROM HM 340E, WALLDORF, Germany). For the cardiac tissue and the kidney tissue, the specimens were stained with standard staining like Haematoxylin and Eosin (H&E). For the proximal aortic tissue, the specimens were stained with Verhoeff-Van Gienson (VVG) staining protocol for elastic fibres. Measurements of media thickness (from the internal to the external elastic laminae) were obtained from five areas. Magnified digital images of the stained aortic slices were obtained using a digital colour attached to an optical microscope. The histological examination was carried out at a magnification of ×200 for the cardiac and kidney tissue and ×400 for the aorta using Leica Scanner, SCN400 and Slide Path Gateway LAN software (Leica Microsystems CMS, Wetzlar, Germany).

3.16 Analysis of data

All data were expressed as means ± standard error of means (SEM). Statistical analysis was done using GraphPadInstat Software (version 5.00, GraphPad software, San Diego, California, USA). Statistical comparison between groups was done using one-way analysis of variance (ANOVA) followed by the Mann Whitney test comparison test. Values of $p < 0.05$ were taken to imply statistical significance.

RESULTS

4.1 Body weight

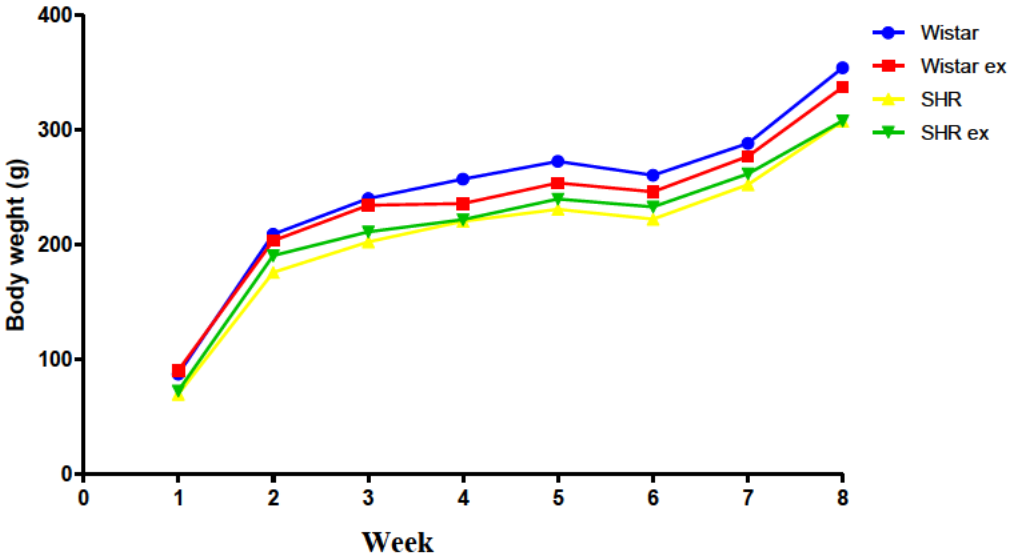


Figure 6. Average body mass.
No significant differences in body weight were observed between all four groups.

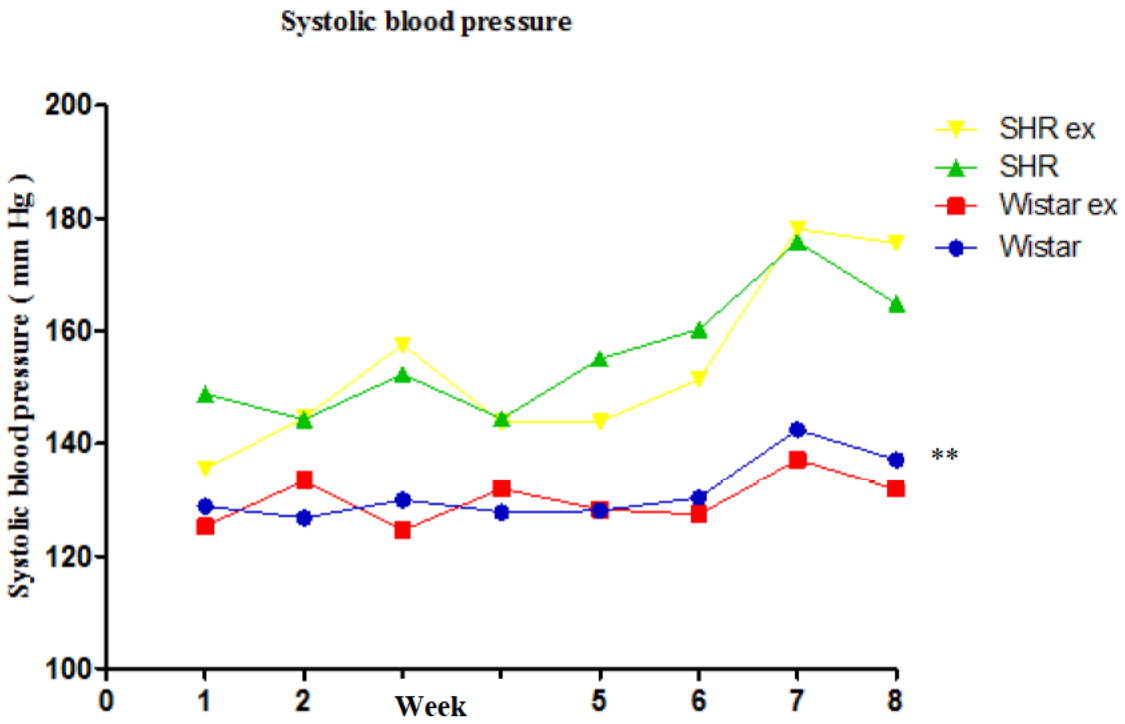


Figure 7. Average Systolic blood pressure.

The systolic BP was significantly lower in the Wistar than in the SHR strain.
The SHR can be classed as Stage 1 hypertensive while the Wistar were normotensive (WHO, 2013).
Exercise had no effect on the systolic BP in both groups
**<0.05 in Wistar and SHR strain

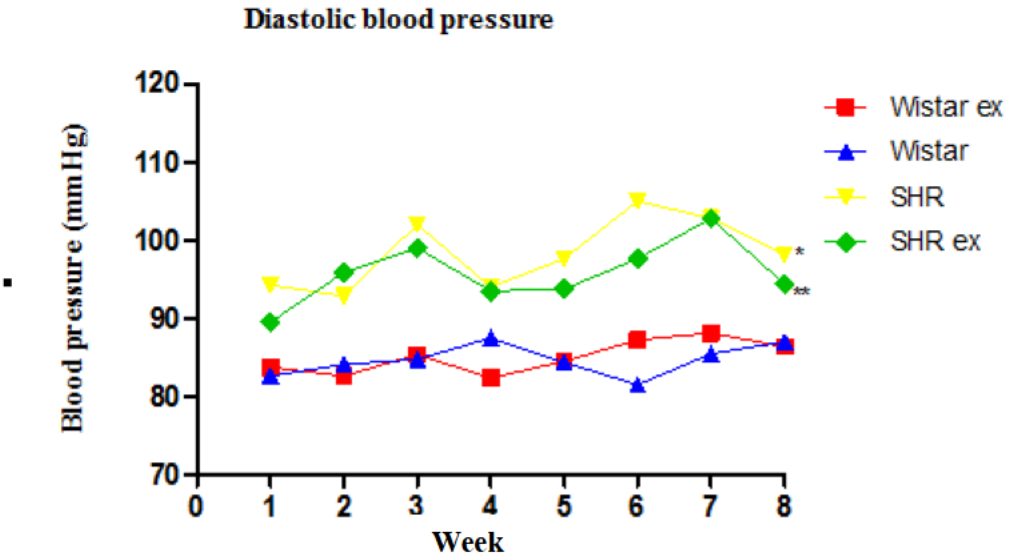


Figure 8. Average diastolic blood pressure.

The diastolic blood pressure was significantly lower in the Wistar than in the SHR strain. The SHR can be classed as Stage 1 hypertensive while the Wistar were normotensive (WHO, 2013). Exercise significantly decreased the diastolic BP in the Wistar EX group but not in the SHR EX group.
*p<0.05 in Wistar group
**<0.05 in Wistar and SHR strain

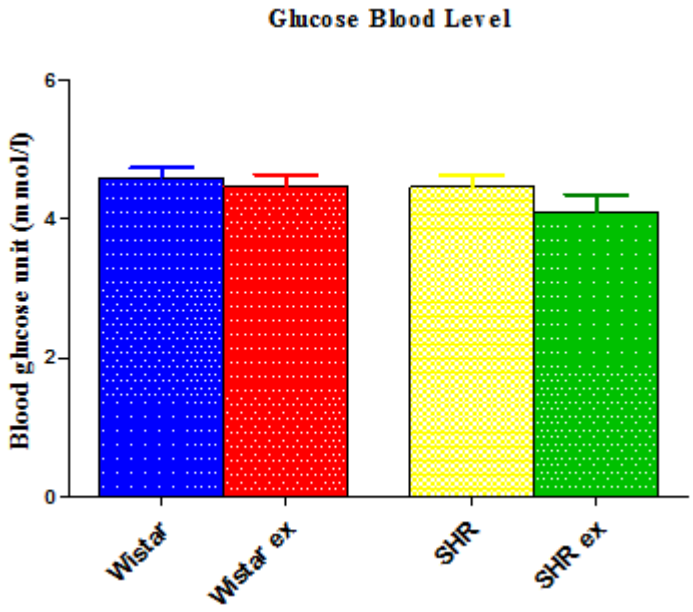


Figure 9 Average blood glucose.

No significant differences in fasting blood glucose were observed between all four groups.

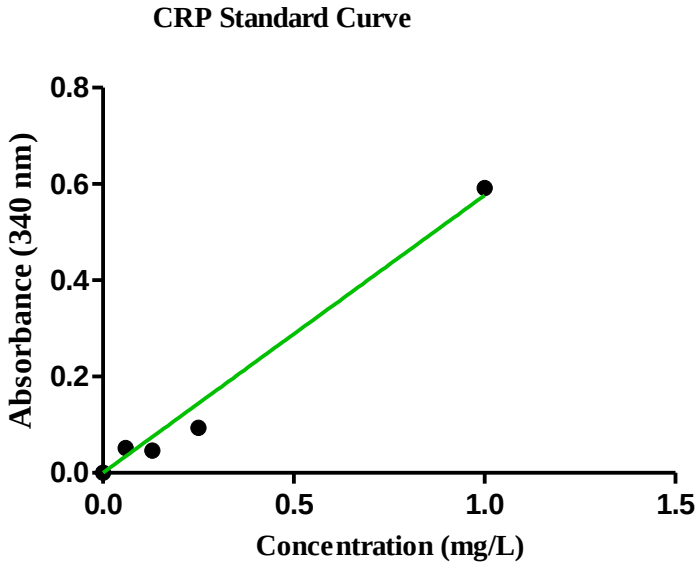


Figure 10. Standard Curve of CRP ($r^2 = 0.99$)

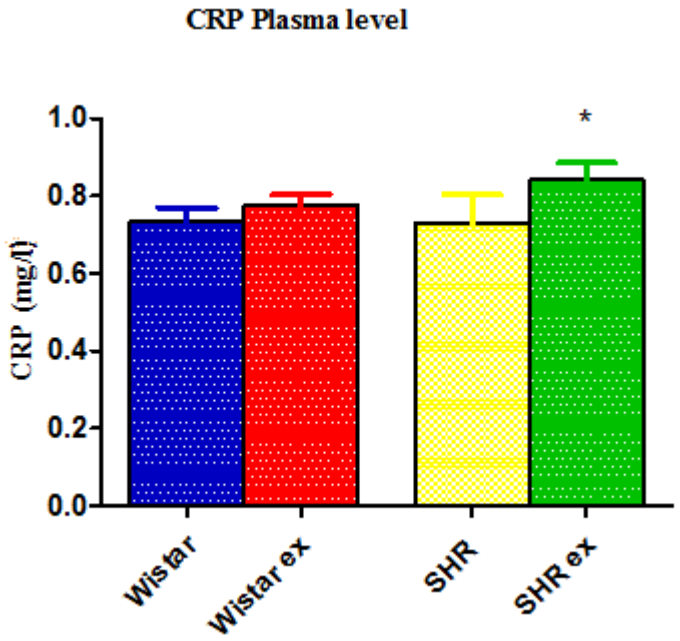


Figure 11. Average plasma CRP level.
* $p < 0.05$ in SHR group

There was no significant difference in plasma CRP levels between the Wistar and SHR strains.
Exercise significantly increased the plasma CRP levels in the SHR EX group but not in the Wistar EX group.

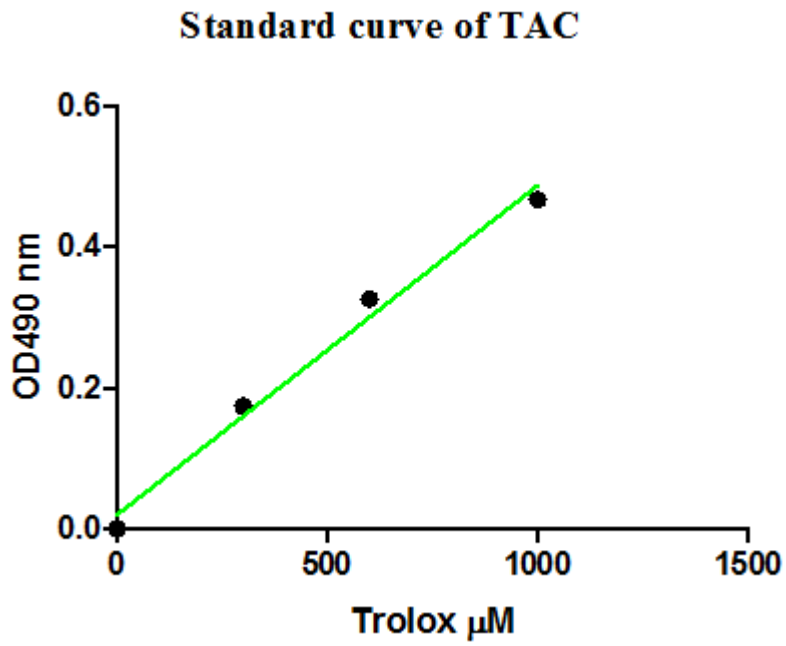


Figure 12. Standard curve of Total Antioxidant Capacity (r^2=0.99)

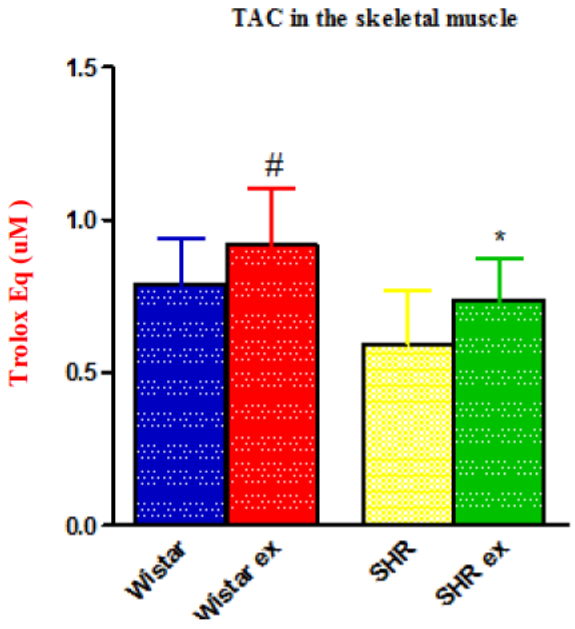


Figure 13. Average TAC in skeletal muscle
# p < 0.05Wistar group
* p< 0.05 in SHR group

The skeletal muscle TAC was significantly higher in the Wistar than the SHR strain.
Exercise significantly increased the muscle TAS in both the SHR EX group and the Wistar EX group.

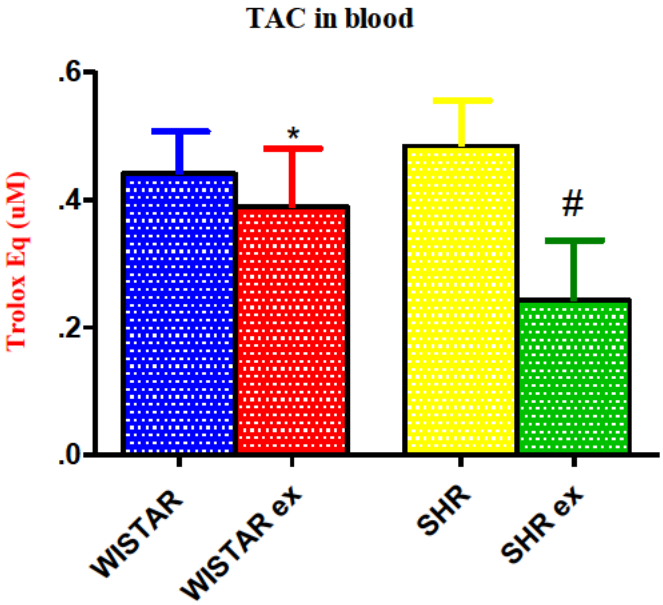


Figure 14. Average TAC blood level
*p < 0.05Wistar group
# p< 0.05 in SHR group

There was no significant difference in blood TAS between the Wistar and SHR strains.
Exercise significantly decreased the blood TAS in both the SHR EX group and the Wistar EX group.

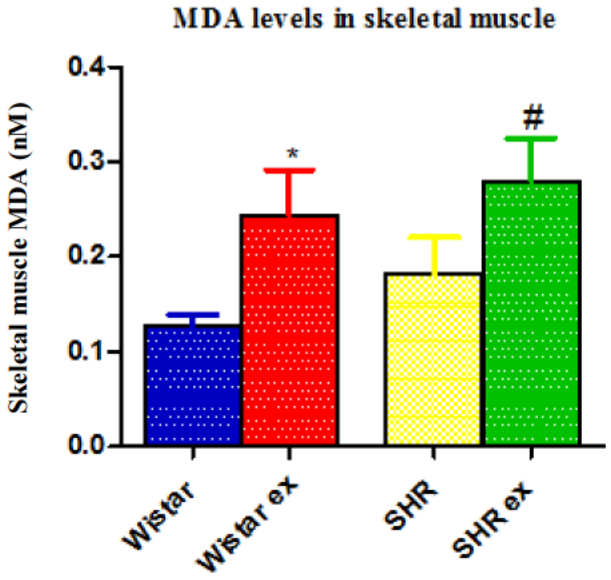


Figure 15. Average MDA in skeletal muscle.
# $p < 0.05$ in the SHR group
* $p < 0.05$ in the Wistar rats group

Skeletal muscle MDA levels were significantly higher in the SHR than the Wistar strain. Exercise significantly increased MDA in both the SHR EX group and the Wistar EX group.

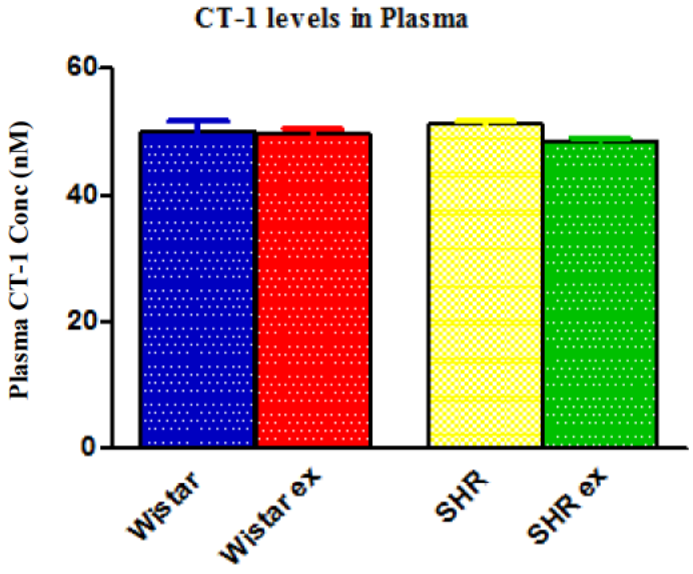


Figure 16. Average plasma CT-1 level
The blood in SHR and Wistar rats exercise group compared to their respective control non-exercise SHR and Wistar rat.

There was no significant difference in plasma CT-1 levels between all four groups.

4.2 Overview

Table 13 shows that over the eight-week period on treadmill activity in SHRs and Wistar there were no significant changes in the body mass (Figure 6), and systolic (Figure 7) blood pressure, blood glucose (Figure 9) and cardiotrophin-1 (Figure 16). However, there was a significant ($p < 0.05$) increase in diastolic blood pressure (Figure 8) and plasma CRP levels in SHR exercise compared the SHR non-exercise groups (Figure 11). The plasma TAC levels was significantly ($p < 0.05$) reduced in both SHR and Wistar rats exercise groups compared to the non-exercise ones (Figure 14), while TAC in the skeletal muscle was significantly ($p < 0.05$) increased in both SHR and Wistar exercise groups compared to the non-exercise ones (Figure 13). The MDA levels was significantly ($p < 0.05$) in both SHR and Wistar rats exercise groups compared to the non-exercise ones (Figure 15).

Table 13. Summary of the effect of the treadmill activity of SHR and Wistar**.**
Data are expressed as Mean ± SEM, n=8 in each group

Parameter	**Groups**			
	Wistar	**Wistar exercise**	**SHR**	**SHR exercise**
Body weight (g)	246.3 ± 27.14	234.9 ± 24.89	210.3 ± 24.29	217.5 ± 24.19
Systolic blood pressure (mmHg)	131.5 ± 1.936	130.0 ± 1.530	155.7 ± 3.843	153.8 ± 5.515
Diastolic blood pressure (mmHg)	85.60 ± 0.7880	**83.36 ± 0.5360** ##	85.60 ± 0.7880	90.11 ± 1.350
Glucose blood (mmol/l)	4.591 ± 0.1300	4.452 ± 0.1778	4.446 ± 0.1746	4.088 ± 0.2537
CRP plasma (mg/l)	0.7332 ± 0.036	0.7759 ± 0.02714	0.7291 ± 0.0743	**0.8421 ± 0.04209** #
TAC in blood (nM)	0.4401 ± 0.06606	**0.3883 ± 0.09216** *	0.4836 ± 0.07106	**0.2417 ± 0.09418** #
TAC in the skeletal muscle (nM)	0.7864 ± 0.1507	**0.9185 ± 0.1815** *	0.5903 ± 0.1788	**0.7352 ± 0.1378** #
MDA in the skeletal muscle (nM)	0.1275 ± 0.01183	**0.2427 ± 0.04885** *	0.1825 ± 0.03817	**0.2495 ± 0.04543** #
CT-1 in plasma (ng/L)	49.98 ± 1.683	49.58 ± 0.8418	51.15 ± 0.4735	48.5 ± 0.4801

4.2 Trace element distribution in the blood, brain and kidney

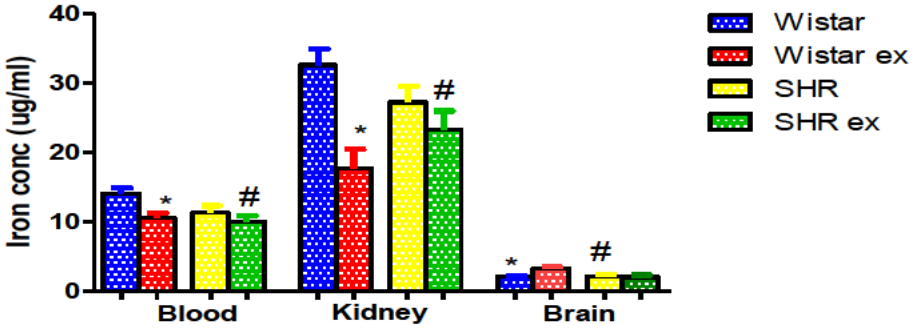


Figure 17. Average of blood, kidney, brain Fe levels of SHR and Wistar rats.
# p < 0.05 in the SHR group
* p< 0.05 in the Wistar rats group

Blood, kidney and brain Fe level was significantly lower in the SHR than the Wistar strain.
Exercise significantly decreased Fe levels in both the SHR EX group and the Wistar EX group in all tissues above.

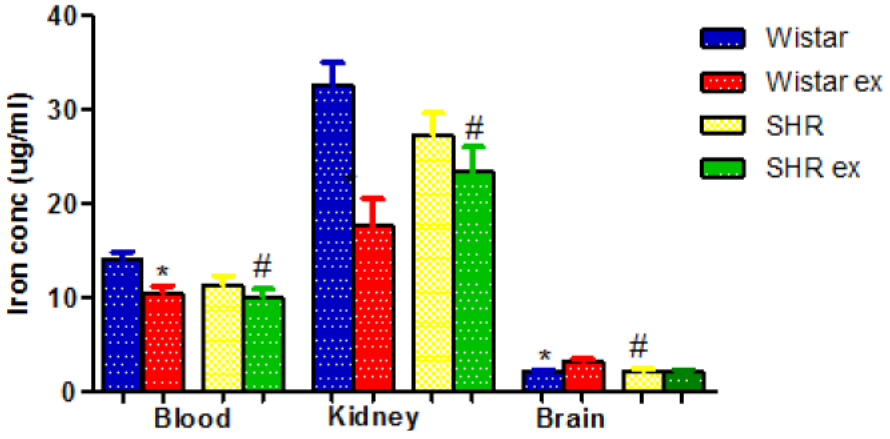


Figure 18. Average of blood and kidney selenium levels of SHR and Wistar rats.
# p < 0.05 in the SHR group
* p< 0.05 in the Wistar rats group

Blood, kidney selenium levels were significantly lower in the SHR than the Wistar strain.
Exercise significantly decreased selenium levels in both the SHR EX group and the Wistar EX group

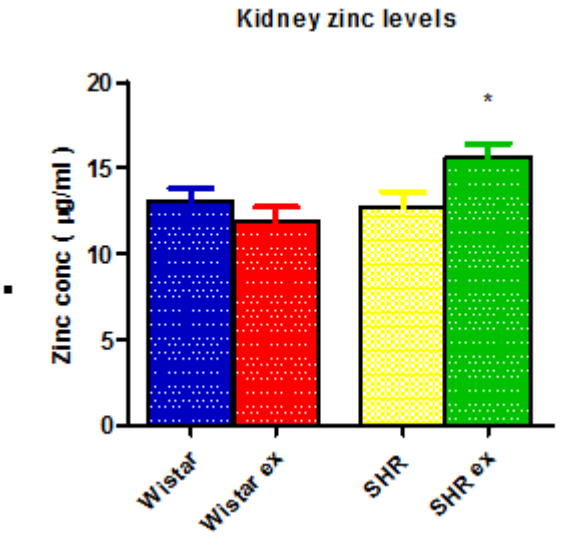


Figure 19. Average of Zn levels in the kidney of SHR and Wistar rats**.**

No significant difference in kidney Zn levels between SHR and Wistar strain.
Exercise significantly increase kidney Zn levels in the SHR EX group only

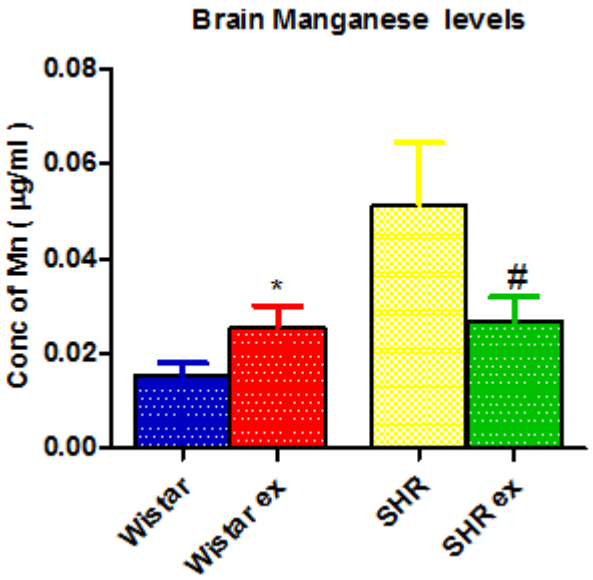


Figure 20. Average of brain Mn level of SHR and Wistar rats.
# $p < 0.05$ in the SHR group
* $p < 0.05$ in the Wistar rats group

Brain Mn levels were significantly higher in the SHR than the Wistar strain.
Exercise significantly increased Mn levels in Wistar EX group but decreased in SHR EX group

4.3 Overview

Table 14. Summary of the effect of the treadmill activity on trace element distribution in SHR and Wistar rats. Data are expressed as mean ± SEM, n=8 in each group

Parameter (μg/g of tissue, g/ml)	**Group**			
	Wistar	**Wistar exercise**	**SHR**	**SHR exercise**
Copper in the blood	0.64 ± 0.069	0.555 ± 0.036	0.543 ± 0.016	0.615 ± 0.126
Copper in brain	1.028 ± 0.057	1.03 ± 0.098	0.966 ± 0.066	0.9093± 0.09
Copper in kidney	4.472 ± 0.131	2.869 ± 0.553	3.819 ± 0.549	3.03 ± 0.589
Iron in blood	17.13 ± 0.748	12.55 ± 0.681	15.34 ± 1.019	**10.11 ± 0.768** #
Iron in brain	2.132 ± 0.153	**3.265 ± 0.243** *	2.07 ± 0.278	2.209 ± 0.212
Iron in kidney	32.64 ± 2.346	**17.69 ± 2.804** #	27.27 ± 2.342	23.34 ± 2.647
Manganese in brain	0.015 ± 0.003	**0.026 ± 0.004** *	0.051 ± 0.013	**0.027 ± 0.005** #
Manganese in kidney	0.573 ± 0.059	0.695± 0.055	0.543 ± 0.036	0.516 ± 0.046
Selenium in blood	0.487 ± 0.057	**0.239 ± 0.012** *	0.364 ± 0.126	**0.269 ± 0.124** #
Selenium in brain	2.127 ± 0.306	1.723 ± 0.094	1.532 ± 0.393	0.871 ± 0.111
Selenium in kidney	2.17 ± 0.306	1.723 ± 0.094	1.532 ± 0.393	0.871 ± 0.111
Zinc in blood	2.804 ± 0.101	2.612 ± 0.1	2.641 ± 0.170	2.531 ± 0.102
Zinc in brain	2.928 ± 0.270	2.401 ± 0.128	2.541 ± 0.311	2.679 ± 0.425
Zinc in kidney	13.09 ± 0.736	11.92 ± 0.808	12.73 ± 0.871	**15.64 ± 0.800** #

# $p < 0.05$ by comparison with the SHR non-exercise group

***** $p < 0.05$ by the comparison with the Wistar rats non-exercise group

4.4 Muscle Liver SOD, NF-kB and IkB gene expression

The results show a down regulation (17.5-fold) of mRNA expression levels of SOD in skeletal muscle of the SHR and Wistar rats exercise groups compared to the SHR and Wistar groups (Figure 24 A, p=0.0003) using simple unpaired T test. While in the liver the mRNA expression levels of SOD in SHR and Wistar rats exercise group compared with the SHR and Wistar exercise group was observed with the same trend (2.6 fold decrease) (Figure 24 B, p=0.02). Up-regulation (15.9 fold) of mRNA expression levels of NF-kB expression in skeletal muscle of SHR exercise compared to the Wistar rats exercise group (Figure 28 C, p=0.014). In the liver up-regulation (7.4 fold) of mRNA expression levels of NK-kB in SHR exercise group compared to SHR group were noted. Steady level of mRNA NF-kB expression in the Wistar rats exercise group compared to Wistar group (Figure 25 D, p=0.00722). Using the unpaired T test, no significant difference was noted in mRNA expression levels of IkB in skeletal muscle of the SHR exercise group compared with the Wistar rats exercise group (Figure 26 E). In the liver no significant difference of mRNA expression levels of IkB was noted in SHR and Wistar rats (Figure 26 F).

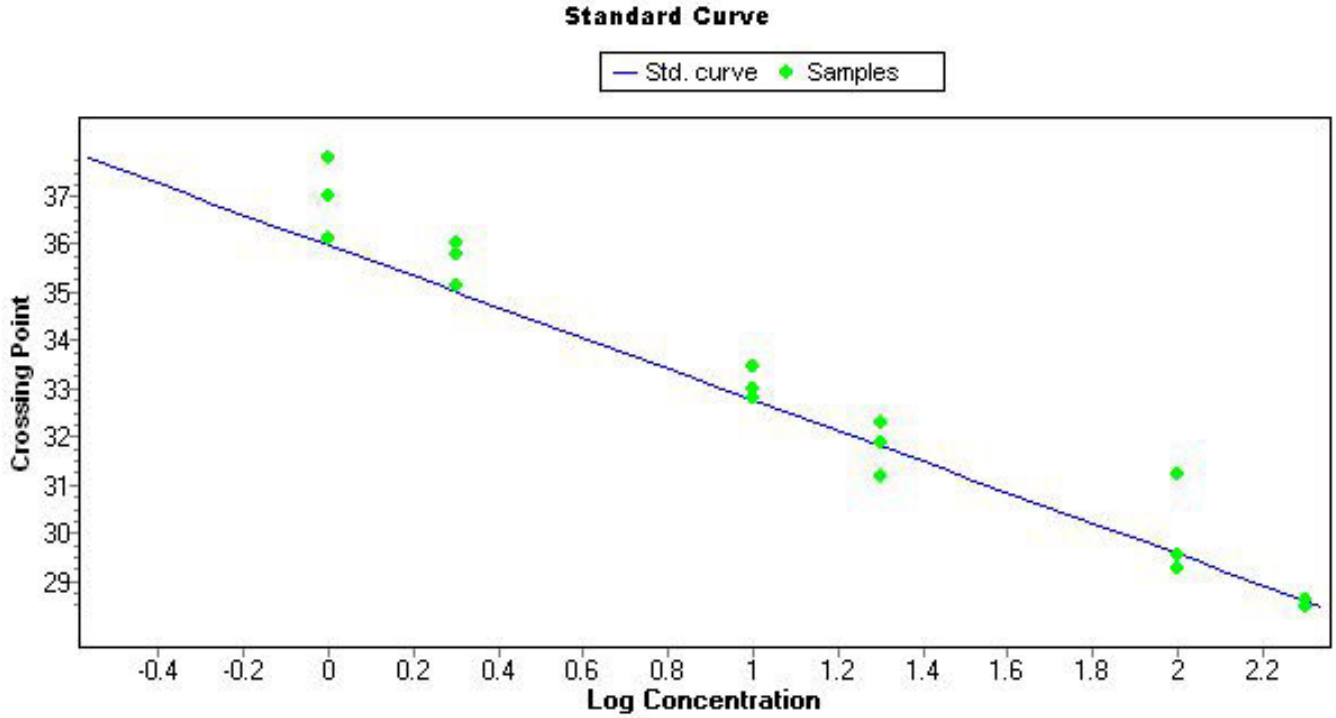


Figure 21. The standard curve constructed for the GAPDH.
The calibration curve shows the crossing point of each standard plotted against the logarithmic concentration to produce a standard curve. A dilution series of GAPDH amplicon was used as a standard template for the reaction

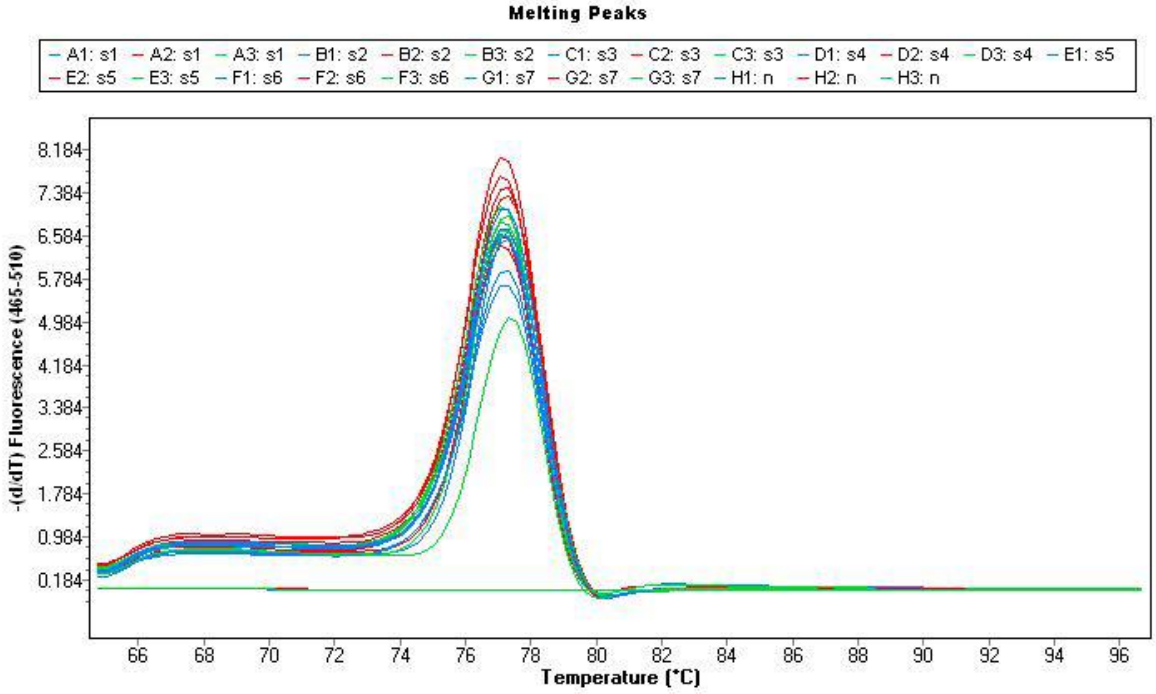


Figure 22. Melting peaks for the GAPDH gene.
Melting curves were converted to melting peaks by plotting the negative derivative of the fluorescence with respect to temperature (-dF/dT) against temperature.

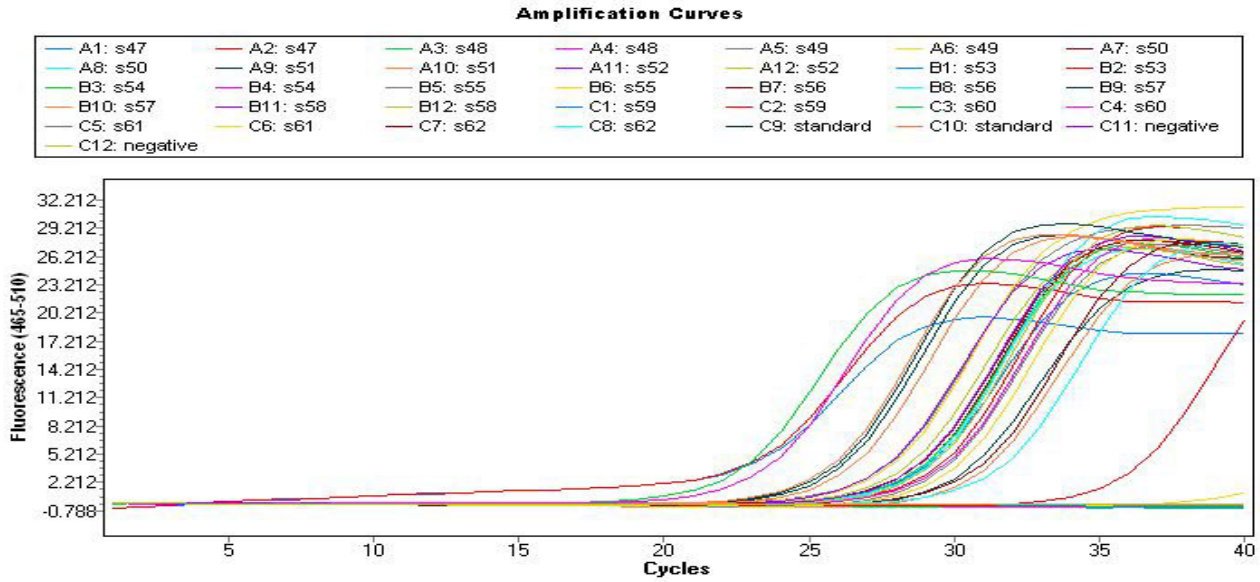


Figure 23. .Representative graphs showing amplification curve for GAPDH using SYBR Green I dye

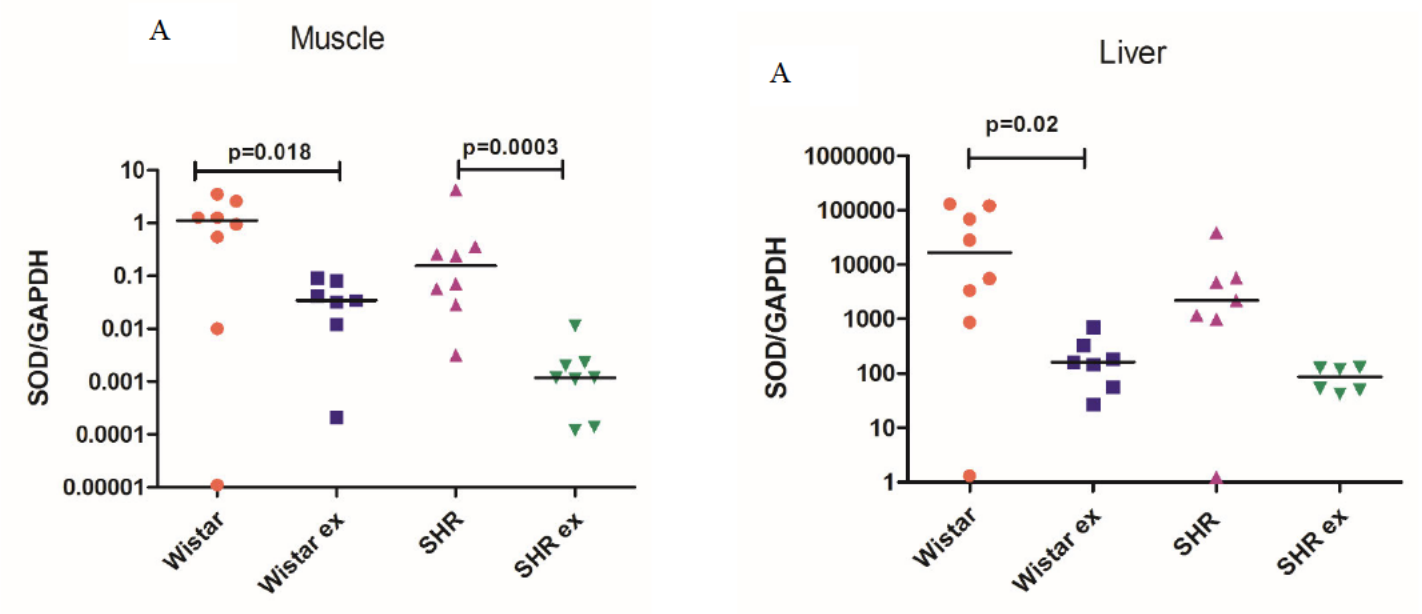


Figure 24. A and B: mRNA expression levels of SOD
SHR and Wistar rats exercise group compared to the SHR and Wistar rats group, n=8. Data are depicted as a normalised as ratio of SOD versus GAPDH. A p value of < 0.05 was considered statistically significant

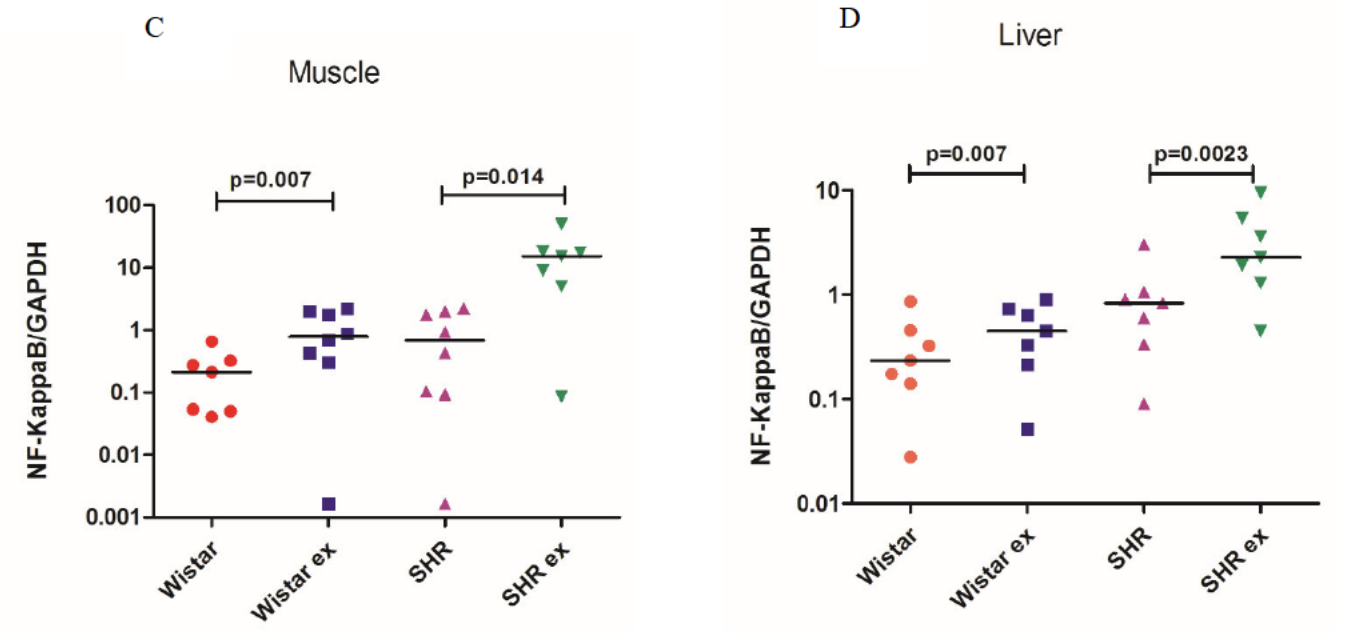
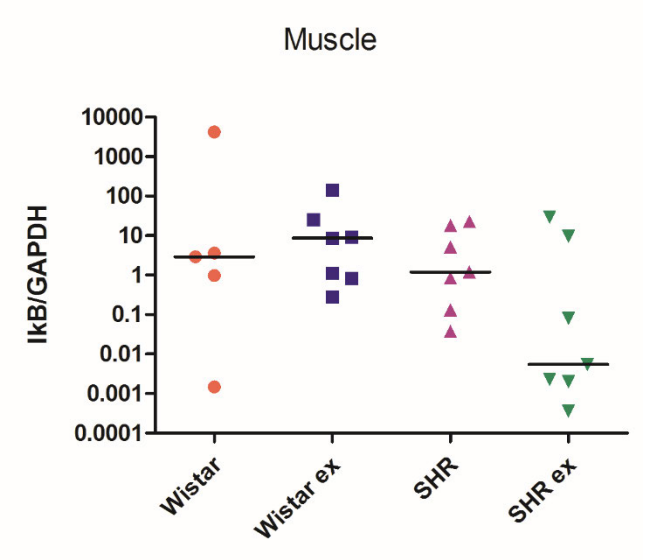


Figure 25. C and D: mRNA expression levels of NF-kB
SHR and Wistar rats exercise group compared to the SHR and Wistar rats group, n=8. Data are depicted as a normalised as ratio of NF-kB versus GAPDH. A p value of < 0.05 was considered statistically significant

E



E

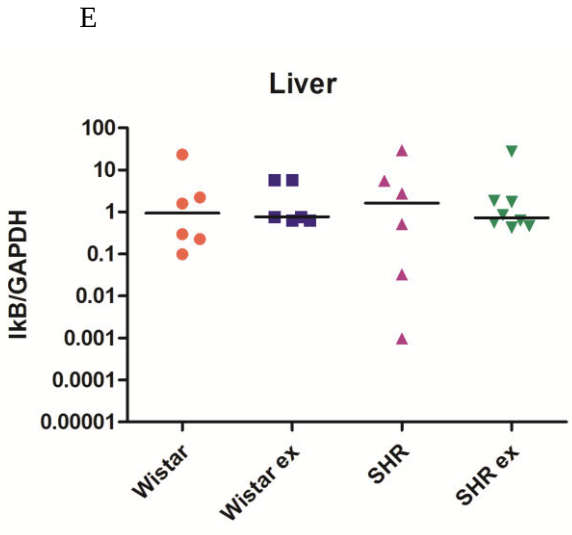


Figure 26. E and F: mRNA expression levels of IkB
SHR and Wistar rats exercise group compared to the SHR and Wistar rats group, n=8. Data are depicted as a normalised as ratio of IkB versus GAPDH. A p value of < 0.05 was considered statistically significant

Histology of the cardiac tissue

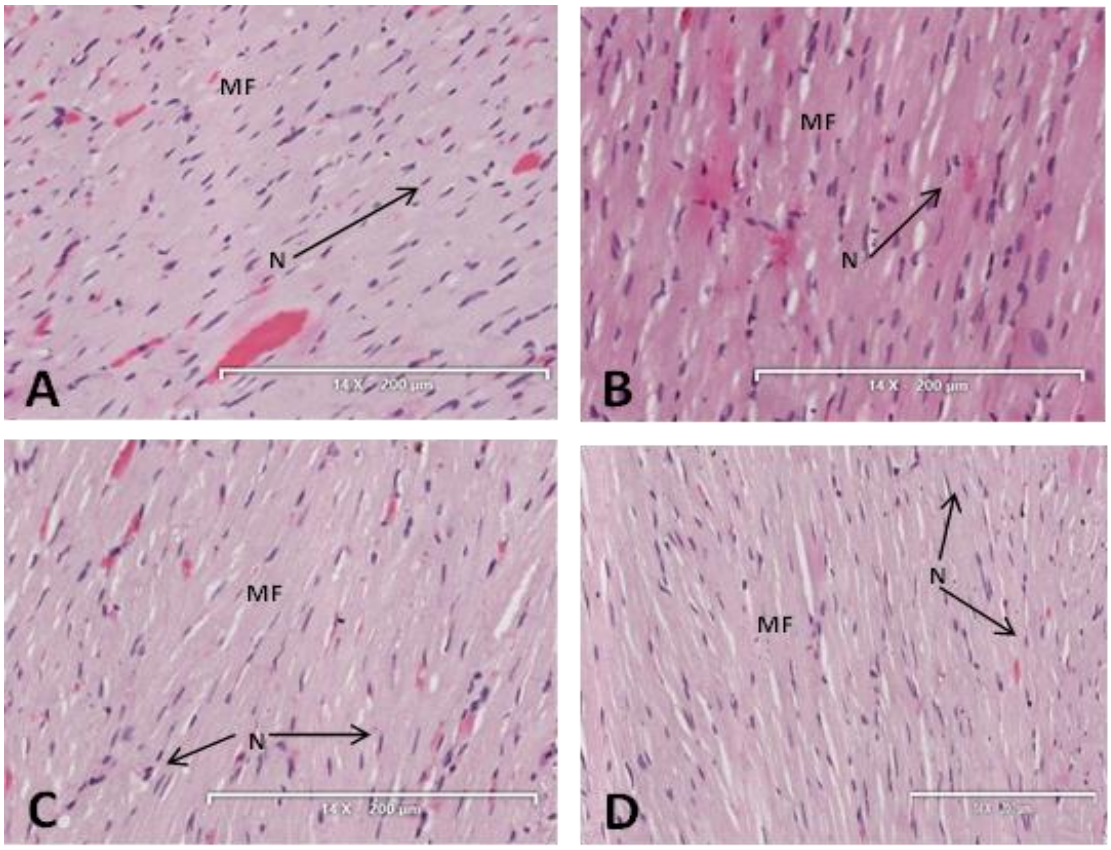


Figure 27. Photomicrograph showing longitudinal section of cardiac tissues
(A) control group of SHR (B) exercise group SHR (C) control group of Wistar rat (D) exercise group of Wistar rat where N: nuclei of cardiomyocytes, MF: myofibres under Haematoxylin and Eosin stain. (LMx200)

Histology of the renal cortex

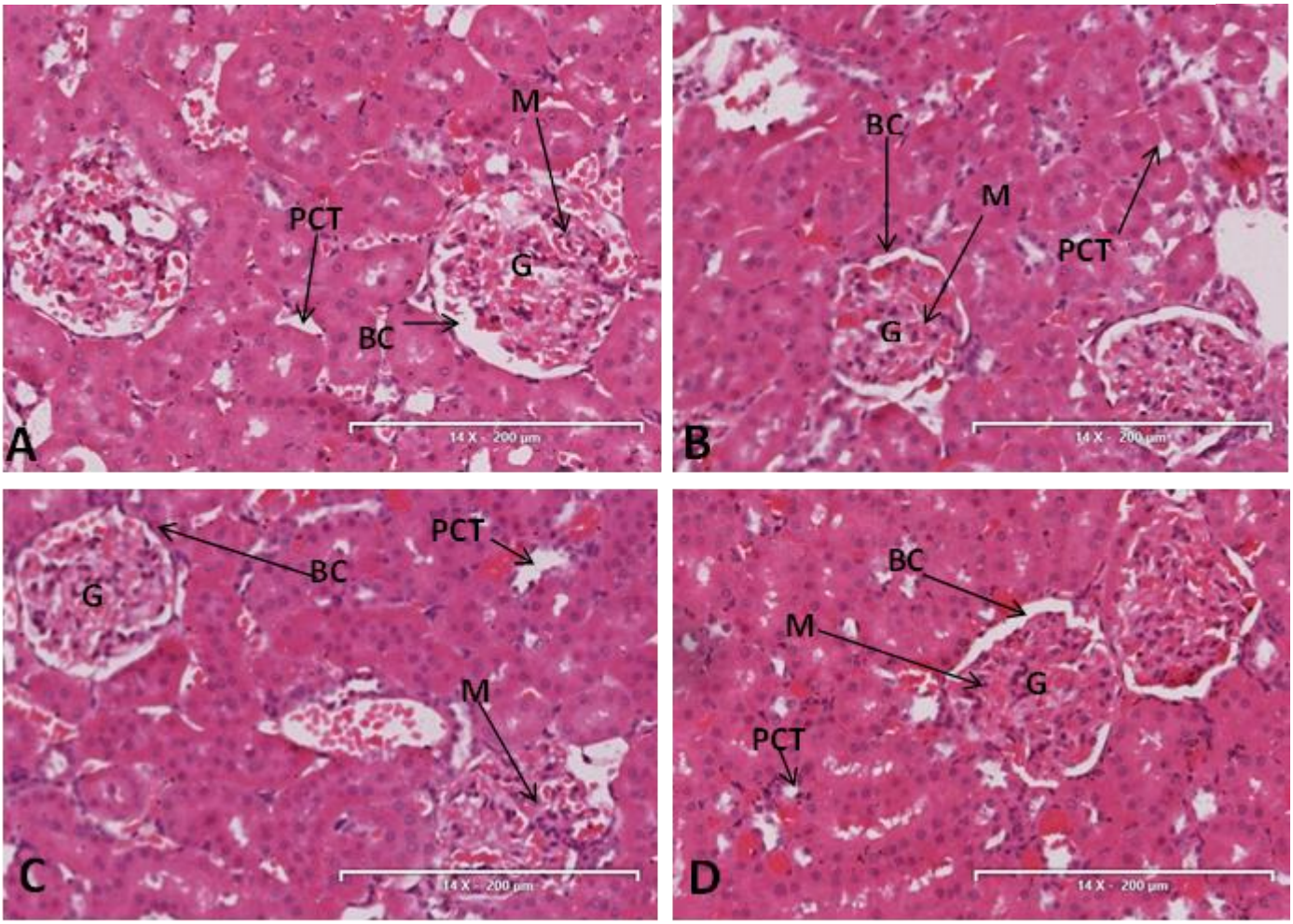


Figure 28. Photomicrograph showing the renal cortex morphology of the kidney
Photomicrograph showing the renal cortex morphology of the kidney
(A) control group of SHR (B) exercise group SHR (C) control group of Wistar rat (D) exercise group of Wistar rat where BC: Bowman's capsule, G: glomerulus, M: mesangium, PCT: proximal convoluted tubule, under Haematoxylin and Eosin stain. (LMx200)

Table 15: Measurements of thickness of the tunica media of the aortic wall (n = 8).
Data are expressed as Mean ± SEM, n=8 in each group

Group	SHR	SHR exercise	Wistar	Wistar exercise
Thickness TM (μm)	394.1 ± 11.66	**417.4 ± 15.16** #	302.3 ± 27.25	**357.1 ± 16.89** *

# $p < 0.05$ by comparison with the SHR group
* $p < 0.05$ by the comparison with the Wistar rats group

Histology of the aorta

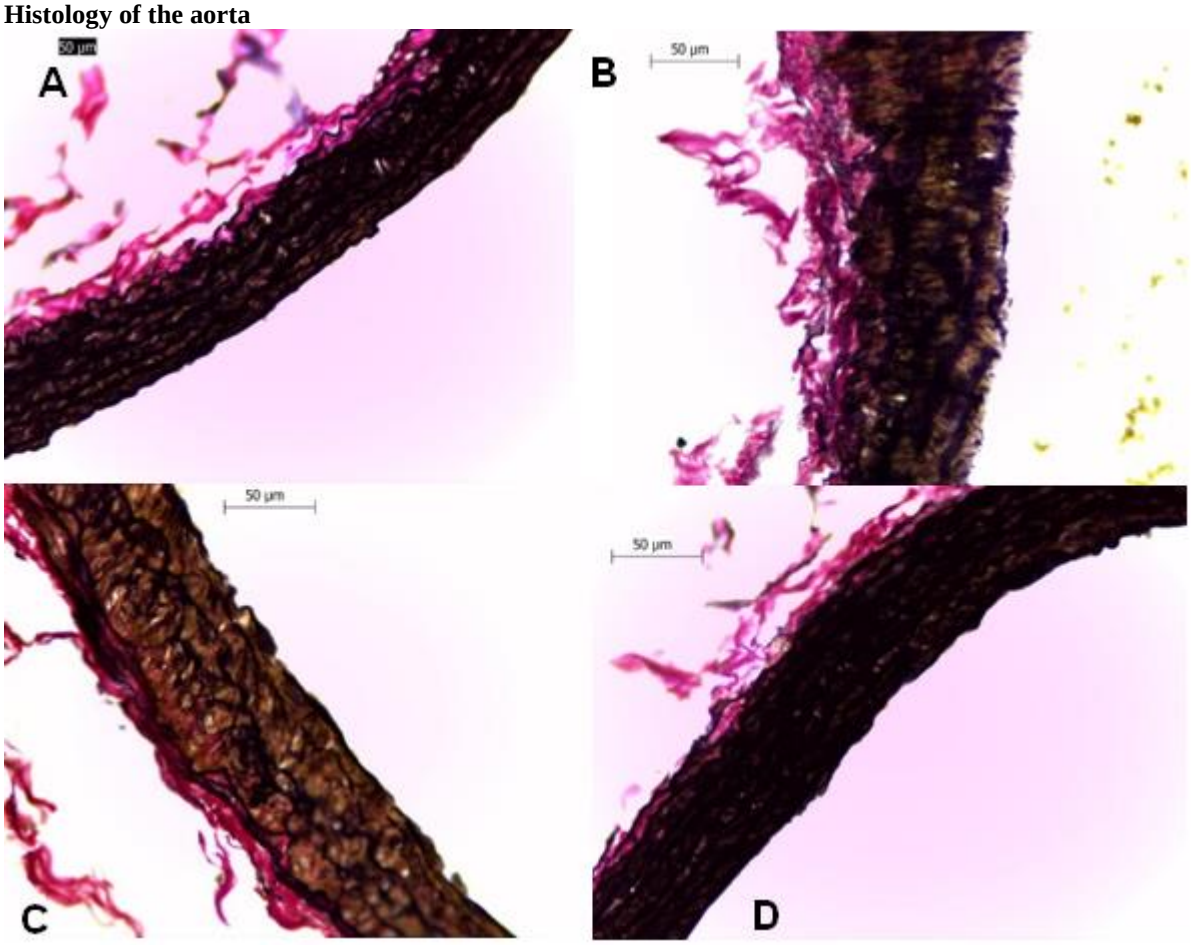


Figure 29 Photomicrograph showing the section of aortic tissues
(A) control group of SHR (B) exercise group SHR (C) control non-exercise group of Wistar rat (D) exercise group of Wistar rat where TM: tunica media

4.5 Overview

For H&E staining, the SHR group showed single, oval and centrally located nuclei of cardiomyocytes with regularly arranged cardiac myofibres (Figure 27 A). However, nuclei of the cardiomyocytes in the spontaneously hypertensive rat exercise group showed deformation in sizes and shapes and the cardiac myofibres in this group were found to be in a disarrayed pattern in comparison to SHR group (Figure 27 B). The single and oval nuclei of the cardiomyocytes were also shown with regularly arranged cardiac myofibres in the Wistar rats group (Figure 26 C) and a similar pattern was observed in Wistar exercise rats (Figure 26 D).

Figure 28 A shows the normal glomerulus (G) of the SHR control group kidney section showing Bowman's capsule, proximal convoluted tubule (PCT), mesangium (M), in comparison to the SHR exercise group which showed thin basement membrane of the Bowman's capsule, hypercellularity of the proximal tubules (Figure 28 B). The Wistar rats control group (Figure 28 C) showed normal Bowman's capsule, PCT and mesangium in comparison to the Wistar rats exercise group in which a similar pattern was observed (Figure 28 D).

Table 15 shows that under VVG staining the increase in elastic fibres was significantly observed ($p < 0.05$) in the SHR exercise group (Figure 29 B) in comparison to the control SHR group (Figure 29 A). Wistar rats exercise group (Figure 29 D) also showed a significant ($p < 0.05$) increase in elastic fibres in comparison to the control Wistar rats (Figure 29 C) group.

DISCUSSION

The aim of this study was to investigate the effect of a moderate treadmill activity on cardiovascular factors in SHR rats, a well-established model of essential hypertension. This study presents strong empirical evidence that the model displays oxidative stress, which is intensified by physical activity. Biochemical, histological and gene expression changes confirm that the aetiology of hypertension is multifactorial in origin.

5.1 Body weight

The data presented here show that the body mass over the eight-week period showed no significant changes in Wistar and SHR strain. Exercise did not show any effect in both SHR and Wistar EX group. Other studies have shown a slight decrease in body mass in the control group that could be due to the adaptation of the Wistar rat to increase in energy consumption (Marques et al., 2001). Free radical production plays a role in alteration of metabolic activities and during physical activity high oxygen intake is needed and this could lead to free radicals production which reflected in SHR EX (Schneider and Oliveira, 2004). Therefore, it is suggested that the unchanged body mass in this study could be due to the duration and intensity of exercise applied to this model.

5.2 Blood pressure parameters

The SBP was significantly elevated in SHR than in the Wistar strain. This difference is due to the genetic differences as expected. As the animal grow older the blood pressure increases as well. The progression of blood pressure started after 4 weeks of the experiment indicating that various hypertensive mechanisms are coming into effect in the SHR and this confirms the validity of the use of this model. The SHR can be classed as comparable Stage 1 hypertensive that has been classified for human values while the Wistar were normotensive (Tran and Giang, 2014). The Factors that impact on the SBP, include, physical, mental and pathological factors. All these factors can be manipulated to lower or raised the SBP. Moreover, physical health has the largest overall impact on the systolic blood pressure (Juliet, 2015). Of all these factors physical activity had no impact on the SBP in both the SHR and Wistar groups.

The diastolic blood pressure was significantly elevated in SHR compared to the Wistar strain. This could be due to genetic differences of the strain. The progression of diastolic pressure in the SHR started around 4 weeks and this can be classed as Stage 1 hypertensive as compared to human guideline of hypertension, while the Wistar were normotensive (Tran and Giang, 2014). Diastolic blood pressure is directly related to the volume of blood left in the aorta at end of diastole. Some of the pathological factors affecting diastolic pressure, include lifestyle, lack of adequate physical activity and, unhealthy diet include. Exercise significantly decreased the diastolic blood pressure in the Wistar EX group but not in the SHR EX group.

Blood pressure changes observed in this study may be explained by two synergistic mechanisms – changes in vascular architecture and oxidative stress related vasodilatory mechanisms.

In the aortic wall, tunica media which is the thickest layer in the arteries, was further increased in the SHR during exercise. While there was a change in the vessel wall thickness we could not pick up a change in blood pressure and this is not comparable to what happened in training humans where the compliance changes and there is a drop in blood pressure This change in distensibility in part could explain why no changes occurred in the DBP in the SHR group after the exercise regimen. The wall to lumen ratio is a very important ratio because it can determine the status of hypertensive patients, this factor could not be verified in the study due to the irregular form that vessels acquired after infiltration. Physical activity may therefore have an effect on the vasculature and peripheral resistance.

Studies have shown that exercise subjects the body to a stress and increased oxygen consumption which may cause organ dysfunction and free radicals generation when the defence mechanism is deficient. At the endothelial level exercise also generates nitric oxide in the cardiovascular system by induction of eNOS. This NO reacts with the superoxide anion to form a very toxic compound peroxynitrite radical which can disturb the vasodilation factor within the vessel by deactivating the nitric oxide which in turn results in vasoconstriction of the vessel that subsequently elevates blood pressure (Husain, 2004). In this study we could not assess the wall to lumen ratio as discussed above. Moderate physical activity did not appear to be beneficial in addressing this mechanism in the SHR. Studies also showed that free radicals act as signalling factors in the vasculature through NAD(P)H oxidase which appears to be the major in vasculature cells(Xu and Touyz, 2006)

Both the blood pressure components, systolic and diastolic, have been altered in SHR. This could have been related to the impaired dilatory mechanism explained above and this could be trigged due to the increased free radical production during aerobic metabolism. Decreased expression of SOD, and TAC in the SHR indicate impaired antioxidant defences and therefore a blunted blood pressure response to exercise observed in the SHR group.

In the Wistar and SHR EX group, a decreased trend in the DBP was shown in this study could be attributed to the beneficial effect of moderate exercise. A previous study over a period of 13 weeks did show a significant increase in the diastolic pressure (Horta et al., 2005). In view of previous studies, it is acknowledged that if the exercise regimen had continued, further changes in blood pressure might have been observed in both exercise groups.

5.3 Biochemical parameters

With regard to the blood glucose levels, there was no significant difference between the all groups; however, there was a trend where the exercise group had a slightly lower blood glucose level

compared to the controls and this could explain that exercise had no effect on the blood glucose levels in this strain. During exercise the muscle experience a metabolic rate increase and this results in an increase need for fuel, therefore an increase expression of Glut receptors. This is one of the reason that glucose uptake is enhanced during physical activity. The SHR does not have this adaptive mechanism to ensure that there is enhanced glucose uptake upon an increase in physical activity. While on the treadmill protocol, it was also noted that the SHR animals had a bigger problem to cope for the duration of the protocol. This is an indication that glucose uptake is not enhanced, the metabolic pathways can not produce energy. In the first week of exercise blood glucose was lower in both Wistar and SHR EX groups and a significant increase in blood glucose was observed at week eight in the exercise groups

It is suggested that the increase in blood glucose at week 8 may be related to the age of the rat. When there is an increase in stress, this results in an increased catecholamine release, which in turn causes an increase in blood pressure, glycolysis and glucose release. Therefore physical activity experienced by the rats could also have caused this trend between DBP and Glucose levels in the Wistar and SHR exercise groups.

There was no significant difference in plasma CRP levels between the Wistar and SHR strains. Physical activity significantly increased the plasma CRP levels in the SHR EX group but not in the Wistar EX group. CRP plasma levels showed no significant difference between Wistar and SHR strain. But physical activity significantly increases plasma CRP levels in SHR EX with 0.12 mmHg while in the Wistar rat group no significant changes occurred. CRP is a component of immune response that the body produces when faced with infection or stress, and is synthesised by the hepatocytes and it is regulated by IL-6,IL-1, and TNF (Salgado et al., 2011). CRP has been used clinically and considered as one of the most potent predictors of cardiovascular diseases. Moreover, CRP was shown to increase the effect of NF-kB activity that was revealed by the cytosolic IkB inhibitors (Jialal et al., 2004) which shows that CRP really participates in many inflammatory factors.

On the other hand, CRP was shown to play a role on endothelial nitric oxide bioavailability by deactivating the NO synthase and free radical production that might shut down the NO production (Fichtlscherer et al., 2004). It is suggested that the increase in plasma CRP level observed in the SHR exercise group could not only be due to the exercise but might also be due to the increase of ROS produced in the blood. It was also shown that plasma CRP level is high in hypertensive subjects (Qamirani et al., 2005) and exercise was found to lower plasma CRP level. Paradoxically in this study it is found that there was an increase in CRP levels in the SHR exercise group and lower plasma TAC levels in both Wistar and SHR EX groups. It is suggested that the increase of CRP plasma levels in SHR EX could be influenced by physical activity and TAC plasma levels in this study.

In the gene expression in skeletal muscle and the liver it was found that NF-kB mRNA levels were significantly different in SHR compared to the exercise ones where the exercise group showed 35.4-fold increased mRNA expression levels of NF-kB in skeletal muscle. While in the liver a 21.1-fold increase of mRNA expression of NF-kB in SHR exercise group was observed compared to the non-exercise one. A similar trend was observed in Wistar rats where a 1.2-fold increase mRNA expression levels of NF-kB was observed in skeletal muscle of the Wistar EX group compared to the non-exercise one. Nuclear factor (NF-kB) is type of protein produced in the body to regulate genes and a broad range of biological processes including innate and adaptive immunity, and inflammation. And in the physiological conditions NF-kB proteins are bound and inhibited by IkB proteins. Kramer and Goodyear (2007) Showed that during physical activity increased NF-kB expression in the skeletal muscle of the rat. And another study in rats showed that NF-kB may play a role to alter the fuel metabolism during exercise through increased transcription of the IL-6 which enhances glucose transport and lipid peroxidation in muscle (Petersen and Pedersen, 2005).

It is suggested that the difference in the NF-kB expression observed in the Wistar and SHR non-exercise could explain that these animals are genetically different. In the SHR exercise group, the increase of NF-kB expression might explain the fact that NF-kB is playing a role in the regulation of numerous genes of the inflammation cascade and studies have shown that NF-kB is a promoter of E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) genes. Also the impact on SHR EX is that NF-kB plays a role in transcription of many vascular genes and angiotensin II participates in the activation of NK-kB in VSMC. Once the NK-kB is activated, this has an impact on the renin angiotensin system (Luft, 2001b) and this could have been one of the causes of the SHR EX group having a high level of NF-kB compared to the Wistar EX. Another observation is that CRP level in the SHR exercise group was found to be high and this could be to the level of NK-kB as explained earlier. In Wistar rat exercise group, the slight increase in NF-kB expression could be due to the normal body response as NF-kB is also activated in healthy conditions (Hughes et al., 1998). Therefore, our study showed that mRNA expression of NF-kB was produced more in skeletal muscle than in the liver of these animals. The skeletal muscle gives us a good indication of NF-kB expression in these animal models since skeletal muscles are metabolically more active.

To assess the antioxidant status, TAC in the blood and skeletal muscle was measured in the SHR and Wistar rat EX groups vs their respective controls. In this study there was no significant difference in blood TAC between the Wistar and SHR strains. While the relative change in enzymatic antioxidant defenses was not monitored, the overall TAC has assessed no changes in the SHR. But studies on high blood pressure monitoring have shown up-regulation of catalase in SHR of 6 weeks of age (Sundaram et al., 2013). This may be a compensatory response mechanism which lead to elevation of CAT. Exercise significantly decreased the blood TAC in both the SHR EX group and the Wistar EX group.

In the skeletal muscle TAC was significantly higher in the Wistar than the SHR strain and exercise significantly increased the muscle TAC in both the SHR EX group and the Wistar EX group. ROS are produced during physical activity and research showed that there is increased oxygen consumption in the mitochondria during exercise and therefore, there is free radical production by these organelles such as superoxide which is increased through complexes I and III of the electron transport chain. Superoxide is usually converted to H_2O_2 by manganese superoxide dismutase (MnSOD) and diffuses into the cytosol (Kramer and Goodyear, 2007). Since exercise improves oxygen utilisation in the body and also increases free radicals production in various tissues a defence mechanism of antioxidant enzymes is also increased in the cardiovascular system. The imbalance of this free radicals production and antioxidants generation results in oxidative stress. Research has shown that NO is produced in the cardiovascular system by induction of eNOS. This NO reacts with superoxide anion to form toxic peroxynitrite radical and this molecule will deactivate the bioavailability of NO and results in vasoconstriction (Husain, 2004). In this study, blood TAC decrease in the SHR EX might be due first to the fact that animal was hypertensive and to the activity of cytosolic enzymes in the blood that is increased after physical activity (Vina et al., 2000) and also due to oxidative stress related vasodilatory mechanisms while in the Wistar this could be a normal response.

The increase of TAC in skeletal muscle in Wistar and SHR exercise groups could be explained by the fact that during physical activity there are metabolic and functional adaptations in the skeletal muscle. Other studies showed a decrease in skeletal muscle antioxidant enzyme activities after exercise training which is linked with the age of animals, and Pansarasa et al. (1999) also demonstrated a decrease in SOD activity in skeletal muscle in aged individuals. However, Gianni et al. (2004) observed an increase of the activities of CAT, total SOD and GPX activities in skeletal muscles from human and rodent. It was also observed that during the ageing process there is an increase of ROS production and antioxidant enzyme activities (Lambertucci et al., 2007) .

These differences found in the literature might be due to differences in the type of animal used. In this study, the significant increase in TAC levels in both Wistar EX and SHR EX in the skeletal muscle could be partially due to the increased expression of antioxidant enzymes in response to exercise training. In the SHR EX group the level of TAC in the skeletal muscle was reduced. This could give us an indication to believe that in the muscle the level of oxidative damage is higher in the SHR EX group than in the Wistar rat exercise group but the compensatory response was shown with an up-regulation in antioxidant status.

The level of oxidative stress was assessed indirectly via MDA levels in the skeletal muscle of SHR and Wistar rats after subjecting the animals to physical activity, by quantifying the *in vivo* concentration of malonyldialdehyde (MDA). MDA is a by-product of lipid peroxidation by free radicals and is regarded as a reasonable indicator of oxidative stress *in vivo* (Pillon and Soulage, 2012). The total MDA concentration in the SHR EX group showed a significant increase compared to

the non-exercise group. A similar trend was observed in the Wistar rats whereby the Wistar exercise group showed a significant increase compared to the Wistar rat non-exercise group. This demonstrates that the lipid peroxidation in the SHR exercise group could be related to the high concentration of free radical in this group or a lower antioxidant concentration in this strain. Physical activity might be the cause of this increase of MDA concentration in the SHR EX group. In the Wistar group the increase in MDA concentration in the skeletal muscle could also be linked to exercise. Some studies showed that ageing causes the imbalance between ROS and antioxidant production which lead to cell damage (Lambertucci et al., 2007). This might also contribute to the increase of MDA concentration in the Wistar rats.

The level of CT-1 in the SHR and Wistar groups showed no significant difference compared to their respective control groups. After subjecting the rat to physical activity no change has been observed. CT-1 is a cytokine member of the interleukin-6 superfamily, produced by cardiomyocytes and non-cardiomyocytes. This unique cytokine induces longitudinal cardiac myocytes hypertrophy with the assembly of sarcomeric units in series in in vitro studies which is physiological hypertrophy (González et al., 2012). Studies showed that pressure overload (afterload) produces a concentric hypertrophy in which cardiac myocytes display an increase in cell diameter with the addition of new myofibrils in parallel. In contrast, volume overload induces eccentric hypertrophy with cardiac chamber dilatation (Takimoto et al., 2002). Therefore CT-1 has been shown to be a potent stimulator of myocytes hypertrophy and this type of myocytes hypertrophy promotes the eccentric form of hypertrophy which is pathological hypertrophy (Takimoto et al., 2002). CT-1 was shown to participate in the progression from left ventricular hypertrophy (LVH) to cardiovascular diseases and studies have demonstrated that the SHR develops pathological hypertrophy. The levels of CT-1 increases during the development of hypertension. However, our results showed no significant difference changes upon physical activity in SHR and Wistar rats. While histological studies have shown changes in cardiac myofibres in SHR. This shows that the type of hypertrophy observed in this study could not be due to an increase expression of CT-1 but due to merely after load which was present in this experimental animal. And there are possibly other signalling factors such as gp 130 and leukaemia inhibitory factor (LIF) (Takimoto et al., 2002) that could be implicated model could be some other signalling factors that participate in these changes and the protocol of exercise was moderate this could also play a role in the unchanged condition.

5.4 Histology

Histological analysis showed no significant changes in SHR compared to wistar rat. In contrast, when the rats were subjected to physical activity, changes in shapes and sizes of nuclei of cardiomyocytes with disarrayed cardiac myofibres were observed in SHR EX compared to the Wistar EX group under H&E staining. However, in the Wistar rats no significant changes were observed. These findings were also reported in a previous study (Cosyns et al., 2007). It is suggested that changes in the SHR EX

group may be related to the level of lipid peroxidation in this model and exercise may also play a part in the activation of the signalling pathways by the CT-1 which activates phosphorylation of the STAT-3 transcription factor then leading to hypertrophic effects (Latchman, 1999). Histology of the kidney showed a thin basement membrane in both SHR exercise and non-exercise groups compared to the Wistar rats group. Earlier studies showed that the damage observed in the SHR could be pressure dependent and show how initial vascular damage leads to a loss of auto regulation and arterial hypertrophy in the juxtamedullarry cortex (Hultström, 2012). It is suggested that in this study the changes could not necessary be due to exercise but could be attributed to the pathology of hypertension since these animals were already hypertensive.

The histological findings on the aortic wall, the tunica media specially showed that an increase in thickness of tunica media in the thoracic aorta in the SHR EX group compared to the non-exercise group under VVG. In the Wistar rats a trend of Wistar EX group being higher in tunica media than the non-exercise group was shown. These findings were consistent with the earlier work that mentioned the smooth muscle cells proliferation in the tunica media layer of the aortic wall and elastic fibres were found to be disordered in hypertensive rats (Thent et al., 2012). It is suggested that the thickness of tunica media found in the study in the SHR exercise group might be related to the level of oxidative stress in this model and exercise could also enhance the hypertrophy of the tunica media. Other studies showed that free radicals are strongly implicated in the modulation of cell proliferation (Dhalla et al., 1996). The trend shown in the Wistar EX group also gives an indication of the vessel adaptation during physical activity. Studies have shown that ROS can cause damage resulting in hypertrophy, apoptosis, and fibrosis and contribute to myocardial remodelling. This occurs when there is a higher level of ROS which leads to pathophysiologic remodelling, apoptosis, and chamber dysfunction via G-protein pathways, where the Gαq is acting and mediates signalling for numerous stimuli such as angiotensin, norepinephrine and mechanical strain and that causes hypertrophy and apoptosis in cardiac myocytes (Qin et al., 2010).

5.5 Trace elements

Trace elements are important components of biological structures. They play a role in the enzymatic profile for antioxidant protection. Any increase or decrease of trace elements in the body can influence the activity of the antioxidant enzymes. Some of these trace elements contain: Cu, Fe, Mn, Se, and Zn. Earlier studies reported these trace elements to also contribute in the regulation of blood pressure (Saltman, 1983). In this study the level of Fe in the blood, kidney and brain was significantly lower in the SHR than the Wistar strain and this could be due the genetic makeup of the strains. Fe as a component of haemoglobin plays an important role in oxygen transport to the organ systems and is an essential trace element that is crucial to normal cell functioning (Pirincci et al., 2013) The lower blood Fe levels in the SHR are consistent with another study where lowered blood haemoglobin level in SHR animals was observed (Hofller, 2015) Physical activity significantly decreased Fe levels in

both the SHR EX and the Wistar EX group in all tissues described above. It is suggested that the decreased Fe level in SHR EX could be due to oxidative stress during aerobic metabolism as it is known that Fe metabolism and Fe proteins can be influenced by oxidative stress (Ozturk et al., 2013). This was clearly shown by the decreased levels of TAC in this model and SOD mRNA expression has also confirmed these metabolic changes. Systemic inflammatory response syndrome (SIRS) is an acute inflammatory state affecting the whole body and is associated with a redistribution on vitamins and trace elements from the circulating compartment to tissues and organs which are involved in protein synthesis and immune cell production (Berger, 2005). Its mediators could also play a pathological role and is noted by the increased level of CRP and NF-kB. Although the exercise regimen coupled with oxidative stress was not sufficient to induce SIRS in this animal, there seems to be a coordinated pattern of trace element redistribution that mirrors what happened in SIRS in both Wistar EX and SHR EX groups.

Mn is an essential element that is needed for the activity of several enzymes (Pirincci et al., 2013). Mn is one of the essential trace elements that plays an important role in antioxidant defence and forms a part of SOD enzyme. Brain Mn levels were significantly higher in the SHR than the Wistar strain. Exercise significantly increased brain Mn levels in the Wistar EX group but decreased in the SHR EX group. The increase in brain Mn in the Wistar rats exercise group might be due to a shift in metabolism due to aerobic activity and could be a normal metabolic response which appears to be reduced in the SHR.

In this study, a lower level of brain Mn concentration in SHR exercise group compared to the non-exercise one was observed. This is in agreement with previous studies where Mn was found to be lower in patients having stress problem and transferrin may be involved in Mn transport into the brain (Takeda, 2003). It is suggested that the low concentration in SHR EX group could also linked to lower SOD levels as indicated by decreased TAC and by the decrease in the expression of mRNA of SOD.

Se is an essential trace element, and plays an important role in antioxidant selenoproteins for protection against oxidative stress initiated by excess ROS (Bleys et al., 2008). It has been linked to an increased risk of various diseases including heart disease (Tinggi, 2008). Blood and kidney Se levels were significantly lower in the SHR than the Wistar strain. Exercise significantly decreased Se levels in both the SHR EX group and the Wistar EX group. This could be a normal response of both groups to exercise but the response was exaggerated in the SHR group, possibly due to overall impaired antioxidant defence mechanisms.

Zn is essential to the function of several transcription factors and proteins that recognise certain DNA sequences and regulate gene transcription. It contributes to the efficiency of neurotransmitters (Yan et al., 2008). Previous studies have demonstrated that Zn modulates innate immune response through NF-KappaB (Bao et al., 2010). No significant difference was found in kidney Zn levels between the

SHR and Wistar strains. Exercise significantly increased kidney Zn levels in the SHR EX group only. It is suggested that the increased Zn kidney level in the SHR EX group may reflect a normal compensatory anti-inflammatory response. Zn mobilisation in response to oxidative stress also protects cells from free radical injury (Yan et al., 2008).

CONCLUSION AND RECOMMENDATIONS

This study has provided further confirmation that the SHR is a well-established model for essential hypertension. Hypertension in the SHR is multifactorial and polygenic in origin but this study highlighted the etiological role of oxidative stress in hypertension. It is demonstrated that the elevation in blood pressure was accompanied by:

- The impaired antioxidant status shown by decreased TAC in blood, increased MDA level was observed in the skeletal muscle, and decreased expression of mRNA SOD level in the skeletal muscle and liver.
- The hypertension in SHR exhibited inflammatory markers showed by CRP levels and expression of mRNA NF-kB.
- Tissue redistribution of trace element such as Fe, Mn, Se and Zn.
- Structural changes of the vessels, renal cortex and myocardium.

During exercise, antioxidant status was further compromised:

- Where TAC in blood was decreased, mRNA expression of SOD level in the skeletal muscle and liver was decreased as well and MDA level in skeletal muscle increased.
- Where inflammatory markers CRP plasma level increased and NF-kB mRNA there was expression upon activity.
- Where trace element redistribution such as Fe level in the blood, kidney and brain decreased, Se level decreased in the blood and kidney, Zn levels in the kidney increased and Mn levels in the brain increased.
- Where there were structural changes of the vessels, the renal cortex and myocardium were altered as well.

From the evidence in this study, it can be seen that a compromised antioxidant and free radical status contributes to the hypertensive state in a number of ways in the SHR. For this 8 week duration moderate activity had no effect on traditional mechanisms and elevate the blood pressure in the SHR.

A histological study coupled with trace element, gene expression, free radical and antioxidant status determination in specific organs showed damage upon physical activity.

This study provides a foundation for further research on the following areas:

Investigating the peripheral vessels' structural changes that occur during hypertension in this model, the full antioxidant profile, the lipid profile, the exercise protocol, supplementation study and liver metabolism of trace elements that would highlight further inverstigation that occur in this model and increase the intensity and duration of the study.

REFERENCES

ADDO, J., SMEETH, L. & LEON, D. A. 2007. Hypertension In Sub-Saharan Africa : a systematic review. *American Heart Association,* 50**,** 1524-4563.

AGARWAL, A., APONTE-MELLADO, A., PREMKUMAR, B. J., SHAMAN, A. & GUPTA, S. 2012. The effects of oxidative stress on female reproduction: a review. *Reproduction Biology Endocrinology,* 10**,** 49.

ANDERSON, T. J. 2006. Arterial stiffness or endothelial dysfunction as a surrogate marker of vascular risk. *Canadian Journal of Cardiology,* 22**,** 72B-80B.

APEL, K. & HIRT, H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Reviews Plant Biology.,* 55**,** 373-399.

ASAI, S., SAITO, Y., KUWAHARA, K., MIZUNO, Y., YOSHIMURA, M., HIGASHIKUBO, C., TSUJI, T., KISHIMOTO, I., HARADA, M., HAMANAKA, I., TAKAHASHI, N., YASUE, H. & NAKAO, K. 2000. The heart is a source of circulating cardiotrophin-1 in humans. *Biochemestry Biophysical Research Communications,* 279**,** 320-3.

AY, Y., AYDıN, C., BASEL, H., BEKTAŞ, H., BÜLÜT, G., INAN, B., AY, N. K. & DEMIR, I. 2013. The Effects of Levosimendan Exposure on Oxidant/Antioxidant Status and Trace Element Levels in the Pulmonary Artery of Rats. *The Journal of Membrane Biology,* 246**,** 473-478.

BALABAN, R. S., NEMOTO, S. & FINKEL, T. 2005. Mitochondria, Oxidants, and Aging. *Cell,* 120**,** 483-495.

BAO, S., LIU, M. J., LEE, B., BESECKER, B., LAI, J. P., GUTTRIDGE, D. C. & KNOELL, D. L. 2010. Zinc modulates the innate immune response in vivo to polymicrobial sepsis through regulation of NF-kappaB. *American Journal of Physiology Lung Cellular and Molecular Physiology,* 298**,** L744-54.

BARNES, P. J. & KARIN, M. 1997. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *New England Journal of Medicine,* 336**,** 1066-71.

BATTELLI, M. G., BOLOGNESI, A. & POLITO, L. 2014. Pathophysiology of circulating xanthine oxidoreductase: New emerging roles for a multi-tasking enzyme. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*.

BELVIRANLı, M. & GÖKBEL, H. 2006. Acute exercise induced oxidative stress and antioxidant changes. *European Journal of General Medicine,* 3**,** 126-131.

BERGER, M. M. 2005. Can oxidative damage be treated nutritionally? *Clinical Nutrition,* 2**,** 172-183.

BLEYS, J., NAVAS-ACIEN, A. & GUALLAR, E. 2008. Serum selenium levels and all-cause, cancer, and cardiovascular mortality among US adults. *Archives of Internal Medicine,* 168**,** 404-10.

BUETTNER, G. R. & JURKIEWICZ, B. A. 1996. Catalytic metals, ascorbate and free radicals: combinations to avoid. *Radiation Research,* 145**,** 532-541.

BUSTIN, S. 2002. Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. *Journal of Molecular Endocrinology,* 29**,** 23-39.

BUSTIN, S., BENES, V., NOLAN, T. & PFAFFL, M. 2005. Quantitative real-time RT-PCR–a perspective. *Journal of Molecular Endocrinology,* 34**,** 597-601.

BUTTAR, H. S., LI, T. & RAVI, N. 2005. Prevention of cardiovascular diseases: Role of exercise, dietary interventions, obesity and smoking cessation. *Experimental and Clinical Cardiology,* 10**,** 229-49.

CABRERA, M. P. & CHIHUAILAF, R. H. 2011. Antioxidants and the integrity of ocular tissues. *Veterinary Medicine International,* 2011.

CALABRO, P., LIMONGELLI, G., RIEGLER, L., MADDALONI, V., PALMIERI, R., GOLIA, E., ROSELLI, T., MASARONE, D., PACILEO, G. & GOLINO, P. 2009. Novel insights into the role of cardiotrophin-1 in cardiovascular diseases. *Journal of Molecular and Cellular Cardiology,* 46**,** 142-148.

CALHOUN, D. A., JONES, D., TEXTOR, S., GOFF, D. C., MURPHY, T. P., TOTO, R. D., WHITE, A., CUSHMAN, W. C., WHITE, W., SICA, D., FERDINAND, K., GILES, T. D., FALKNER, B. & CAREY, R. M. 2008. Resistant hypertension: diagnosis, evaluation, and treatment: a scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Circulation,* 117**,** 189141.

CAMPBELL, N. & REECE, J. 2005. Cellular respiration: harvesting chemical energy.

CHALMERS, J. 2011. Is a blood pressure target of <130/80 mm Hg still appropriate for high-risk patients? *Circulation,* 124**,** 1700-2.

CONVERSE, R. L., JR., JACOBSEN, T. N., TOTO, R. D., JOST, C. M., COSENTINO, F., FOUAD-TARAZI, F. & VICTOR, R. G. 1992. Sympathetic overactivity in patients with chronic renal failure. *New England Journal of Medicine,* 327**,** 1912-8.

COSYNS, B., DROOGMANS, S., WEYTJENS, C., LAHOUTTE, T., VAN CAMP, G., SCHOORS, D. & FRANKEN, P. R. 2007. Effect of streptozotocin-induced diabetes on left ventricular function in adult rats: an in vivo Pinhole Gated SPECT study. *Cardiovascular Diabetology,* 6**,** 30.

DAVIS, G. E. & SENGER, D. R. 2005. Endothelial extracellular matrix: biosynthesis, remodeling, and functions during vascular morphogenesis and neovessel stabilization. *Circulation Research,* 97**,** 1093-107.

DELLES, C., MILLER, W. H. & DOMINICZAK, A. 2008. Targeting Reactive Oxygen Species *Antioxidants & Redox Signaling,* 10**,** 1061-1071.

DEVASAGAYAM, T., TILAK, J., BOLOOR, K., SANE, K. S., GHASKADBI, S. S. & LELE, R. 2004. Free radicals and antioxidants in human health: current status and future prospects. *Japi,* 52**,** 794-804.

DHALLA, A. K., HILL, M. F. & SINGAL, P. K. 1996. Role of oxidative stress in transition of hypertrophy to heart failure. *Journal of the American College of Cardiology,* 28**,** 506-514.

DORNAS, W. C. & SILVA, M. E. 2011. Animal models for the study of arterial hypertension. *Journal of Biosciences,* 36**,** 731-7.

DRÖGE, W. 2002b. Free Radicals in the Physiological Control of Cell Function. *Physiological Reviews,* 82**,** 47-95.

DRUMMOND, G. R. & SOBEY, C. G. 2014. Endothelial NADPH oxidases: which NOX to target in vascular disease? *Trends in Endocrinology & Metabolism,* 25**,** 452-463.

ELAHI, M. M., KONG, Y. X. & MATATA, B. M. 2009. Oxidative stress as a mediator of cardiovascular disease. *Oxididative Medicine Cellular Longevity,* 2**,** 259-69.

FELICE, F., LUCCHESI, D., DI STEFANO, R., BARSOTTI, M. C., STORTI, E., PENNO, G., BALBARINI, A., DEL PRATO, S. & PUCCI, L. 2010. Oxidative stress in response to high glucose levels in endothelial cells and in endothelial progenitor cells: Evidence for differential glutathione peroxidase-1 expression. *Microvascular Research,* 80**,** 332-338.

FICHTLSCHERER, S., BREUER, S., SCHÄCHINGER, V., DIMMELER, S. & ZEIHER, A. M. 2004. C-reactive protein levels determine systemic nitric oxide bioavailability in patients with coronary artery disease. *European Heart Journal,* 25**,** 1412-1418.

FLORA, S. J. 2009a. Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. *Oxidative Medicine and Cellular Longevity,* 2**,** 191-206.

FLORA, S. J. 2009b. Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. *Oxidative of Medicine and Cellular Longevity,* 2**,** 191-206.

FÖRSTERMANN, U., CLOSS, E. I., POLLOCK, J. S., NAKANE, M., SCHWARZ, P., GATH, I. & KLEINERT, H. 1994. Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. *Hypertension,* 23**,** 1121-1131.

FÖRSTERMANN, U. & SESSA, W. C. 2012. Nitric oxide synthases: regulation and function. *European Heart Journal,* 33**,** 829-837.

FULDA, S., GORMAN, A. M., HORI, O. & SAMALI, A. 2010. Cellular stress responses: cell survival and cell death. *International Journal of Cell Biology,* 2010.

GHOSH, S., NG, L. L., TALWAR, S., SQUIRE, I. B. & GALINANES, M. 2000. Cardiotrophin-1 protects the human myocardium from ischemic injury. Comparison with the first and second window of protection by ischemic preconditioning. *Cardiovascular Research,* 48**,** 440-7.

GIANNI, P., JAN, K. J., DOUGLAS, M. J., STUART, P. M. & TARNOPOLSKY, M. A. 2004. Oxidative stress and the mitochondrial theory of aging in human skeletal muscle. *Experimental Gerontology,* 39**,** 1391-1400.

GIBSON, U. E., HEID, C. A. & WILLIAMS, P. M. 1996. A novel method for real time quantitative RT-PCR. *Genome Research,* 6**,** 995-1001.

GIELEN, S., SCHULER, G. & ADAMS, V. 2010. Cardiovascular effects of exercise training: molecular mechanisms. *Circulation,* 122**,** 1221-38.

GOMES, E. C., SILVA, A. N. & OLIVEIRA, M. R. D. 2012. Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. *Oxidative medicine and cellular longevity,* 2012.

GOMEZ-CABRERA, M. C., FERRANDO, B., BRIOCHE, T., SANCHIS-GOMAR, F. & VIÑA, J. 2013. Exercise and antioxidant supplements in the elderly. *Journal of Sport and Health Science,* 2**,** 94-100.

GONZÁLEZ, A., LÓPEZ, B., RAVASSA, S., BEAUMONT, J., ZUDAIRE, A., GALLEGO, I., BRUGNOLARO, C. & DÍEZ, J. 2012. Cardiotrophin-1 in Hypertensive Heart Disease. *Endocrine,* 42**,** 9-17.

GOSKER, H. R., WOUTERS, E. F., VAN DER VUSSE, G. J. & SCHOLS, A. M. 2000. Skeletal muscle dysfunction in chronic obstructive pulmonary disease and chronic heart failure: underlying mechanisms and therapy perspectives. *American Journal of Clinical Nutrition,* 71**,** 1033-47.

GUSTAFSON, C. E., ALM, R. A. & TRUST, T. J. 1993. Effect of heat denaturation of target DNA on the PCR amplification. *Gene,* 123**,** 241-244.

GUTIERREZ, J., BALLINGER, S. W., DARLEY-USMAR, V. M. & LANDAR, A. 2006. Free radicals, mitochondria, and oxidized lipids: the emerging role in signal transduction in vascular cells. *Circulation Research,* 99**,** 924-32.

GUTTERIDGE, J. 1995. Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clinical Chemistry,* 41**,** 1819-1828.

GUYENET, P. G. 2006. The sympathetic control of blood pressure. *Nature Reviews Neuroscience,* 7**,** 335-346.

HALLIWELL, B. 1994. Free Radicals and Antioxidants: A Personal View. *Nutrition Reviews,* 52**,** 253-265.

HALLIWELL, B. 2006. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiology,* 141**,** 312-22.

HANCOCK, J. T., DESIKAN, R. & NEILL, S. J. 2001. Role of reactive oxygen species in cell signalling pathways. *Biochemical Society Transactions,* 29**,** 345-50**.**

HENDRIKS, M. E., WIT, F. W. N. M., ROOS, M. T. L., BREWSTER, L. M., AKANDE, T. M., DE BEER, I. H., MFINANGA, S. G., KAHWA, A. M., GATONGI, P., VAN ROOY, G., JANSSENS, W., LAMMERS, J., KRAMER, B., BONFRER, I., GAEB, E., VAN DER GAAG, J., RINKE DE WIT, T. F., LANGE, J. M. A. & SCHULTSZ, C. 2012. Hypertension in Sub-Saharan Africa: Cross-Sectional Surveys in Four Rural and Urban Communities. *PLoS ONE,* 7**,** e32638**.**

HIGASHI, Y. & YOSHIZUMI, M. 2004. Exercise and endothelial function: role of endothelium-derived nitric oxide and oxidative stress in healthy subjects and hypertensive patients. *Pharmacology & Therapeutics,* 102**,** 87-96**.**

HIGUCHI, R., FOCKLER, C., DOLLINGER, G. & WATSON, R. 1993. Kinetic PCR Analysis: Real-time Monitoring of DNA Amplification Reactions. *Natural Biotechnology,* 11**,** 1026-1030**.**

HOFLLER. 2015. *The Effect of Multivitamin-Multimineral Supplementation on the Health Status of Inbred Wistar and Spontaneously Hypertensive Rat Strains.* Masters, University of KwaZulu Natal.

HOPKINS, N. & MCLOUGHLIN, P. 2002. The structural basis of pulmonary hypertension in chronic lung disease: remodelling, rarefaction or angiogenesis? *Journal of Anatomy,* 201**,** 335-48**.**

HORTA, P. P., DE CARVALHO, J. J. & MANDARIM-DE-LACERDA, C. A. 2005. Exercise training attenuates blood pressure elevation and adverse remodeling in the aorta of spontaneously hypertensive rats. *Life Sci,* 77**,** 3336-43**.**

HU, L. S., GEORGE, J. & WANG, J. H. 2013. Current concepts on the role of nitric oxide in portal hypertension. *World Journal of Gastroenterology,* 19**,** 1707**.**

HUGHES, K., ANTONSSON, A. & GRUNDSTROM, T. 1998. Calmodulin dependence of NFkappaB activation. *FEBS Lett,* 441**,** 132-6**.**

HULTSTRÖM, M. 2012. Development of structural kidney damage in spontaneously hypertensive rats. *Journal of Hypertension,* 30**,** 1087-1091**.**

HUSAIN, K. 2004. Interaction of regular exercise and chronic nitroglycerin treatment on blood pressure and rat aortic antioxidants. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease,* 1688**,** 18-25**.**

HYBERTSON, B. M., GAO, B., BOSE, S. K. & MCCORD, J. M. 2011. Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. *Molecular Aspects of Medicine,* 32**,** 234-246**.**

JENNER, P. 2003. Oxidative stress in Parkinson's disease. *Annals Neurology,* 53**,** S36-8**.**

JIALAL, I., DEVARAJ, S. & VENUGOPAL, S. K. 2004. C-reactive protein: risk marker or mediator in atherothrombosis? *Hypertension,* 44**,** 6-11**.**

JIN, R. C. & LOSCALZO, J. 2010. Vascular nitric oxide: formation and function. *Journal of Blood Medicine,* 1**,** 147**.**

JONES, D. P. 2002. Redox potential of GSH/GSSG couple: assay and biological significance. *Methods in Enzymology,* 348**,** 93-112**.**

JOYNER, M. J., CHARKOUDIAN, N. & WALLIN, B. G. 2008. A sympathetic view of the sympathetic nervous system and human blood pressure regulation. *Experimental Physiology,* 93**,** 715-24**.**

JULIET, W. 2015. *Factors Effecting Systolic Blood Pressure* [Online]. http://www.livestrong.com/article/67792-factors-effecting-systolic-blood-pressure/.

KACHHAWA, K., VARMA, M., SAHU, A., KACHHAWA, P. & JHA, R. K. 2014. Oxidative stress and Antioxidant Enzyme levels In Hypertensive Chronic Kidney Disease patients. *International Journal of Biomedical and Advance Research,* 5**,** 488-490**.**

KASHYAP, M., YADAV, V., SHERAWAT, B., JAIN, S., KUMARI, S., KHULLAR, M., SHARMA, P. & NATH, R. 2005. Different antioxidants status, total antioxidant power and free radicals in essential hypertension. *Molecular and Cellular Biochemistry,* 277**,** 89-99**.**

KORGE, P., PING, P. & WEISS, J. N. 2008. Reactive oxygen species production in energized cardiac mitochondria during hypoxia/reoxygenation: modulation by nitric oxide. *Circulation Research,* 103**,** 873-80**.**

KOSAKA, N., IGUCHI, H. & OCHIYA, T. 2010. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. *Cancer science,* 101**,** 2087-2092**.**

KOZIAN, D. H. & KIRSCHBAUM, B. J. 1999. Comparative gene-expression analysis. *Trends Biotechnol,* 17**,** 73-8**.**

KRAMER, H. F. & GOODYEAR, L. J. 2007. *Exercise, MAPK, and NF-κB signaling in skeletal muscle*.

KULLER, L. H. 2007. Epidemic hypertension in Sub-Saharan Africa. *Hypertension,* 50**.**

KUO, S.-M., TAN, C.-H., DRAGAN, M. & WILSON, J. X. 2005. Endotoxin increases ascorbate recycling and concentration in mouse liver. *The Journal of Nutrition,* 135**,** 2411-2416**.**

KUWAHARA, K., SAITO, Y., HARADA, M., ISHIKAWA, M., OGAWA, E., MIYAMOTO, Y., HAMANAKA, I., KAMITANI, S., KAJIYAMA, N., TAKAHASHI, N., NAKAGAWA, O., MASUDA, I. & NAKAO, K. 1999. Involvement of cardiotrophin-1 in cardiac myocyte-nonmyocyte interactions during hypertrophy of rat cardiac myocytes in vitro. *Circulation,* 100**,** 1116-24**.**

LACY, F., O'CONNOR, D. T. & SCHMID-SCHÖNBEIN, G. W. 1998. Plasma hydrogen peroxide production in hypertensives and normotensive subjects at genetic risk of hypertension. *Journal of Hypertension,* 16**,** 291-303.

LAMBERTUCCI, R. H., LEVADA-PIRES, A. C., ROSSONI, L. V., CURI, R. & PITHON-CURI, T. C. 2007. Effects of aerobic exercise training on antioxidant enzyme activities and mRNA levels in soleus muscle from young and aged rats. *Mechanisms of Ageing and Development,* 128**,** 267-275.

LANDMESSER, U., DIKALOV, S., PRICE, S. R., MCCANN, L., FUKAI, T., HOLLAND, S. M., MITCH, W. E. & HARRISON, D. G. 2003. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *The Journal of Clinical Investigation,* 111**,** 1201-1209.

LATCHMAN, D. S. 1999. Cardiotrophin- 1 (CT- 1): a novel hypertrophic and cardioprotective agent. *International journal of experimental pathology,* 80**,** 189-196.

LATCHMAN, D. S. 2000. Cardiotrophin-1: a novel cytokine and its effects in the heart and other tissues. *Pharmacology and Therapeutics,* 85**,** 29-37.

LEE, M. Y. & GRIENDLING, K. K. 2008. Redox signaling, vascular function, and hypertension. *Antioxidants & Redox Signaling,* 10**,** 1045-1059.

LERMAN, L. O., CHADE, A. R., SICA, V. & NAPOLI, C. 2005. Animal models of hypertension: an overview. *Journal of Laboratory and Clinical Medicine,* 146**,** 160-73.

LI, M. & FUKAGAWA, N. K. 2010. Age-related changes in redox signaling and VSMC function. *Antioxidants & Redox Signaling,* 12**,** 641-655.

LIN, C. P., LIN, F. Y., HUANG, P. H., CHEN, Y. L., CHEN, W. C., CHEN, H. Y., HUANG, Y. C., LIAO, W. L., HUANG, H. C., LIU, P. L. & CHEN, Y. H. 2013. Endothelial progenitor cell dysfunction in cardiovascular diseases: role of reactive oxygen species and inflammation. *BioMedical Research International,* 845037**,** 30.

LIOCHEV, S. I. & FRIDOVICH, I. 2000. Copper- and zinc-containing superoxide dismutase can act as a superoxide reductase and a superoxide oxidase. *Journal of Biological Chemestry,* 275**,** 38482-5.

LOBO, V., PATIL, A., PHATAK, A. & CHANDRA, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev,* 4**,** 118-26.

LOYKE, H. 2002a. Effects of elements in human blood pressure control. *Biological Trace Element Research,* 85**,** 193-209.

LOYKE, H. F. 2002b. Effects of elements in human blood pressure control. *Biological Trace Element Research,* 85**,** 193-209.

LUFT, F. C. 2001a. Workshop: mechanisms and cardiovascular damage in hypertension. *Hypertension,* 37**,** 594-8.

LUFT, F. C. 2001b. Workshop: mechanisms and cardiovascular damage in hypertension. *Hypertension,* 37**,** 594-598.

MADAMANCHI, N. R., VENDROV, A. & RUNGE, M. S. 2005. Oxidative stress and vascular disease. *Arteriosclerosis Thrombosis Vascular Biology,* 25**,** 29-38.

MAIRBAURL, H. 2013. Red blood cells in sports: effects of exercise and training on oxygen supply by red blood cells. *Frontiers in Physiology,* 4**,** 332.

MANOLOV, D., KOENIG, W., HOMBACH, V. & TORZEWSKI, J. 2003. C-reactive protein and atherosclerosis. Is there a causal link?

MARK, A. L. 1996. The sympathetic nervous system in hypertension: a potential long-term regulator of arterial pressure. *Journal of Hypertension Supplement,* 14**,** S159-65.

MARQUES, C., LAWFORD, P., COCHRANE, T. & SIMONE, R. P. 2001. Effects of Moderate Exercise on Biomechanical Function of Femur Bones in Wistar Male Rats.

MATÉS, J. M., PEREZ-GOMEZ, C. & BLANCA, M. 2000. Chemical and biological activity of free radical 'scavengers' in allergic diseases. *Clinica Chimica Acta,* 296**,** 1-15.

MENESHIAN, A. & BULKLEY, G. B. 2002. The physiology of endothelial xanthine oxidase: from urate catabolism to reperfusion injury to inflammatory signal transduction. *Microcirculation,* 9**,** 161-75.

MITSUMOTO, H., KLINKOSZ, B., PIORO, E. P., TSUZAKA, K., ISHIYAMA, T., O'LEARY, R. M. & PENNICA, D. 2001. Effects of cardiotrophin- 1 (CT- 1) in a mouse motor neuron disease. *Muscle & nerve,* 24**,** 769-777.

MOCELLIN, S., ROSSI, C. R., PILATI, P., NITTI, D. & MARINCOLA, F. M. 2003. Quantitative real-time PCR: a powerful ally in cancer research. *Trends in Molecular Medicine,* 9**,** 189-95.

MONSERRAT, L., LOPEZ, B., GONZALEZ, A., HERMIDA, M., FERNANDEZ, X., ORTIZ, M., BARRIALES-VILLA, R., CASTRO-BEIRAS, A. & DIEZ, J. 2011a. Cardiotrophin-1 plasma levels are associated with the severity of hypertrophy in hypertrophic cardiomyopathy. *Eur Heart J,* 32**,** 177-83.

MONSERRAT, L., LÓPEZ, B., GONZÁLEZ, A., HERMIDA, M., FERNÁNDEZ, X., ORTIZ, M., BARRIALES-VILLA, R., CASTRO-BEIRAS, A. & DÍEZ, J. 2011b. Cardiotrophin-1 plasma levels are associated with the severity of hypertrophy in hypertrophic cardiomyopathy. *European Heart Journal,* 32**,** 177-183.

MONTECUCCO, F. & MACH, F. 2008. New evidences for C-reactive protein (CRP) deposits in the arterial intima as a cardiovascular risk factor. *Clinical Interventions in Aging,* 3**,** 341.

MORRISON, T. B., WEIS, J. J. & WITTWER, C. T. 1998. Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification. *Biotechniques,* 24**,** 954-8, 960, 962.

MULVANY, M. J. 1999. Vascular remodelling of resistance vessels: can we define this? *Cardiovasc Res,* 41**,** 9-13.

NETHERY, D., STOFAN, D., CALLAHAN, L., DIMARCO, A. & SUPINSKI, G. 1999. *Formation of reactive oxygen species by the contracting diaphragm is PLA2dependent*.

NISHIKAWA, T., EDELSTEIN, D., DU, X. L., YAMAGISHI, S.-I., MATSUMURA, T., KANEDA, Y., YOREK, M. A., BEEBE, D., OATES, P. J. & HAMMES, H.-P. 2000. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature,* 404**,** 787-790.

OPIE, L. H. & SEEDAT, Y. K. 2005. Hypertension in sub-Saharan African populations. *Circulation,* 112**,** 3562-8.

OZTURK, P., KURUTAS, E. B. & ATASEVEN, A. 2013. Copper/zinc and copper/selenium ratios, and oxidative stress as biochemical markers in recurrent aphthous stomatitis. *Journal of Trace Elements in Medicine and Biology,* 27**,** 312-316.

PAL, S., RADAVELLI-BAGATINI, S. & HO, S. 2013. Potential benefits of exercise on blood pressure and vascular function. *Journal of the American Society of Hypertension,* 7**,** 494-506.

PALMIERI, B. & SBLENDORIO, V. 2007. Oxidative stress tests: overview on reliability and use. *European Review for Medical and Pharmacological Sciences,* 11**,** 308-42.

PANSARASA, O., BERTORELLI, L., VECCHIET, J., FELZANI, G. & MARZATICO, F. 1999. Age-dependent changes of antioxidant activities and markers of free radical damage in human skeletal muscle. *Free Radical Biology and Medicine,* 27**,** 617-622.

PARAVICINI, T. M. & TOUYZ, R. M. 2008. NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities. *Diabetes Care,* 31 Suppl 2**,** S170-80.

PEPYS, M. B. & HIRSCHFIELD, G. M. 2003. C-reactive protein: a critical update. *J Clin Invest,* 111**,** 1805-12.

PEROU, C. M., JEFFREY, S. S., VAN DE RIJN, M., REES, C. A., EISEN, M. B., ROSS, D. T., PERGAMENSCHIKOV, A., WILLIAMS, C. F., ZHU, S. X., LEE, J. C., LASHKARI, D., SHALON, D., BROWN, P. O. & BOTSTEIN, D. 1999. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. *Proceedings of the National Academy of Sciences of the United States of America,* 96**,** 9212-7.

PETRULEA, M., MURESAN, A. & DUNCEA, I. 2012. Oxidative Stress and Antioxidant Status in Hypo-and Hyperthyroidism.

PFAFFL, M. 2001. Development and Validation of an Externally Standardised Quantitative Insulin-like Growth Factor-1 RT-PCR Using LightCycler SYBR Green I Technology. *In:* MEUER, S., WITTWER, C. & NAKAGAWARA, K.-I. (eds.) *Rapid Cycle Real-Time PCR.* Springer Berlin Heidelberg.

PILLON, N. J. & SOULAGE, C. O. 2012. *Lipid Peroxidation by-Products and the Metabolic Syndrome*.

PIRINCCI, N., GECIT, I., GUNES, M., KABA, M., TANIK, S., YUKSEL, M. B., ARSLAN, H. & DEMIR, H. 2013. Levels of serum trace elements in renal cell carcinoma cases. *Asian Pacific Journal Cancer Prevention,* 14**,** 499-502.

POWERS, S. K. 1999. Analysis of cellular responses to free radicals: focus on exercise and skeletal muscle. *Proceedings of the Nutrition Society,* 58**,** 1025-1033.

POWERS, S. K. & JACKSON, M. J. 2008a. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiological reviews,* 88**,** 1243-76.

POWERS, S. K. & JACKSON, M. J. 2008b. *Exercise-Induced Oxidative Stress: Cellular Mechanisms and Impact on Muscle Force Production*.

POWERS, S. K. & JACKSON, M. J. 2008c. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiological Reviews,* 88**,** 1243-1276.

QAMIRANI, E., REN, Y., KUO, L. & HEIN, T. W. 2005. C-reactive protein inhibits endothelium-dependent NO-mediated dilation in coronary arterioles by activating p38 kinase and NAD (P) H oxidase. *Arteriosclerosis, Thrombosis, and Vascular Biology,* 25**,** 995-1001.

QIN, F., LENNON-EDWARDS, S., LANCEL, S., BIOLO, A., SIWIK, D. A., PIMENTEL, D. R., DORN, G. W., KANG, Y. J. & COLUCCI, W. S. 2010. Cardiac-specific overexpression of catalase identifies hydrogen peroxide-dependent and -independent phases of myocardial remodeling and prevents the progression to overt heart failure in G(alpha)q-overexpressing transgenic mice. *Circulation of Heart Failiure,* 3**,** 306-13.

RADOMSKI, M. W. & MONCADA, S. 1993. Regulation of vascular homeostasis by nitric oxide. *Thrombosis and Haemostasis-Stuttgart-,* 70**,** 36-36.

RAHMAN, K. 2007a. Studies on free radicals, antioxidants, and co-factors. *Clin Interv Aging,* 2**,** 219-36.

RAHMAN, K. 2007b. Studies on free radicals, antioxidants, and co-factors. *Clinical Interventions in Aging,* 2**,** 219.

REHMAN, A., LEIBOWITZ, A., YAMAMOTO, N., RAUTUREAU, Y., PARADIS, P. & SCHIFFRIN, E. L. 2012. Angiotensin type 2 receptor agonist compound 21 reduces vascular injury and myocardial fibrosis in stroke-prone spontaneously hypertensive rats. *Hypertension,* 59**,** 291-299.

RENNA, N. F., DE LAS HERAS, N. & MIATELLO, R. M. 2013. Pathophysiology of vascular remodeling in hypertension. *International journal of hypertension,* 2013.

RIRIE, K. M., RASMUSSEN, R. P. & WITTWER, C. T. 1997. Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. *Analytical Biochemistry,* 245**,** 154-60.

RODRIGO, R., LIBUY, M., FELIÚ, F. & HASSON, D. 2013. Oxidative Stress-Related Biomarkers in Essential Hypertension and Ischemia-Reperfusion Myocardial Damage. *Disease Markers,* 35**,** 773-790.

RODRIGO, R., PRAT, H., PASSALACQUA, W., ARAYA, J., GUICHARD, C. & BACHLER, J. P. 2007. Relationship between Oxidative Stress and Essential Hypertension. *The Japanese Society of Hypertension,* 30**,** 1159-1167.

RUBATTU, S., PAGLIARO, B., PIERELLI, G., SANTOLAMAZZA, C., CASTRO, S. D., MENNUNI, S. & VOLPE, M. 2015. Pathogenesis of target organ damage in hypertension: role of mitochondrial oxidative stress. *International journal of molecular sciences,* 16**,** 823-39.

SALGADO, F. J., ARIAS, P., CANDA-SÁNCHEZ, A., NOGUEIRA, M. & VEAS, F. 2011. Acute phase proteins as biomarkers of disease: from bench to clinical practice. *Acute Phase Proteins as Early non-Specific Biomarkers of Human and Veterinary Diseases.* .

SALTMAN, P. 1983. Trace elements and blood pressure. *Annals of Internal Medicine,* 98**,** 823-7.

SARIKONDA, K. V., WATSON, R. E., OPARA, O. C. & DIPETTE, D. J. 2009. Experimental animal models of hypertension. *Journal of American Society of Hypertension,* 3**,** 158-65.

SAVOIA, C. & SCHIFFRIN, E. L. 2007. Reduction of C-reactive protein and the use of anti-hypertensives. *Vascular Health Risk Management* 3**,** 975-83.

SCHALASTA, G., ARENTS, A., SCHMID, M., BRAUN, R. & ENDERS, G. 2000. Fast and type-specific analysis of herpes simplex virus types 1 and 2 by rapid PCR and fluorescence melting-curve-analysis. *Infection,* 28**,** 85-91.

SCHNEIDER, C. D. & OLIVEIRA, A. R. D. 2004. Oxygen free radicals and exercise: mechanisms of synthesis and adaptation to the physical training. *Revista Brasileira de Medicina do Esporte,* 10**,** 308-313.

SCOTT, P. K. & MALCOLM, J. J. 2008. Exercise-Induced Oxidative Stress: Cellular Mechanisms and Impact on Muscle Force Production. *Physiological reviews,* 88**,** 1243-1276.

SHARMA, P., JHA, A. B., DUBEY, R. S. & PESSARAKLI, M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany,* 2012.

SHREE, S., KUMAR, K., ITAGAPPA, M. & RAMESH, G. 2013. Study of Oxidative Stress and Antioxidant Status in Oral Cancer Patients. *International Journal of Oral and Maxillofacial Pathology,* 4**,** 2-6.

SINGH, R. B., MENGI, S. A., XU, Y. J., ARNEJA, A. S. & DHALLA, N. S. 2002. Pathogenesis of atherosclerosis: A multifactorial process. *Experimental and Clinical Cardiology,* 7**,** 40-53.

SOMANI, S., FRANK, S. & RYBAK, L. 1995. Responses of antioxidant system to acute and trained exercise in rat heart subcellular fractions. *Pharmacology Biochemistry and Behavior,* 51**,** 627-634.

SOMOVA, L., KHAN, M. & CHETTY, S. 1998. Stress and salt intake: experimental data on Dahl saltresistant and saltsensitive rats. *Stress and Health,* 14**,** 125-134.

SPEICH, M., PINEAU, A. & BALLEREAU, F. 2001. Minerals, trace elements and related biological variables in athletes and during physical activity. *Clinica Chimica Acta,* 312**,** 1-11.

STEINBERG, D. 2009. The LDL modification hypothesis of atherogenesis: an update. *Journal of Lipid Research,* 50**,** 15.

STOCKER, R. & KEANEY JR, J. F. 2004. Role of oxidative modifications in atherosclerosis. *Physiological Reviews,* 84**,** 1381-1478.

SUNDARAM, A., SIEW KEAH, L., SIRAJUDEEN, K. N. & SINGH, H. J. 2013. Upregulation of catalase and downregulation of glutathione peroxidase activity in the kidney precede the development of hypertension in pre-hypertensive SHR. *Hypertens Res,* 36**,** 213-8.

SZABO, C., ISCHIROPOULOS, H. & RADI, R. 2007. Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. *Nature Reviews Drug Discovery,* 6**,** 662-680.

TAKEDA, A. 2003. Manganese action in brain function. *Brain Research Reviews,* 41**,** 79-87.

TAKIMOTO, Y., AOYAMA, T., IWANAGA, Y., IZUMI, T., KIHARA, Y., PENNICA, D. & SASAYAMA, S. 2002. *Increased expression of cardiotrophin-1 during ventricular remodeling in hypertensive rats*.

TARPEY, M. M., WINK, D. A. & GRISHAM, M. B. 2004. Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 286**,** R431-R444.

TAULAVUORI, E., HELLSTRÖM, E. K., TAULAVUORI, K. & LAINE, K. 2001. Comparison of two methods used to analyse lipid peroxidation from Vaccinium myrtillus (L.) during snow removal, reacclimation and cold acclimation. *Journal of experimental botany,* 52**,** 2375-2380.

THENT, Z. C., LIN, T. S., DAS, S. & ZAKARIA, Z. 2012. Histological changes in the heart and the proximal aorta in experimental diabetic rats fed with Piper sarmentsoum. *African Journal Traditional Complement Alternative Medicines,* 9**,** 396-404.

THIRIET, M. 2015. Pathogenesis of Cardiac Diseases. *Diseases of the Cardiac Pump.* Springer International Publishing.

TINGGI, U. 2008. Selenium: its role as antioxidant in human health. *Environmental Health and Preventive Medicine,* 13**,** 102-8**.**

TOUYZ, R. & SCHIFFRIN, E. 2004. Reactive oxygen species in vascular biology: implications in hypertension. *Histochemistry and Cell Biology,* 122**,** 339-352**.**

TOUYZ, R. M. 2004. Reactive oxygen species and angiotensin II signaling in vascular cells -- implications in cardiovascular disease. *Brazilian Journal of Medical and Biology Research,* 37**,** 1263-73**.**

TRAN, T. M. & GIANG, N. M. 2014. Changes in blood pressure classification, blood pressure goals and pharmacological treatment of essential hypertension in medical guidelines from 2003 to 2013. *International Journal of Cardiology Metabolic & Endocrine,* 2**,** 1-10**.**

TSEVELEKI, V., RUBIO, R., VAMVAKAS, S.-S., WHITE, J., TAOUFIK, E., PETIT, E., QUACKENBUSH, J. & PROBERT, L. 2010. Comparative gene expression analysis in mouse models for multiple sclerosis, Alzheimer's disease and stroke for identifying commonly regulated and disease-specific gene changes. *Genomics,* 96**,** 82-91**.**

TURRENS, J. F. 2003. Mitochondrial formation of reactive oxygen species. *The Journal of Physiology,* 552**,** 335-344**.**

URSO, M. L. & CLARKSON, P. M. 2003. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology,* 189**,** 41-54**.**

UTTARA, B., SINGH, A. V., ZAMBONI, P. & MAHAJAN, R. 2009. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacology,* 7**,** 65**.**

VALKO, M., LEIBFRITZ, D., MONCOL, J., CRONIN, M. T., MAZUR, M. & TELSER, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell biology,* 39**,** 44-84**.**

VALKO, M., RHODES, C. J., MONCOL, J., IZAKOVIC, M. & MAZUR, M. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions,* 160**,** 1-40**.**

VICTOR, V. M., ROCHA, M. & ESPLUGUES, J. V. 2005. Role of free radicals in sepsis: antioxidant therapy. *Current Pharmaceutical Design,* 11**,** 3141-3158**.**

VINA, J., GOMEZ-CABRERA, M. C., LLORET, A., MARQUEZ, R., MINANA, J. B., PALLARDO, F. V. & SASTRE, J. 2000. Free radicals in exhaustive physical exercise: mechanism of production, and protection by antioxidants. *IUBMB Life,* 50**,** 271-7**.**

VIRDIS, A., DURANTI, E. & TADDEI, S. 2011. Oxidative stress and vascular damage in hypertension: role of angiotensin II. *International Journal of Hypertension,* 2011.

WILKENING, S. & BADER, A. 2004. Quantitative real-time polymerase chain reaction: methodical analysis and mathematical model. *Journal of Biomolecular Techniques,* 15**,** 107-11.

WILSON, D. W., METZ, H. N., GRAVER, L. M. & RAO, P. S. 1997. Direct method for quantification of free malondialdehyde with high-performance capillary electrophoresis in biological samples. *Clinical Chemestry,* 43**,** 1982-4.

WU, D. & CEDERBAUM, A. I. 2003. Alcohol, oxidative stress, and free radical damage. *Alcohol Research and Health,* 27**,** 277-284.

XU, S. & TOUYZ, R. M. 2006. Reactive oxygen species and vascular remodelling in hypertension: still alive. *Canadian Journal of Cardiology,* 22**,** 947-951.

YAN, M., SONG, Y., WONG, C. P., HARDIN, K. & HO, E. 2008. Zinc deficiency alters DNA damage response genes in normal human prostate epithelial cells. *Journal of Nutrition,* 138**,** 667-73.

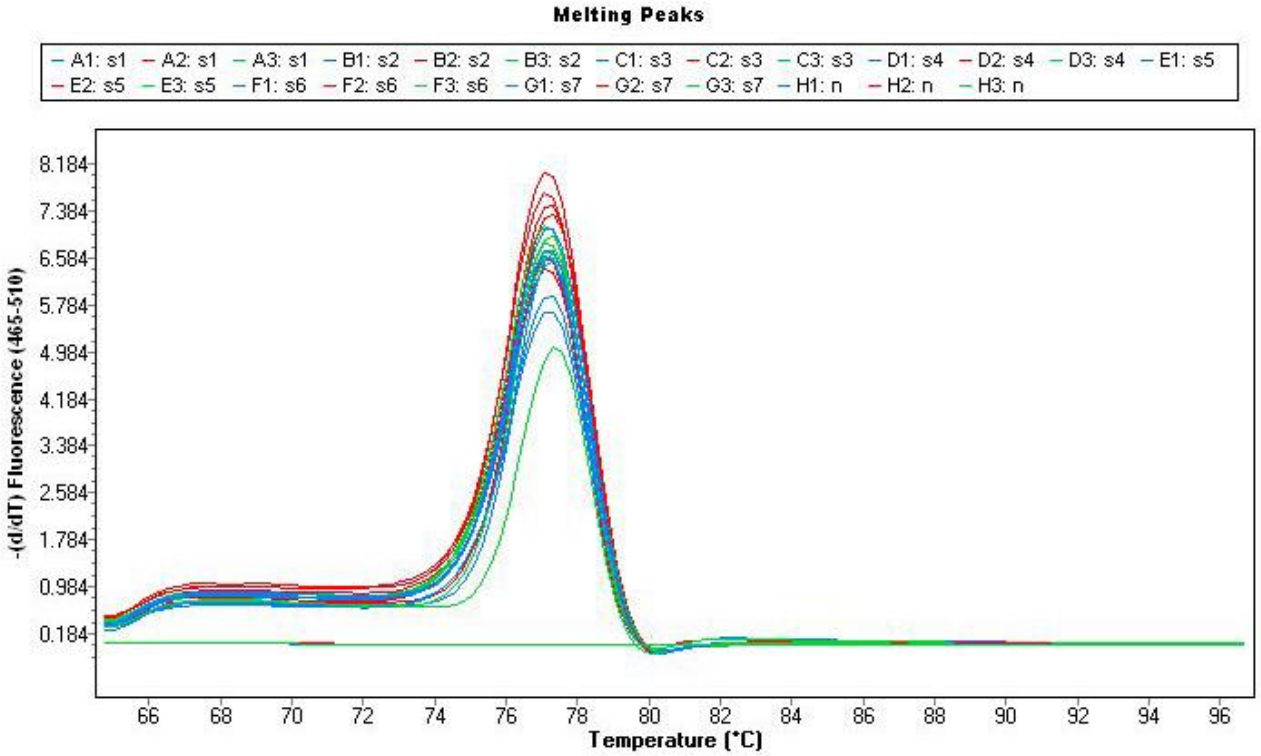
YOKOYAMA, M. & HIRATA, K.-I. 2007. Endothelial nitric oxide synthase uncoupling: Is it a physiological mechanism of endothelium-dependent relaxation in cerebral artery? *Cardiovascular Research,* 73**,** 8-9.

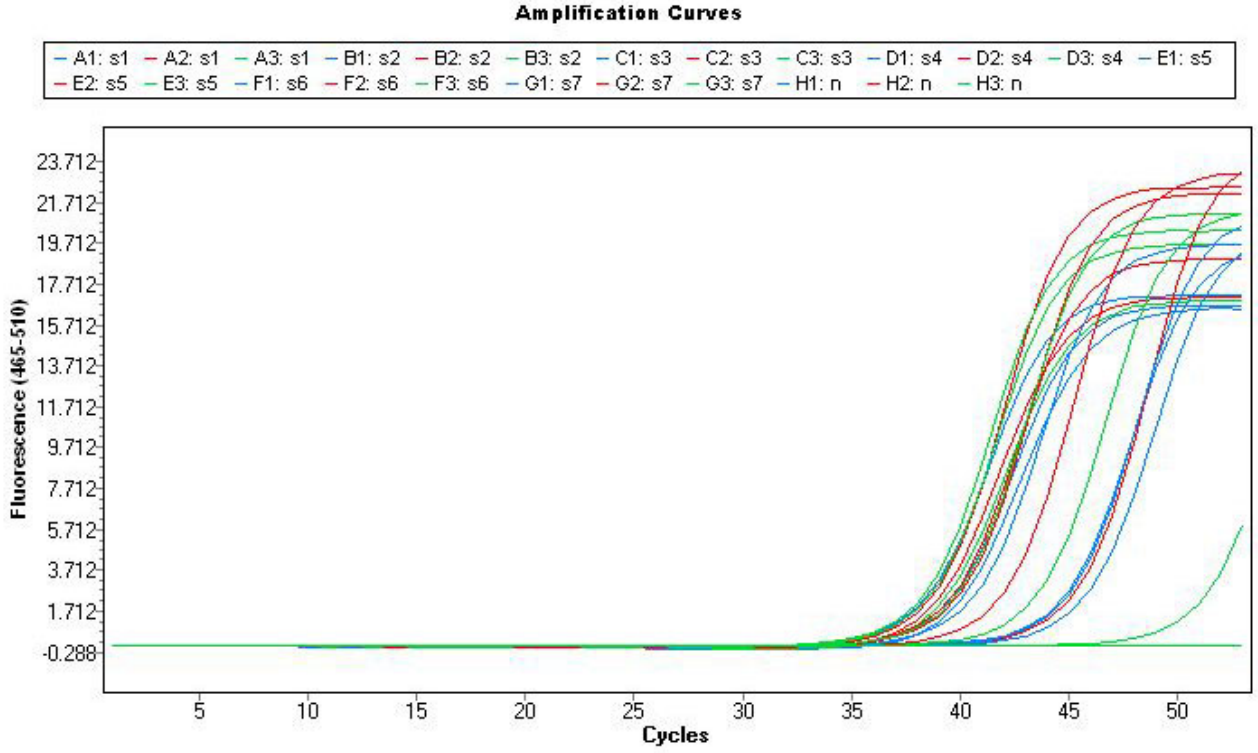
ZENG, C., VILLAR, V. A. M., YU, P., ZHOU, L. & JOSE, P. A. 2009. Reactive oxygen species and dopamine receptor function in essential hypertension. *Clinical and Experimental Hypertension,* 31**,** 156-178.

APPENDICES

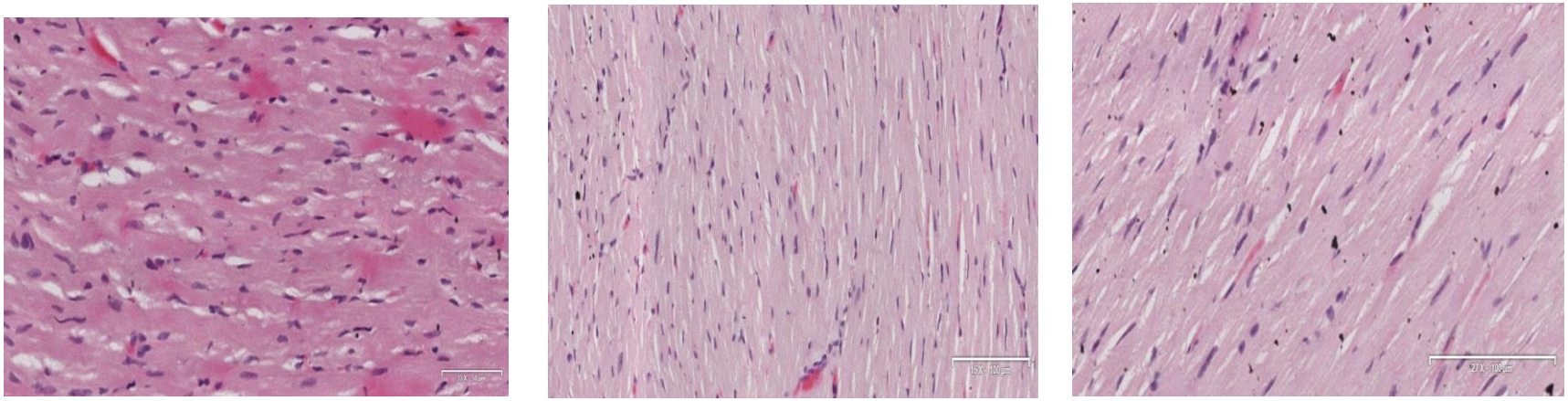
- **Appendix 1**
  - **Melting peaks for the GAPDH gene**
  - **Amplification**
- **Appendix 2**
  - **Photomicrograph showing longitudinal section of cardiac tissues**
- **Appendix 3**
  - **Photomicrograph showing the renal cortex morphology of the kidney**
- **Appendix 4**
  - **Photomicrograph showing the section of aortic tissues**

APPENDIX 1

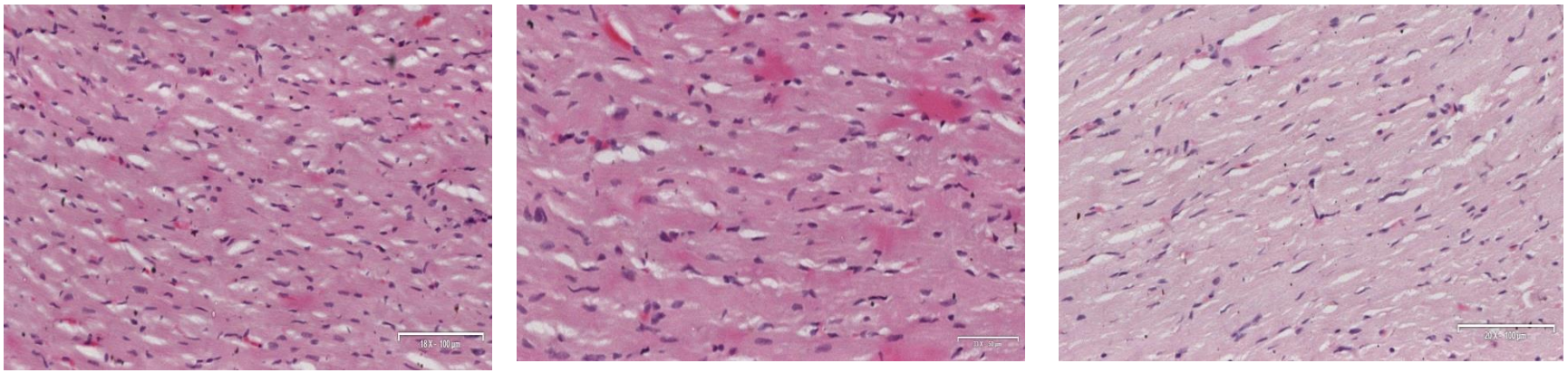

Melting Peaks
A1: s1 A2: s1 A3: s1 B1: s2 B2: s2 B3: s2 C1: s3 C2: s3 C3: s3 D1: s4 D2: s4 D3: s4 E1: s5
E2: s5 E3: s5 F1: s6 F2: s6 F3: s6 G1: s7 G2: s7 G3: s7 H1: n H2: n H3: n
-(d/dT) Fluorescence (465-510)
8.184
7.384
6.584
5.784
4.984
4.184
3.384
2.584
1.784
0.984
0.184
66 68 70 72 74 76 78 80 82 84 86 88 90 92 94 96
Temperature (°C)


Amplification Curves
A1: s1 A2: s1 A3: s1 B1: s2 B2: s2 B3: s2 C1: s3 C2: s3 C3: s3 D1: s4 D2: s4 D3: s4 E1: s5
E2: s5 E3: s5 F1: s6 F2: s6 F3: s6 G1: s7 G2: s7 G3: s7 H1: n H2: n H3: n
Fluorescence (465-510)
23.712
21.712
19.712
17.712
15.712
13.712
11.712
9.712
7.712
5.712
3.712
1.712
-0.288
5 10 15 20 25 30 35 40 45 50
Cycles

APPENDIX 2



WISTAR NEX



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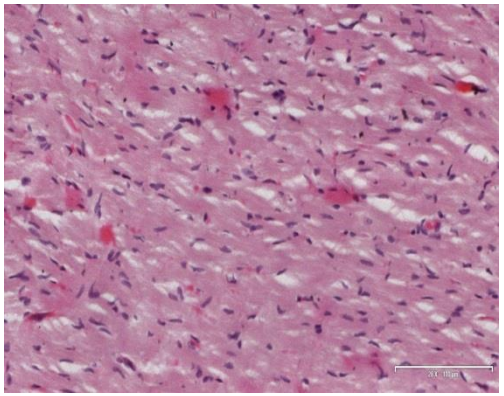

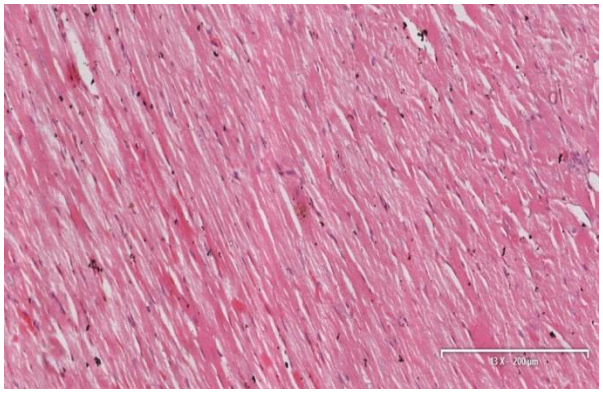

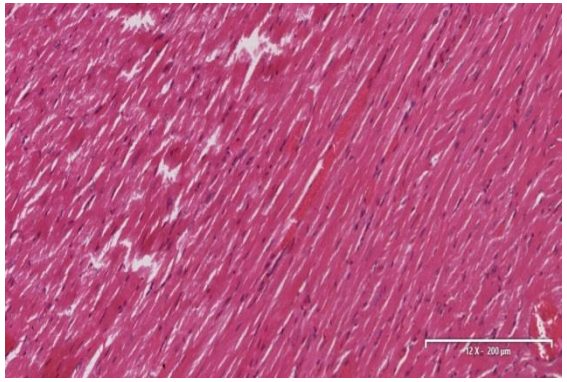

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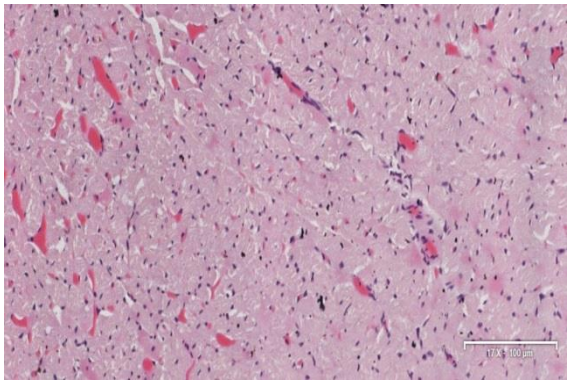
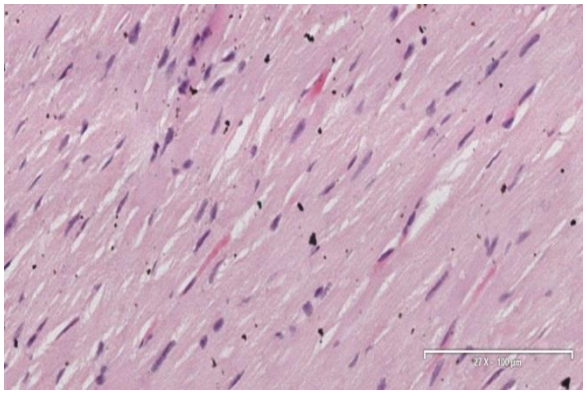
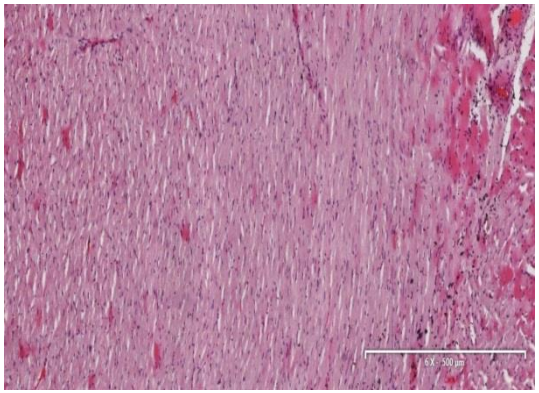
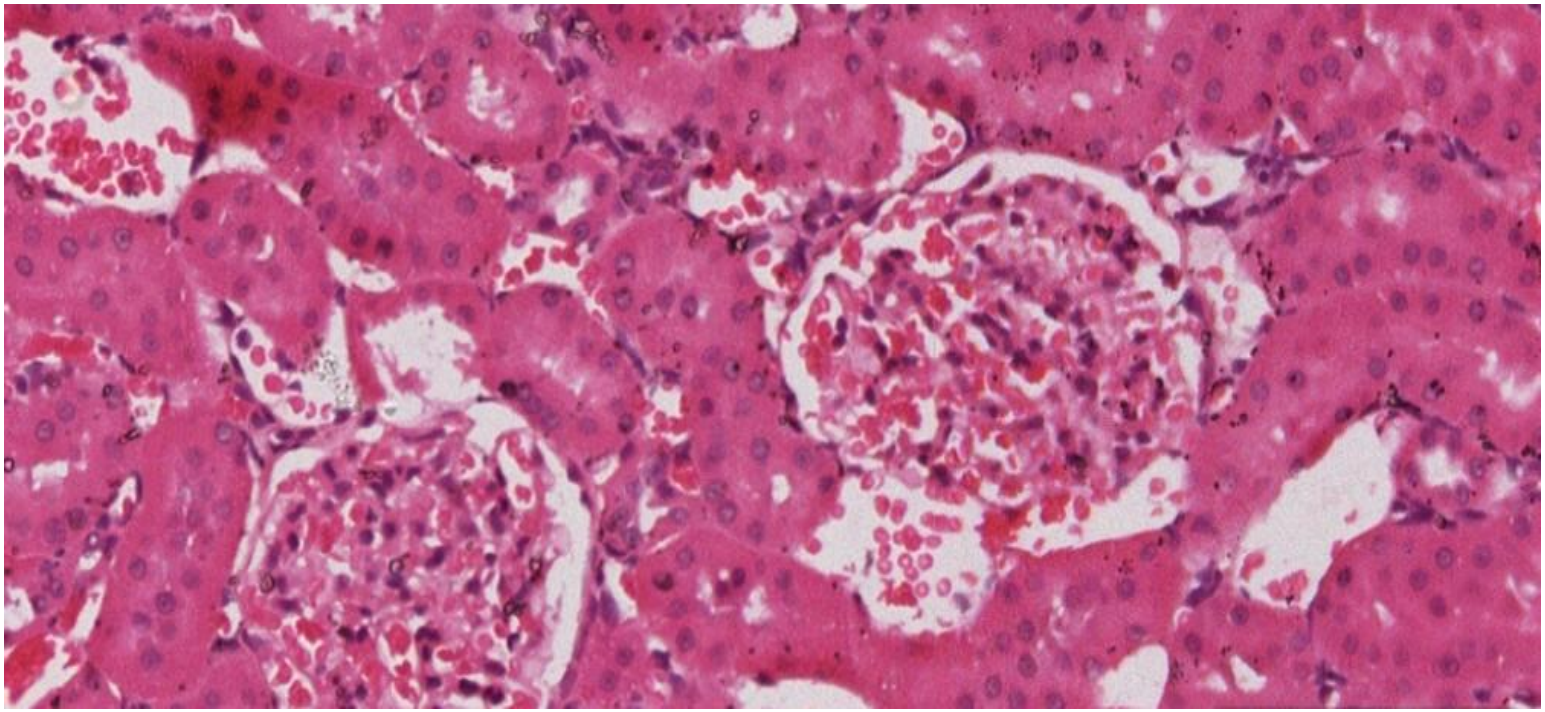






SHR EX

APPENDIX 3



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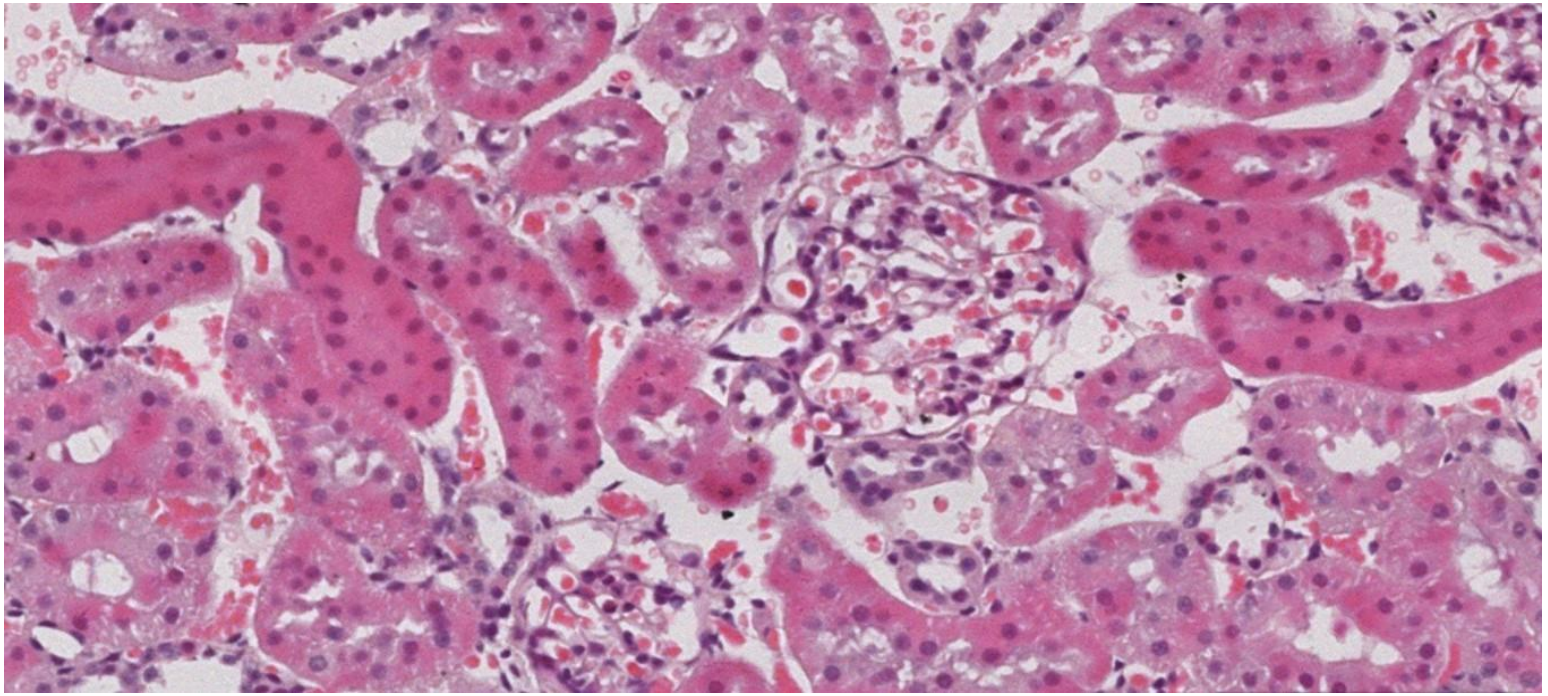

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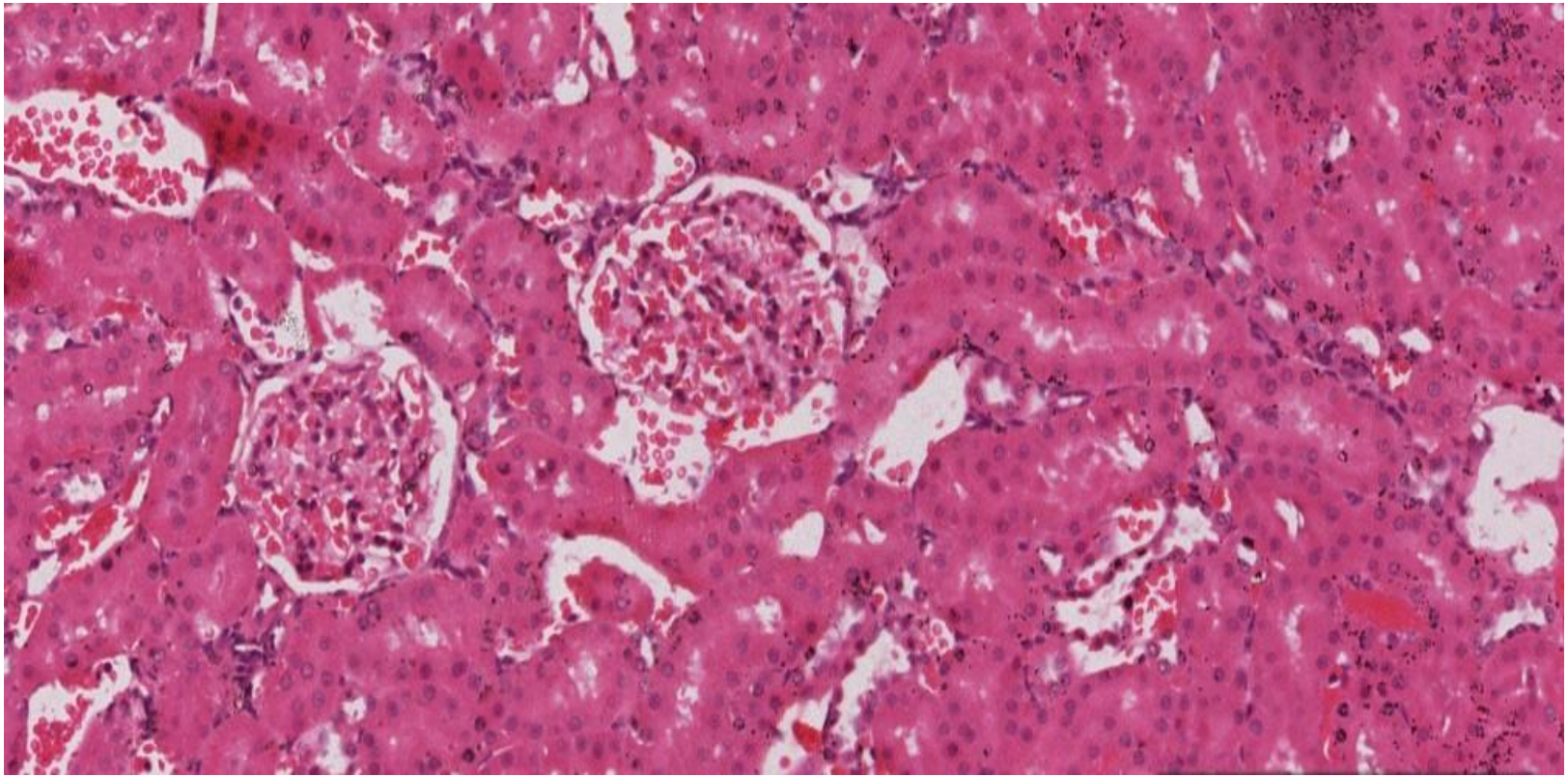

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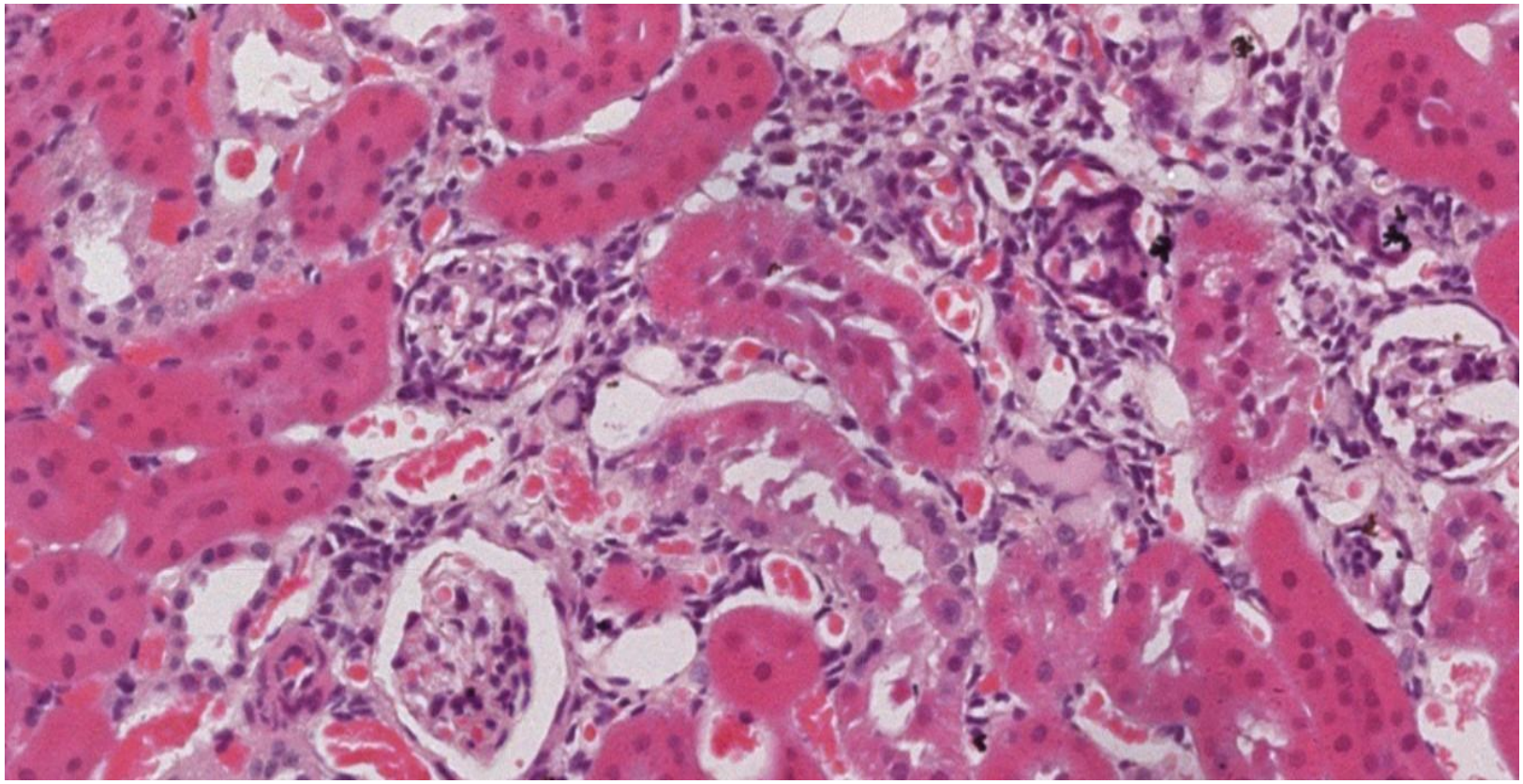
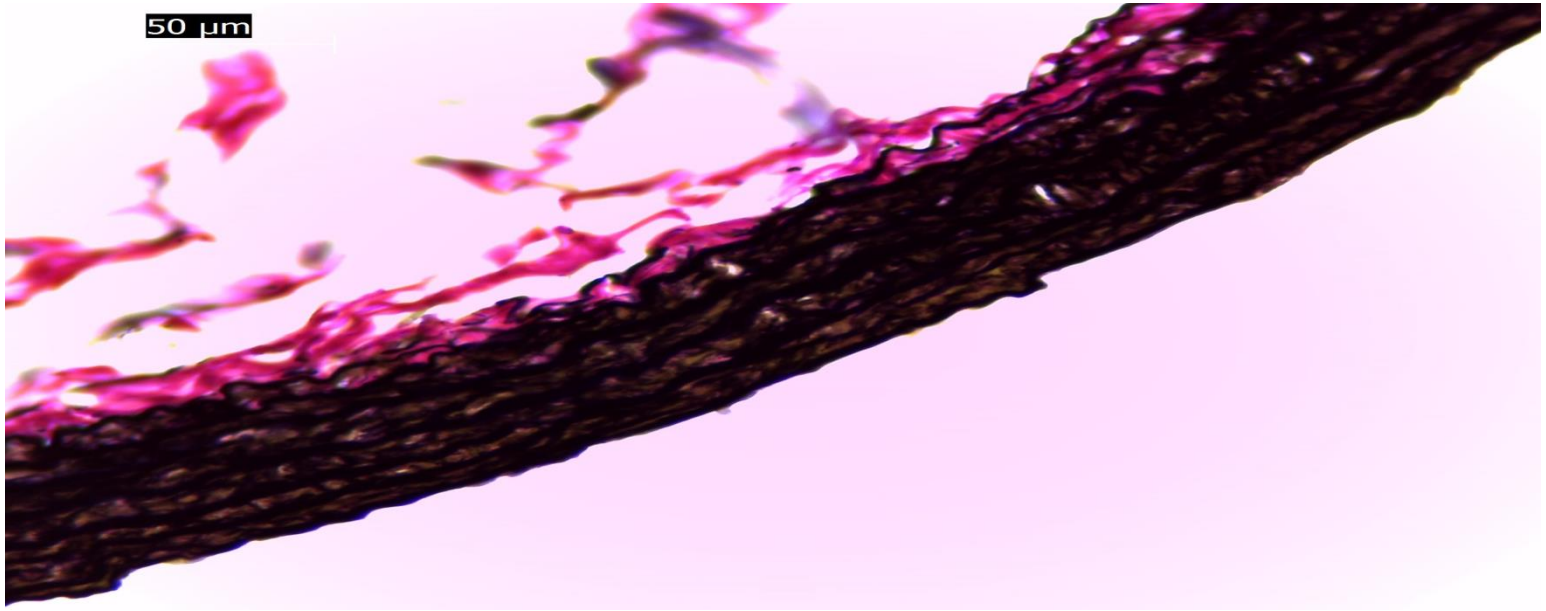


SHR EX

APPENDIX 4



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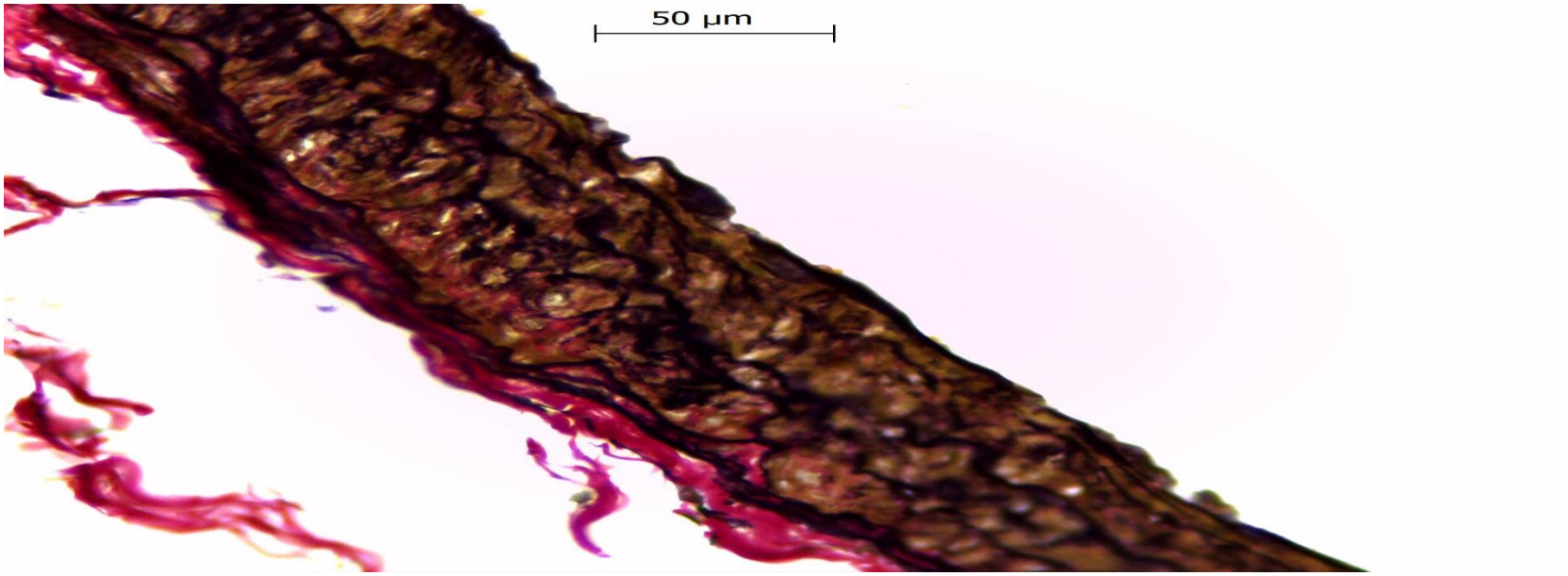

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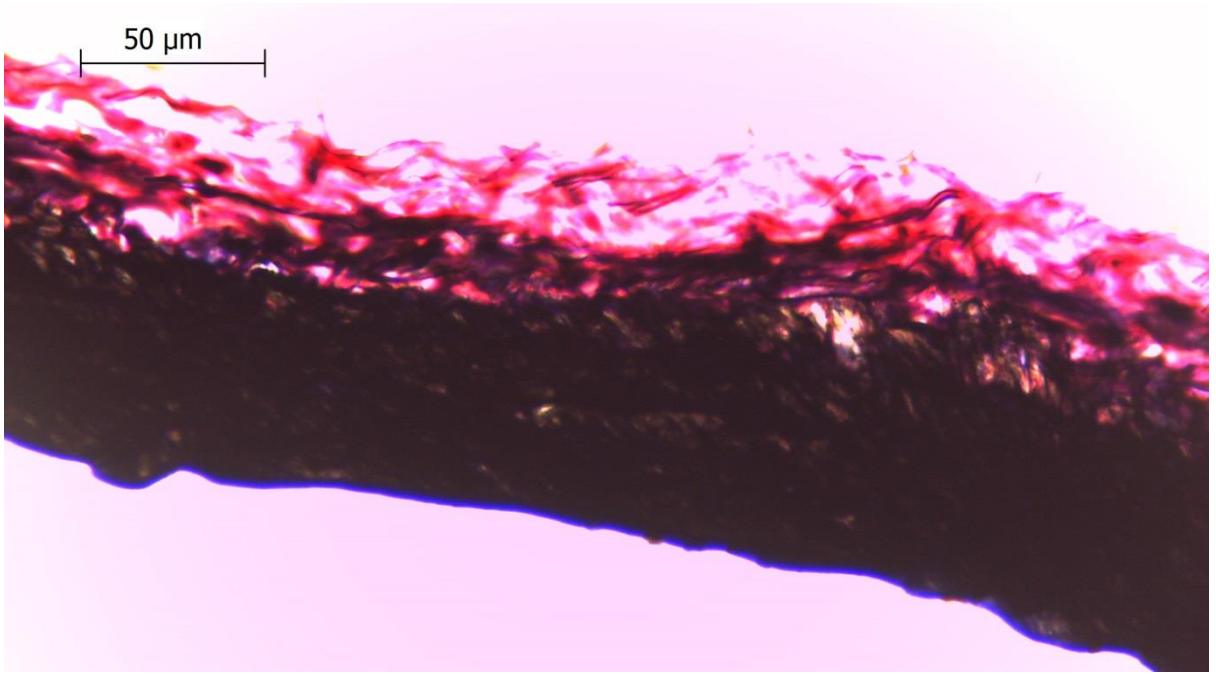

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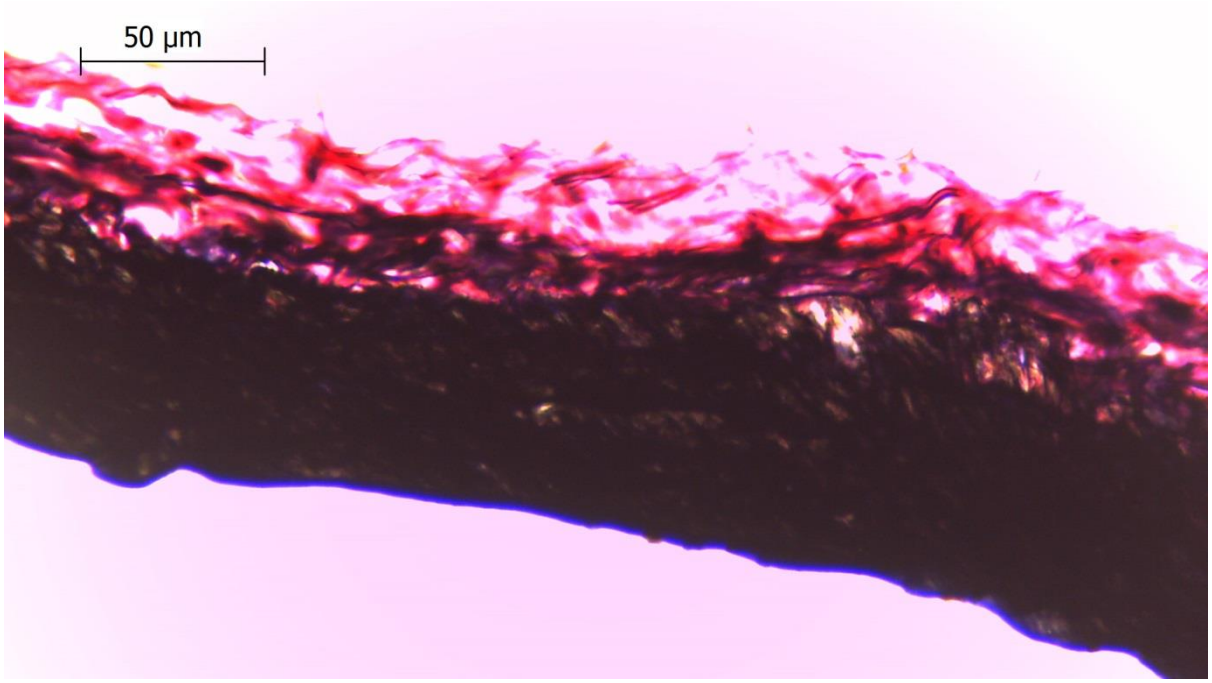

SHR EX

Table 16

STANDARD PREPARATION FOR TRACE ELEMENT					
Cu Zn Mn Se (multi-element)					
0.1	0.5	1	2	5	mg/ml
5	25	50	100	250	µ l
Fe					
0.5	1	5	10	50	mg/ml
25	50	250	500	2500	µ l

Trace element concentration was diluted with deionised water in 50ml volumetric tube.

Conc µg/g of tissue = µg/ml x total volume of sample ⁄ Mass of tissue (g)