PROTECTIVE EFFECTS OF APOCYNIN AND CATALASE IN OXIDATIVE STRESS OF RENAL FAILURE, HYPERTENSION AND HYPOTENSION RAT MODELS

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LIST OF ABBREVIATIONS

$1O_2^-$	Singlet oxygen
ACE	Angiotensin converting enzyme
Ang I	Angiotensin I
Ang II	Angiotensin II
Ang III	Angiotensin III
Ang IV	Angiotensin IV
Ang VII	Angiotensin VII
Apocynin	Acetovanillone or 4-hydroxy-3methoxy-acetophenone
AQP2	Aquaporin-2
ARBs	Angiotensin receptor blockers
AT1	Angiotensin II type 1 receptor
AT2	Angiotensin II type 2 receptor
AT3	Angiotensin II type 3 receptor
AT4	Angiotensin II type 4 receptor
ATP	Adenosine triphosphate
BP	Blood pressure
cDNA	complementary DNA
cfPWV	Carotid to femoral PWV

CKD	Chronic kidney disease
CNIs	Calcineurin inhibitors
CNS	Central nervous system
CrCl	Creatinine clearance
CRF	Chronic renal failure
CsA	Cyclosporine A
Cu^{2+}	Copper ion
CVDs	Cardiovascular diseases
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
EGFR	Epidermal growth factor receptor
ENaCs	Epithelial sodium channels
ERK	Extracellular signal-regulated kinase
ESRD	End-stage renal disease
ETC	Electron transport chain
FAK	Focal adhesion kinase
FasL	Fas ligand
Fe^{2+}	Ferrous ion
Fe^{3+}	Ferric ion
FE_{K^+}	Fractional excretion potassium
FE_{Na^+}	Fractional excretion of sodium

FFA	Free fatty acid
GFR	Glomerular filtration rate
GSK-3β	Glycogen synthase kinase-3β
GSSH	Oxidized glutathione
H&E	Haematoxylin and eosin
H_2O	Water
H_2O_2	Hydrogen peroxide
HIV	Human Immunodeficiency Virus
HOCl	Hypochlorous acid
HONOO	Alkyl peroxynitrites
HOO^-	Hydroperoxy
HR	Heart rate
IFN-γ	Interferon-gamma
IL-2	Interleukin-2
iNOS 2	inducible nitric oxide synthase 2
IPS	Institute of Postgraduate Studies
JAKs	Janus kinases
JNC8	Joint National Committee 8
JNK	c-Jun N-terminal kinase
KCl	Potassium chloride
L-Arginine	Nω-Monomethyl-L-arginine

L-NAME	Nω-Nitro-L-arginine methyl ester hydrochloride
MAP	Mean arterial blood pressure
MAPK	Mitogen-activated protein kinase;
MAPs	Mitogen-activated proteins
MDA	Malondialdehyde
MMPs	Matrix metalloproteinases;
N_2O_3	Dinitrogen trioxide
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NFAT-1	Nuclear factor of activated T cells
NO	Nitric oxide
NO_2^-	Nitrate
$NO_2^{\cdot}$	Nitrogen dioxide radicals
NO_3^-	Nitrite
NOO	Nitrogen dioxide
Nox	NADPH oxidase
O_2	Molecular oxygen
O_2^-	Superoxide anion
O_3	Ozone
OH^-	Hydroxyl

$ONOO^-$	Peroxynitrite
p130Cas	p130 Crk-associated substrate
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
PGE2	prostaglandin E2
PNS	Peripheral nervous system
PTK	Protein tyrosine kinases
PTP	Protein tyrosine phosphatases
PWV	Pulse wave velocity
qPCR	quantitative PCR
RAAS	Renin-angiotensin aldosterone system
RCBP	Renal cortical blood perfusion
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
RO^-	Alkoxyl
ROO^-	Peroxyl
ROS	Reactive oxygen species
RT-PCR	Real time PCR
SBP	Systolic blood pressure
SHR	Spontaneously hypertensive rat
SOD	Superoxide dismutase

Src	Proto-oncogene tyrosine-protein kinase
STAT	Signal transducer and activator of transcription proteins
T-AOC	Total antioxidant capacity
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TGF-β	Transforming growth factor-beta
TNF	Tumor necrosis factor
TNF-α	Tumour necrosis factor –alpha
TPR	Total peripheral resistance
$U_{K^+}V$	Absolute potassium excretion
$U_{Na^+}V$	Absolute sodium excretion
USM	Universiti Sains Malaysia
VPR	Volume pressure recording
WKY	Wistar-Kyoto

KESAN PERLINDUNGAN APOSININ DAN KATALASE PADA MODEL TIKUS KEGAGALAN GINJAL, HIPERTENSI DAN HIPOTENSI TERHADAP STRES OXIDATIF

ABSTRAK

Stres oksidatif terlibat dalam patogenesis sejumlah penyakit ginjal dan kardiovaskular. Di dalam kebanyakkan penyakit, terdapat peningkatan ekspresi oksidase nikotinamida adenina dinukleotida (oksidase NADPH atau Nox) yang menyebabkan pengeluaran anion superoksida (O_2^-) dan akumulasi hidrogen peroksida (H_2O_2) sepanjang progressi penyakit. Kajian ini menyelidik kesan aposinin, iaitu perencat oksidase NADPH, dan katalase, iaitu pengaut H_2O_2, terhadap fungsi ginjal dan hemodinamik yang terjejas pada model-model tikus Wistar-Kyoto siklosporin A (CsA), Wistar-Kyoto L-NAME dan L-arginina. Tikus menerima CsA (25 mg/kg/hari p.o.), L-NAME (15 mg/kg/hari p.o.) dan L-arginina (12.5 mg/ml p.o.) masing-masing dan dibahagikan kepada pembawa, aposinin (2.5 mmol/L p.o.), katalase (10,000 U/kg/hari i.p.) atau gabungan apocynin dan katalase selama 14 hari. Fungsi dan hemodinamik ginjal, dan penanda tekanan oksidatif plasma telah diukur. Selain itu, kajian histologi dan ekspresi molekul oksidase NADPH 4 (Nox 4) mRNA telah dinilai pada penghujung tempoh rawatan. Kedua-dua kumpulan CsA dan L-NAME mempunyai tekanan darah yang lebih tinggi sementara kumpulan L-arginine mempunyai tekanan darah rendah berbanding dengan kumpulan kawalan. Tambahan pula, perkumuhan kreatinina lebih rendah di dalam kumpulan CsA dan L-NAME dan lebih tinggi di dalam kumpulan L-arginina jika berbanding dengan kumpulan kawalan. Aktiviti Nox 4 mRNA tisu ginjal

dan paras malondialdehyde plasma (MDA) lebih tinggi manakala jumlah superoksida dismutase (T-SOD), dan jumlah kapasiti antioksidan (T-AOC) lebih rendah pada ketiga-tiga model jika berbanding dengan kumpulan kawalan. Rawatan terhadap tikus CsA atau L-NAME dengan aposinin, katalase atau gabungan kedua-duanya memulihkan tekanan darah dan perkumuhan kreatinina hampir ke nilai rujukan. Walau bagaimanapun, di dalam kalangan model tikus L-arginine, rawatan dengan aposinin atau katalase dapat menghalang penurunan tekanan darah dan memulihkan pelepasan kreatinin tetapi bukan kombinasi tersebut. Aktiviti Nox 4 mRNA telah dikurangkan selepas rawatan aposinin, katalase dan gabungan aposinin dan katalase berbanding dengan model tikus CsA atau L-NAME yang tidak dirawat. Sebaliknya, hanya aposinin atau gabungan aposinin dan katalase dapat memulihkan paras Nox 4 mRNA pada model L-arginina. Kerosakan histologi pada ketiga-tiga model tersebut telah diperbaiki susulan rawatan dengan aposinin dan katalase. Kesimpulannya, rawatan dengan menggunakan aposinin dan katalase pada model penyakit ginjal yang disebabkan oleh CsA, L-NAME atau L-arginina dapat mengimbangi perubahan tekanan darah dan disfungsi ginjal, dan menurunkan status stres oksidatif, berkemungkinan disumbang oleh pengurangan ekspresi Nox 4. Semua penemuan ini menunjukkan bahawa sebatian antioksidan seperti aposinin dan katalase berpotensi dalam merawat penyakit kardiovaskular. Kajian sewajarnya yang menggunakan subunit Nox lain Akan berguna dalam memahami mekanisme stres oksidatif di dalam penyakit.

PROTECTIVE EFFECT OF APOCYNIN AND CATALASE IN OXIDATIVE STRESS OF RENAL FAILURE, HYPERTENSION AND HYPOTENSION RAT MODELS

ABSTRACT

Oxidative stress is involved in the pathogenesis of several renal and cardiovascular diseases. In many of these diseases, there is an enhanced expression of nicotinamide adenine dinucleotide oxidase (NADPH oxidase or Nox) leading to the production of superoxide anion (O_2^-) and accumulation of hydrogen peroxide (H_2O_2) during disease progression. This study investigated the effects of apocynin, an NADPH oxidase inhibitor, and catalase, an H_2O_2 scavenger, on impaired renal function and haemodynamic in cyclosporine A (CsA), L-NAME and L-arginine Wistar-Kyoto rat models. Rats received CsA (25 mg/kg/day p.o.), L-NAME (15 mg/kg/day p.o.) and L-arginine (12.5 mg/ml p.o.) respectively and were assigned to the vehicle, apocynin (2.5 mmol/L p.o.), catalase (10,000 U/kg/day i.p.) or a combination of apocynin and catalase for 14 days. Renal function and haemodynamic, and plasma oxidative stress markers were measured. In addition, histological study and molecular expression of NADPH oxidase 4 (Nox 4) mRNA were assessed at the end of the treatment period. Both CsA and L-NAME groups had higher while L-arginine group had lower blood pressure compared to control. Moreover, creatinine clearance was lower in CsA and L-NAME groups was higher in L-arginine group compared to control. The renal tissue Nox 4 mRNA activity and the plasma malondialdehyde (MDA) levels were higher while total superoxide dismutase (T-SOD), and total antioxidant capacity (T-AOC) were all lower

in the three models compared to control. Treatment of CsA or L-NAME rats with apocynin, catalase or a combination of both resulted in restored blood pressure and creatinine clearance to near control values. However, in the L-arginine rat model, treatment with either apocynin or catalase but not their combination prevented the decrease in blood pressure and restored creatinine clearance. The Nox 4 mRNA activity was reduced after apocynin, catalase and combined treatment using both apocynin and catalase compared to untreated CsA or L-NAME rats. On another hand, only apocynin or apocynin combined with catalase treatment restored Nox 4 mRNA levels in the L-arginine model. The histological damage in the three models was ameliorated following treatment with apocynin and catalase. In conclusion, treatment using apocynin and catalase in models of renal disease due to CsA, L-NAME or L-arginine offset blood pressure changes and renal dysfunction, and reduced oxidative stress status, possibly contributed by a reduction in Nox 4 expression. These findings suggest that antioxidant compounds such as apocynin and catalase have potential in treating cardiovascular diseases. Further studies utilizing other subunits of Nox will be useful in understanding the underlying oxidative stress mechanism in disease.

CHAPTER 1

INTRODUCTION

1.1 Role of oxidative stress and antioxidants in pathogenesis and therapeutic strategies of cardiovascular and renovascular systems

Despite extensive research and the absolute efforts of health care professionals, cardiovascular and renal diseases are still major leading causes of morbidity and mortality (Ahmad *et al*., 2014). However, dramatic increase in patients with renal failure secondary to the deleterious cardiovascular effects of oxidative stress is a point of great concern. Essentially, both systems are interrelated and often prognosis of one terminates to other system. This study investigates the influence of oxidative stress on the cardiovascular and renovascular systems. Furthermore, the physiology and pathophysiology of oxidative stress in CsA-induced renal failure, L-NAME-induced hypertension and L-arginine-induced hypotension models are investigated using rat's models.

In both cardiovascular and renovascular systems, oxidative stress is playing its role in the pathogenesis of cardiovascular complications to renal failure. In physiological conditions, the balance between prooxidant and antioxidant substances is kept slightly in favour of prooxidant products, thus favouring a mild oxidative stress. NADPH oxidases are enzymes whose biological function is electron transport. Nox enzymes in mammalian organisms have received most attention due to their ability to generate reactive oxygen species (ROS). Therefore, Nox plays a key role in the onset of many cardiovascular related diseases and inhibition of Nox plays a central role in the

pharmacological treatment of many diseases (Babior, 2004). There are several underlying mechanisms co-exist in counteracting the down-regulation of antioxidant mechanisms during a disease condition. Moreover, pathophysiology of kidney and vascular system are largely dependent on antioxidant enzymes such as superoxide dismutase, catalase and pro-oxidant like malondialdehyde (Tanir *et al.*, 2005; Chia *et al.*, 2013b). At present, few inhibitors are available commercially to thwart Nox activity and adopted as therapeutic options in hypertension and renal diseases (Chia *et al.*, 2018). Therefore, understanding of the cardiovascular and renal physiology system is important to acquire the information on the development, complications and prognosis of hypertension, hypotension and renal failure diseases. Hence, the present study investigates the protective effects of apocynin and catalase during hypertension state and further explores their renoprotective role caused by oxidative stress.

1.2 Cardiovascular system

The cardiovascular system is a closed system of the heart and blood vessels. The heart is an anatomical pump connected to intricate conduits which consists of arteries, veins, and capillaries (SEER Training Modules, 2019a). As the name implies, blood contained in the circulatory system is pumped by the heart around a closed circuit of vessels as it passes repeatedly through the various "circulations" of the body as depicted in Figure 1.1.

The vital role of the cardiovascular system in maintaining homeostasis depends on the continuous and controlled movement of blood through the thousands of miles of capillaries that permeate every tissue and reach every cell in the body. It is in the microscopic capillaries that blood performs its ultimate transport function. Nutrients and other essential materials including the release of several hormones passes from capillary blood into fluids surrounding the cells and at the same time removing the waste products produced during metabolism (SEER Training Modules, 2019a).

There are various control mechanisms exist to regulate and integrate the diverse functions of the cardiovascular system in order to supply blood to specific body areas according to the demands. These mechanisms ensure a constant internal environment surrounding each body cell regardless of differing demands for nutrients or production of waste products (SEER Training Modules, 2019a). In order to understand how that happens, it is necessary to understand the anatomy and physiology of the heart and the kidney thoroughly.

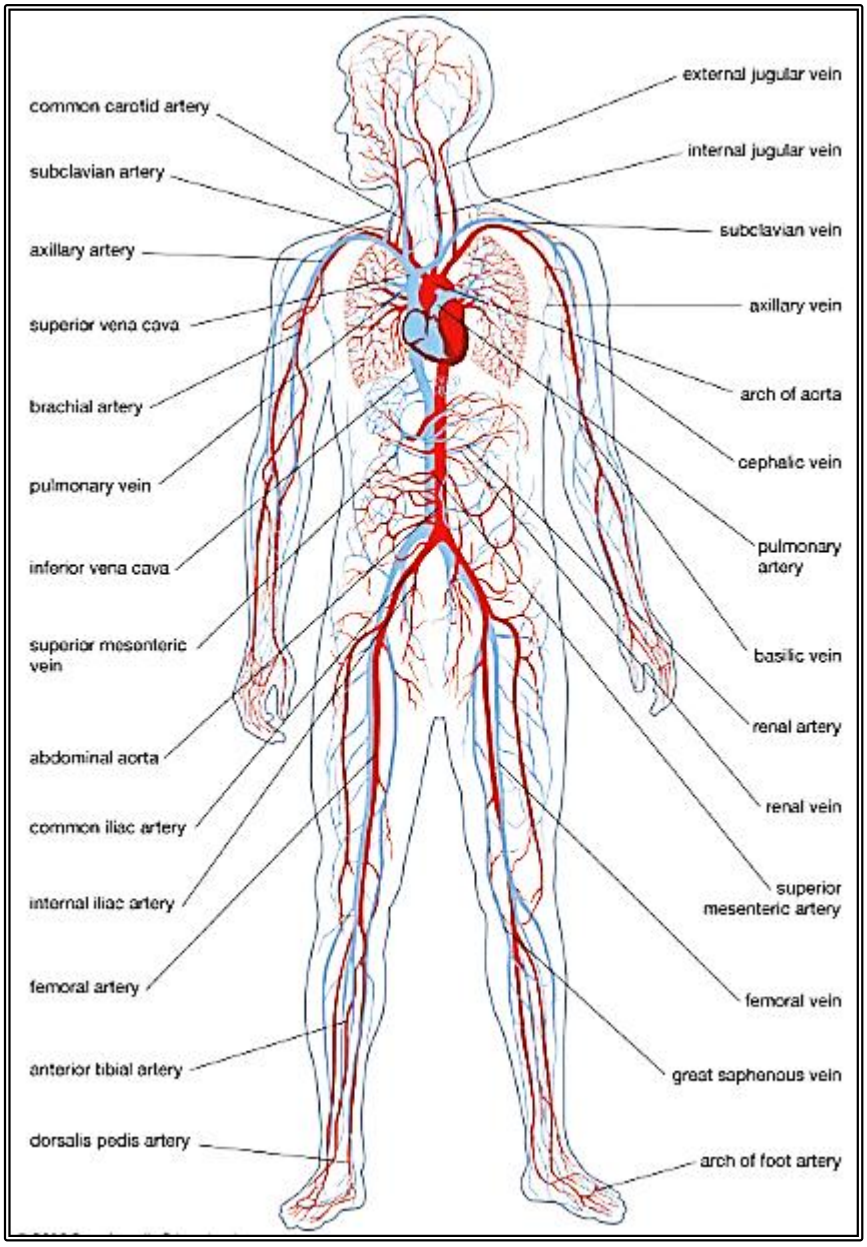

Figure 1.1: The cardiovascular system. Red color indicates oxygenated blood carried in arteries whereas blue indicates deoxygenated blood carried in veins and capillaries which join the arteries and veins.

Diagram adapted from (Principles of Human Physiology), Pearson Education Subscription and End-User License Agreement. Access code: USWSP4-FROMM-STEAK-NEMAN-PLANT-MINES; Expire date: Dec 30, 2020).

1.3 Heart

1.3.1 The functional and gross anatomy of the heart

As implies, the word “Cardiac” is derived from Greek word “Kardia” which mean “Heart”. The heart is the organ that helps supply blood and oxygen to the entire body via blood vessels by rhythmic contraction. It is divided by a septum into two halves, and the halves are in turn divided into four chambers. The heart is situated within the chest cavity and surrounded by a fluid-filled sac called the pericardium. This amazing muscle produces electrical impulses that cause the heart to contract, pumping blood throughout the body. The heart and the circulatory system together form the cardiovascular system (Bailey, 2019).

Essentially, the human heart is located within the thoracic cavity, medially between the lungs in the space known as the mediastinum. Within the mediastinum, the heart is separated from the other mediastinal structures by a tough membrane known as the pericardium that sits in its own space called the pericardial cavity. The dorsal surface of the heart lies closes the bodies of the vertebrae, and its anterior surface lies deep to the sternum and costal cartilages. The great veins, the superior and inferior venae cavae, together with the great arteries, the aorta and pulmonary trunk, are attached to the superior surface of the heart. The base of the heart is located at the level of the third costal cartilage, as seen in Figure 1.2. The inferior tip of the heart, the apex, located just to the left of the sternum between the junction of the fourth and fifth ribs near their articulation with the costal cartilages. Likewise, the right side of the heart is deflected anteriorly, and the left side is deflected posteriorly. The slight deviation of the apex to

the left is reflected in a depression in the medial surface of the inferior lobe of the left lung, called the cardiac notch (CNX, 2019).

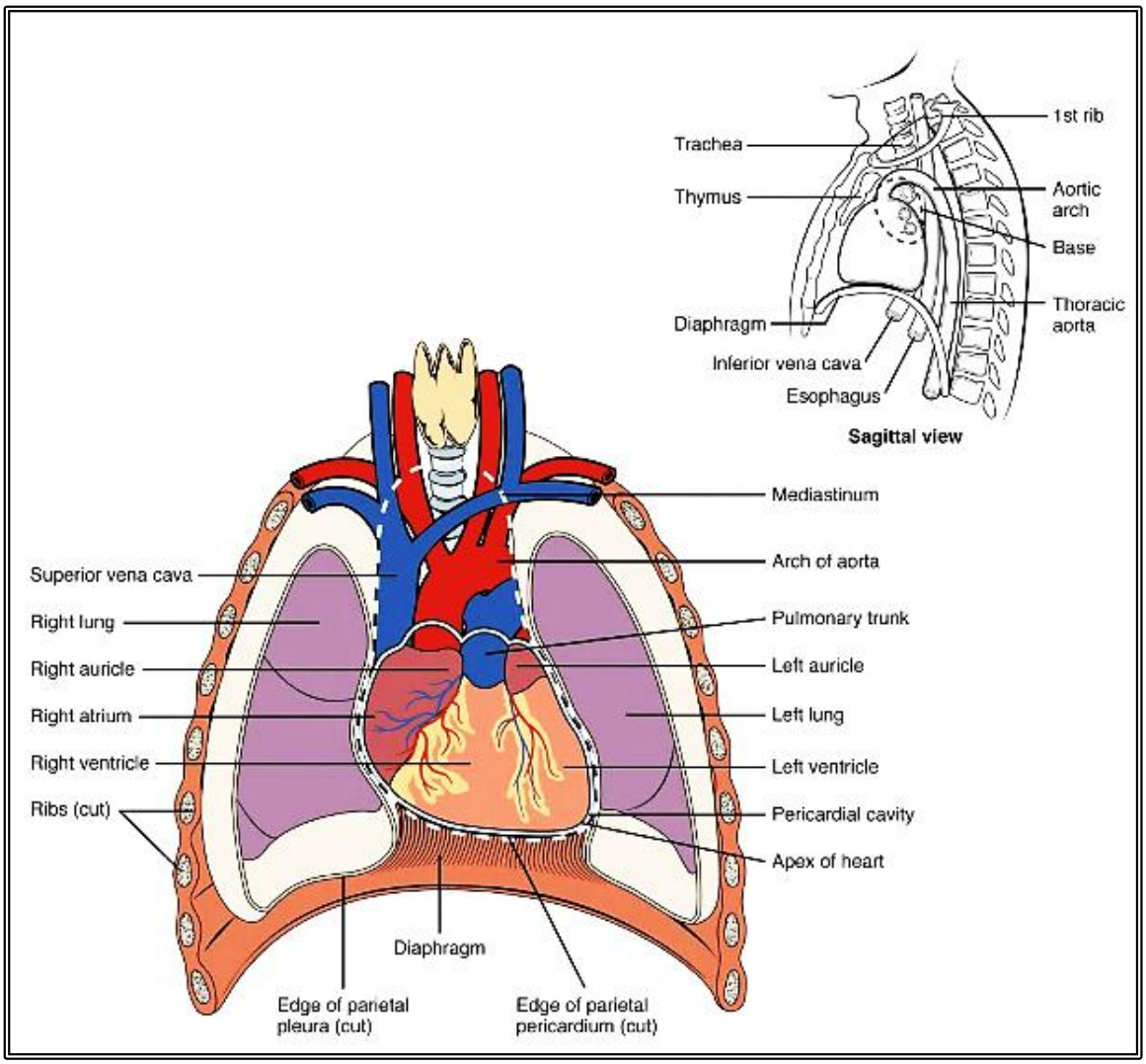

Figure 1.2: The gross anatomy and position of the heart. The heart is located within the thoracic cavity, medially between the lungs in the mediastinum. It is about the size of a fist, is broad at the top, and tapers toward the base.

Diagram adapted from (Anatomy & Physiology), OpenStax CNX. Available at: https://openstax.org/books/anatomy-and-physiology/pages/19-1-heart-anatomy.
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In human, a typical heart is approximately the size of a fist: 12 cm in length, 8 cm wide, and 6 cm in thickness. Given the size difference between the sexes where the weight of a female heart is approximately 250–300 grams and the weight of a male heart is approximately 300–350 grams. The heart contracts at different rates depending on many factors. At rest, a healthy heart beats around 60 to 80 times a minute, but it can increase to 100 beats a minute or more as exercise, emotions, fever, diseases, and some medications may also influence the heart rate (Hall, 2015a).

As presented in Figure 1.3, the human heart consists of four chambers. The left and right side of each have one atrium and one ventricle. Each of the upper chambers, the right atrium and the left atrium principally acts as a receiving chamber and contracts to push blood into the lower chambers, the right ventricle and the left ventricle. The ventricles serve as the primary pumping chambers of the heart, propelling blood to the lungs or to the rest of the body (Germann *et al.*, 2005a).

There are two linked circuits in the human circulation called the systemic and pulmonary circuits. The systemic circuit transports oxygenated blood to virtually all of the tissues of the body and returns relatively deoxygenated blood and carbon dioxide to the heart to be sent back to the pulmonary circulation whereas the pulmonary circuit transports blood to and from the lungs, where it picks up oxygen and delivers carbon dioxide for exhalation. The right ventricle pumps deoxygenated blood into the pulmonary trunk, which leads toward the lungs and bifurcates into the left and right pulmonary arteries. These vessels in turn branch many times before reaching the pulmonary capillaries, where gaseous exchange occurs. Here, carbon dioxide exits the

blood likewise oxygen also enters the blood too. Highly oxygenated blood returning from the pulmonary capillaries in the lungs passes through a series of vessels that join together to form the pulmonary veins in the body that carry highly oxygenated blood. The pulmonary veins conduct blood into the left atrium, which pumps the blood into the left ventricle, which in turn pumps oxygenated blood into the aorta and on to the many branches of the systemic circuit. Eventually, these vessels will lead to the systemic capillaries, where exchange with the tissue fluid and cells of the body occurs. In this case, oxygen and nutrients exit the systemic capillaries to be used by the cells in their metabolic processes, and carbon dioxide and waste products will enter the blood (Germann *et al.*, 2005a).

The blood exits the systemic capillaries is lower in oxygen concentration than when it entered. The capillaries will ultimately unite to form venous, joining to form ever-larger veins, eventually flowing into the two major systemic veins, the superior vena cava and the inferior vena cava, which return blood to the right atrium. The blood in the superior and inferior venae cavae flows into the right atrium, which pumps blood into the right ventricle. This process of blood circulation continues as long as the individual remains alive (Hall, 2015a).

The membrane that directly surrounds the heart and defines the pericardial cavity is called the pericardium or pericardial sac. It also surrounds the “roots” of the major vessels, or the areas of closest proximity to the heart. Generally, the heart wall is made up of three layers consists of an epicardium (outermost layer connective tissue); a middle layer called myocardium and an inner most layer called endocardium. The heart

pumps by the continuous contraction (systole) and relaxation (diastole) rhythmic of the myocardium due to the presence of connective tissue with contractile properties. When the cardiac muscle in the atrium and ventricle walls contracts, the mechanical force moves the wall inwards and squeezes the blood into the chamber. As the contraction increases, the pressure within both atrium and ventricle force the blood out while the atrium and ventricle expands and fill with blood when muscle cardiac muscle relaxes to complete a cardiac cycle (Anderson, 2000). This mechanical action creates pressure in the heart chambers to fluctuate; therefore, it is very important that blood flow in the heart is only unidirectional. The presence of four types of valves which consists of left and right atrioventricular valves called bicuspid or mitral valve and a tricuspid valve which allow the blood flow only from atrium to ventricle. On the other hand, the aortic semilunar valve and pulmonary semilunar valve also perform the same function to allow blood flow forward at the same time prevent the blood flowing backward (Marieb & Hoehn, 2007a).

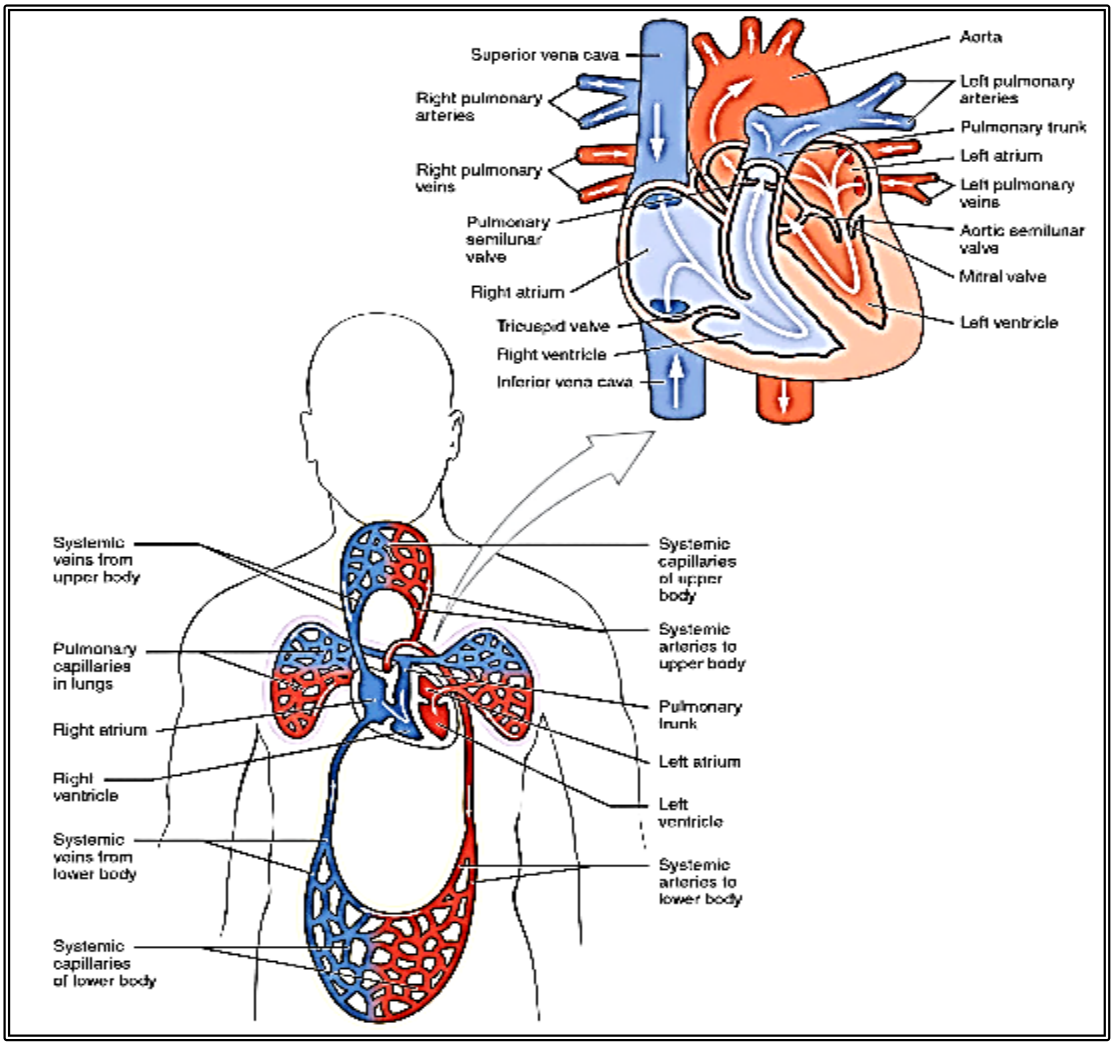

Figure 1.3: The blood circulation. Blood flows from the right atrium to the right ventricle, which is pumped into the pulmonary circuit. The blood in the pulmonary artery branches is low in oxygen but proportionately high in carbon dioxide. Gaseous exchange occurs in the pulmonary capillaries with oxygen enter the blood, carbon dioxide exit via diffusion, and blood high in oxygen and low in carbon dioxide is returned to the left atrium. The oxygenated blood is then enters the left ventricle, which is pumps into the systemic circuit. Blood returns to the right atrium and the cycle is repeated.

Diagram adapted from (Anatomy & Physiology), OpenStax CNX. Available at: https://openstax.org/books/anatomy-and-physiology/pages/19-1-heart-anatomy.
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1.3.2 Physiology of the heart

To pump blood throughout the body, the cardiac muscles must be coordinated perfectly in squeezing the blood in the right direction, time and right pressure. The heart's activity is coordinated by an elaborated conduction system with electrical impulses that determines the series of excitation of cardiac muscle cells. The heartbeat is modulated by the electrical signal begins at the sinoatrial node or the heart's pacemaker, positioned at the top of the right atrium where it join with superior vena cava and the atrioventricular node which is located near the tricuspid valve in the interatrial septum. This signal causes the atria to contract, pushing blood down into the ventricles. These cells act as a gate where they slow the signal down so that the atria and ventricles do not contract concurrently with a slight delay. Succeeding each action potential, the pacemaker cells exhibit slow spontaneous depolarization and eventually triggers the next action potential which causes the left ventricle to pump oxygenated blood into the aorta whose branches transport blood to capillary beds of all tissues and organs in the systemic circuit (Germann *et al.*, 2005a; Chia, 2013).

A complete cardiac cycle is associated with the ventricular contraction termed as systole and relaxation diastole. The pressure exerted by blood on unit area of blood vessels is termed as blood pressure (BP) which consists of the systolic blood pressure (SBP) and diastolic blood pressure (DBP). A normal blood pressure of a healthy adult is around 120-140 mmHg for SBP and 70-90 mmHg for DBP while the average pressure required to pump the blood into the blood vessels is known as mean arterial blood pressure (MAP) with value between 90-110 mmHg. In addition to that, the difference between the SBP and the DBP is termed as pulse pressure. Generally, a pulse pressure

should be at least 25% of the systolic blood pressure. A pulse pressure below this level is described as narrow where this may occur for instance, in patients with a low stroke volume, which may be seen in congestive heart failure, stenosis of the aortic valve or significant blood loss post trauma. In opposite, a wide pulse pressure is common seen in healthy people following strenuous exercise when their resting pulse pressure of 30–40 mmHg may increase temporarily to 100 mmHg as stroke volume increases. However, a persistently high pulse pressure at or above 100 mmHg may indicate excessive resistance in the arteries and can be caused by a variety of disorders as chronic high resting pulse pressures can degrade the heart, brain, and kidneys, and warrant medical treatment. Nevertheless, the duration of this event is not equal. For instances, the heart rate (HR) at the normal resting human is 60 to 80 beat per minutes. Therefore, one cardiac cycle is 0.8 second and systole lasts about 0.3 second whereas diastole lasts for only 0.5 second (Hall & Guyton, 2006a).

Blood pressure; defined as the forces originating in the pumping action of the heart, exerted by the blood against the walls of the blood vessels; the stretching of the vessels in response to this force and their subsequent contraction are important in maintaining blood flow through the vascular system. When the heart rate is increased above 100 beat per minute, it results a condition called tachycardia and in contrary, when the HR is decreased below 60 beat per minute, it causes bradycardia. Normally, the chronotropic action of the HR is modulated by the β1-adrenoceptors (Brodde *et al.*, 2006). The amount of blood being pumped by the heart in one minute is referred as cardiac output. In a healthy individual, the average cardiac output in males is approximately 5.6 L/min while in female is around 4.9 L/min (Fox, 2006).

1.4 The blood vessels

1.4.1 The gross anatomy and physiology

The human vasculature systems are conduits through which blood is distributed to body tissues. These vessels make up of two closed systems that begin and end at the heart. The pulmonary vessels mainly transport blood from the right ventricle to the lungs and back to the left atrium. Whereas, the systemic vessels carries blood from the left ventricle to the tissues in all parts of the body and then returns the blood to the right atrium. Depending on their structure and function, blood vessels are classified into arteries, veins and capillaries (Marieb & Hoehn, 2007a) as shown in Figure 1.4.

1.4.2 Artery

Essentially, all blood vessels possess a hollow interior called lumen with its surface lined by a layer of epithelium cells called endothelium. Surrounding the lumen is a wall that has different composition and thickness from one type of vessels to another. The wall of an artery consists of three layers. The innermost layer is termed as tunica intima also called tunica interna, is composed by simple squamous epithelium surrounded by a connective tissue basement membrane with elastic fibers. The middle layer called tunica media which is primarily made up by smooth muscle and is usually the thickest layer. It not only provides support for the vessel but also changes vessel diameter to regulate blood flow and blood pressure. However, the outermost layer, which attaches the vessel to the surrounding tissue, is called as tunica externa or tunica adventitia. This layer is connective tissue with varying amounts of elastic and collagenous fibers. The connective tissue in this layer is quite dense where it is adjacent

to the tunic media, but it changes to loose connective tissue near the periphery of the vessel (SEER Training Modules, 2019b).

1.4.3 Vein

The walls of veins also made up of three layers as in the arteries. Although all the layers are present, there is less smooth muscle and connective tissue. This makes the walls of veins thinner than those of arteries, which is related to the fact that blood in the veins has less pressure than in the arteries. Because the walls of the veins are thinner and less rigid than arteries, veins can hold more blood. Even slight increases in venous pressure cause the veins to store 0.5 to 1.0 liter of extra blood. Almost 70 percent of the total blood volume is in the veins at any given time. Medium and large veins have venous valves, similar to the semilunar valves associated with the heart that helps to keep the blood flowing toward the heart. Venous valves are especially important in the arms and legs, where they prevent the backflow of blood in response to the pull of gravity. Therefore, the veins provide a reservoirs function for storing large quantity of extra blood that can be called into use whenever necessary elsewhere in the circulation (Hall, 2015b).

1.4.4 Capillary

Capillaries are the smallest and most numerous of the blood vessels in the circulatory system which formed the connection between the vessels that carry blood away from the heart (arteries) and the vessels that return blood to the heart (veins). The primary function of capillaries is the exchange of materials between the blood and tissue cells. The thin walls of capillaries provide small diffusion distance between blood and

surrounding interstitial fluid for gaseous exchange such as oxygen, carbon dioxide as well as electrolytes exchange with the metabolic activity of body tissues. Smooth muscle cells in the arterioles where they branch to form capillaries regulate blood flow from the arterioles into the capillaries. Tissues such as heart and kidney have extensive capillary networks because they are metabolically active and require an abundant supply of oxygen and nutrients. Other tissues, such as connective tissue, have a less abundant supply of capillaries (Martin, 2015) .

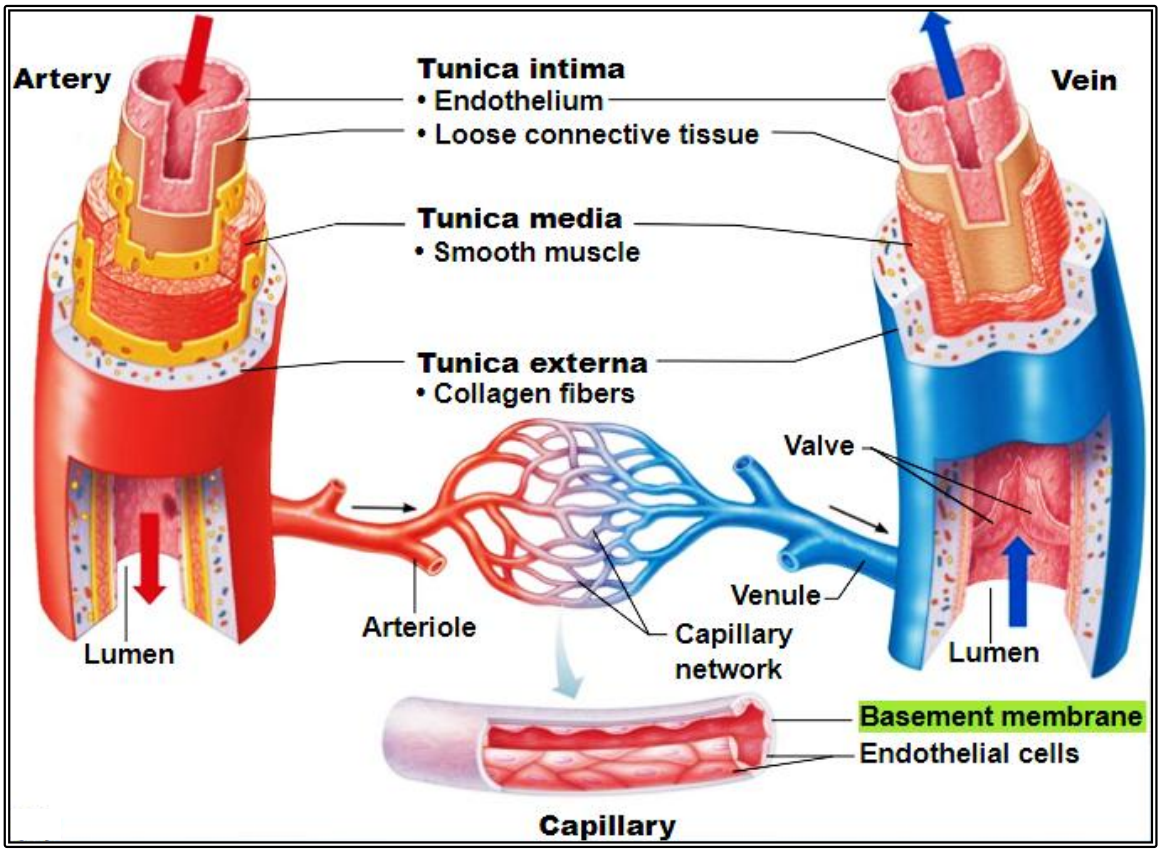

Figure 1.4: Anatomy and structure of blood vessels.

Diagram adapted from (Blood vessels: organization and microscopic anatomy), Pearson Education, Inc, 2015. Available at: https://slideplayer.com/slide/7887079/. License: CC BY: Attribution. License Terms: Download for free at https://slideplayer.com/slide/7887079/. Accessed on [16/10/2019].

1.5 Kidney

1.5.1 The functional and gross anatomy of the kidney

The kidneys are the primary organs of the urinary system. It is an organ that filters the blood, remove the wastes, and excrete the wastes in the urine. Essentially, the human adult kidneys are popularly described as being bean-shaped and reddish in color and each kidney is about 12 cm in length, 7.5 cm in breadth and rather more than 2.5 cm in thickness with an indentation, called the hilum, on the medial side. The hilum leads to a large cavity called the renal sinus, within the kidney. Whereas, the ureter and renal vein leave the kidney and the renal artery enters the kidney at the hilum. Each kidney weighs about 125–175 g in males and 115–155 g in females where its weight is less than 1% of the total body weight of an adult human (Germann *et al.*, 2005b).

1.5.2 The external anatomy of the kidney

The exact positions of the human kidney are located between the 12^{tt} thoracic and 3^{rd} lumbar vertebrae with one on each side of the vertebral column. The right kidney usually is slightly lower than the left because the liver displaces it downward. The kidneys are protected by the lower ribs that lie in shallow depressions against the posterior abdominal wall and behind the parietal peritoneum or retroperitoneal position. Each kidney is adhered in place by the connective tissue called renal fascia and surrounded by a thick layer of adipose tissue called peri-renal fat which helps to protect it from mechanical sheers. A tough fibrous connective tissue called renal capsule closely encapsulates each kidney and provides support for the soft tissue that is inside. On the superior side of each kidney is the adrenal gland. The adrenal cortex directly influences

renal function through the production of the hormone aldosterone to stimulate sodium reabsorption (Richard L. Drake, 2014) such that presented in Figure 1.5.

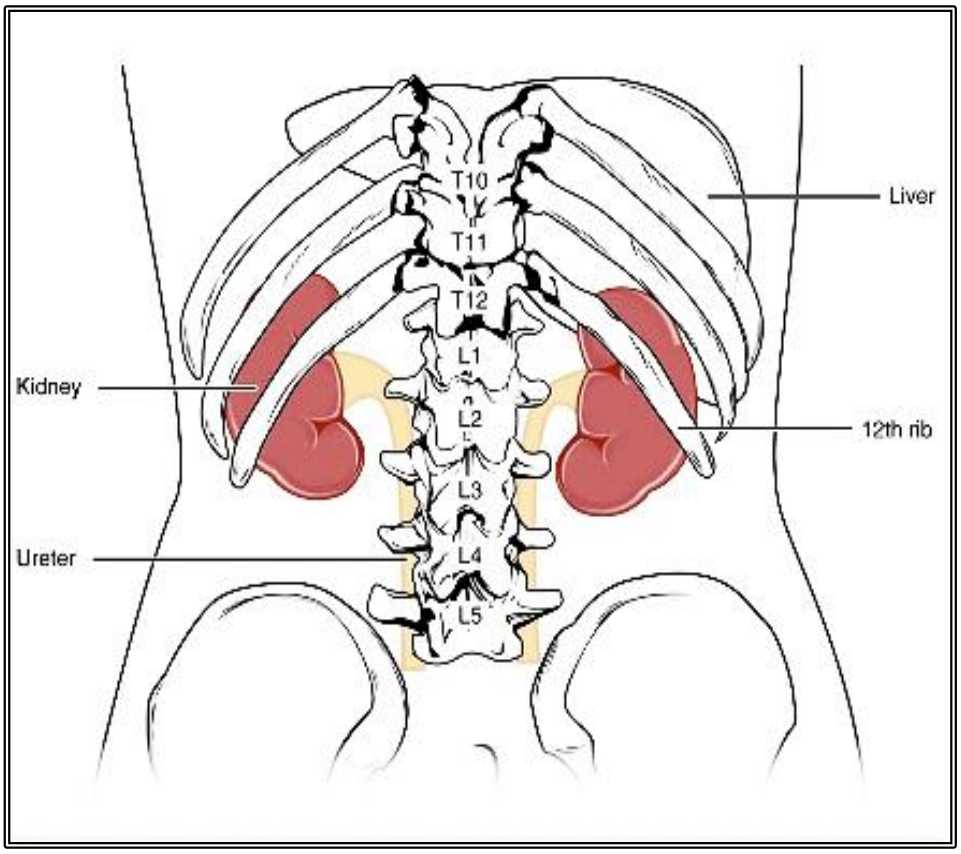

Figure 1.5: Anatomy and position of the pair of kidneys.

Diagram adapted from (Anatomy & Physiology), OpenStax CNX. Available at: https://courses.lumenlearning.com/nemcc-ap/chapter/gross-anatomy-of-the-kidney/. License: CC BY: Attribution. License Terms: Download for free at http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@8.25. Accessed on [16/10/2019].

1.5.3 The internal anatomy of the kidney

The outer reddish zone next to the capsule is the renal cortex that surrounds a darker reddish-brown region called the renal medulla. Inside the renal medulla consists of a series of renal pyramids which appear striated as it consists of straight tubular structures and blood vessels. The wide bases of the pyramids that are adjacent to the cortex and the pointed ends called renal papillae are directed toward the center of the kidney. Portions of the renal cortex extend into the spaces between adjacent pyramids to form renal columns. The cortex and medulla make up the parenchyma, or functional tissue, of the kidney. The central area of the kidney contains the renal pelvis which is located in the renal sinus and is continuous with the ureter. This renal pelvis is a large cavity that collects the urine as it is produced. The periphery of the renal pelvis is interrupted by cuplike projections called calyces. A minor calyx surrounds the renal papillae of each pyramid and collects urine from that pyramid. Several minor calyces converge to form a major calyx. From the major calyces, the urine flows into the renal pelvis and from here it flows into the ureter (SEER Training Modules, 2019c) which is depicted in Figure 1.5 (a) .

Deep down to the renal pyramids, there are over a million microscopic functional subunits called nephrons in the parenchyma (cortex and medulla). Generally, a nephron consists of two parts: a renal corpuscle and a renal tubule by which they perform the work of filtering the blood and forming urine respectively and ultimately the filtrate is modified to be excreted in the form of urine. The renal corpuscle consists of a cluster of capillaries called the glomerulus surrounded by a double-layered epithelial cup called the Bowman’s capsule by which this is the initial site where blood filtration and tubular

fluid formation. Blood enter the glomerular capillaries via the afferent arteriole leads into the renal corpuscle and an efferent arteriole leaves the renal corpuscle to produce protein free plasma filtrate by a process called glomerular ultrafiltration. This filtrate flows into the first part of the tubular region of the nephron called proximal convoluted tubule and further empties into the hairpin loop tubules located in the medulla region known as descending and ascending limbs of loop of Henle's. From the ascending loop of Henle's, the filtrate then enters the distal convoluted tubules which actually resembles the proximal one but just by this tubule is shorter. Following this, the fluid enters a short straight terminal segment called the collecting tubule that joins the nephron to the collecting duct which will empty the filtrates into the calyces and the filtrate is excreted in the form of highly concentrated urine through the urinary system (Martini, 2001; Chia, 2013).

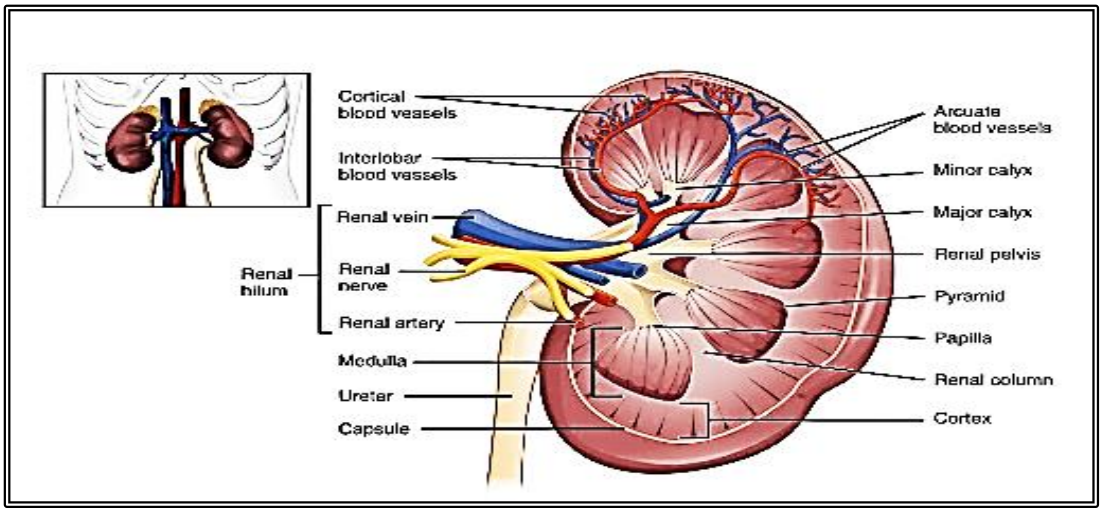

Figure 1.5 (a): The internal anatomy of the left kidney.

Diagram adapted from (Anatomy & Physiology), OpenStax CNX. Available at: https://courses.lumenlearning.com/nemcc-ap/chapter/gross-anatomy-of-the-kidney/. License: CC BY: Attribution. License Terms: Download for free at http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@8.25. Accessed on [16/10/2019].

Although the size of the kidney which is relatively small. However, the blood supply to this organ occupied approximately 22% of the cardiac output. The blood flow into the renal artery and branches into other segmental arteries which is then flow into another type of artery called interlobar arteries. Succeeding here, the blood is further fed into the arcuate arteries and branch into the last segment called interlobular arteries where the blood is supplied to individual nephron. The renal glomerular ultrafiltration process in the renal capsules is driven by the starling forces exert across the walls of the glomerular capillaries. Following this, the blood is transported into small capillary beds namely peritubular capillaries which is branched from the efferent arterioles which are located close to renal tubules and vasa recta that run along the loop of Henle's and collecting duct deep into the renal medulla. As for the arteriole distribution, the filtered blood is then circulated into the interlobular veins from the peritubular capillaries and vasa recta. From here, blood is carried away from the nephrons by arcuate veins to the interlobar veins and renal vein hereafter return to inferior vena cava (Meyer *et al.*, 2004; Germann *et al.*, 2005b; Chia, 2013) such that illustrated in Figure 1.5(b).

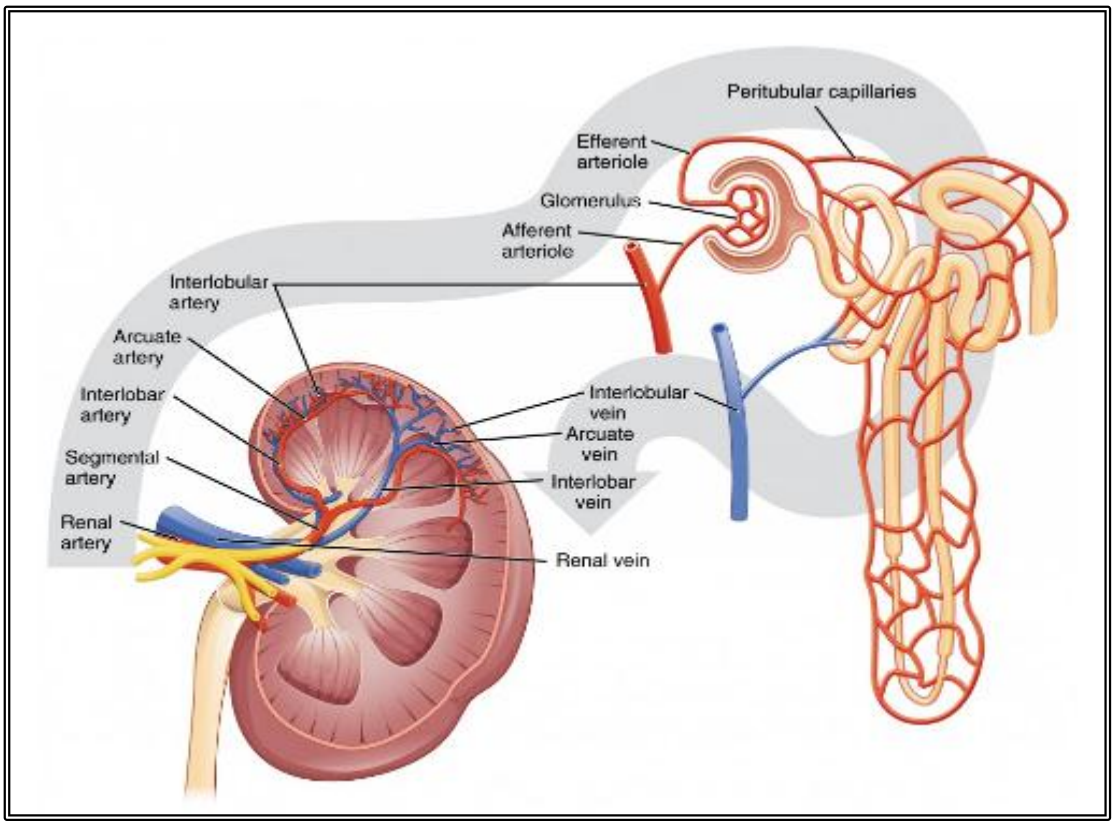

Figure 1.5 (b): The route of blood flow and ultrafiltration mechanism in the kidney.

Diagram adapted from (Anatomy & Physiology), OpenStax CNX. Available at: https://courses.lumenlearning.com/nemcc-ap/chapter/gross-anatomy-of-the-kidney/. License: CC BY: Attribution. License Terms: Download for free at http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@8.25. Accessed on [16/10/2019].

1.5.4 The physiology of the kidney

The kidney performs their principal functions by filtering the plasma and removing substances from the filtrate at variable rates depending on the demands from the body. Subsequently, the kidney clears unwanted substances such as urea from the metabolism of amino acids, creatinine from muscle creatine, uric acid from nucleic acid and metabolites of various hormones from the filtrate and therefore from the blood by excreting them in urine and while returning needed substances back to blood circulations. In addition to that, the kidneys also regulate the electrolyte contents, volume and acid-base balance of the extracellular fluid due to the consequence of varying internal and external environments including the regulation of arterial pressure (Hall, 2015c).

Under normal condition, the kidney of a resting adult receive 1.2 to 1.3 liters of blood per minute as it receives almost 25% of the cardiac output from more than hundred liters of plasma daily and performs its task via simple mechanisms such as filtration, reabsorption and secretion which take place in the nephron. Each kidney in the human contains about 800,000 to 1,000,000 nephron, each capable of forming urine. Unfortunately, kidney cannot regenerate new nephrons and hence, with renal injury, disease and aging will cause a gradual decrease in nephron number. The glomerular filtration rate (GFR) in a healthy human adult is approximately 125mL/min where its magnitude is correlates fairly well with surface kidney surface area. A rate of 125 mL/min is equivalence to 7.5 L/hour or 180L/day which represent the normal urine volume is about 1.0 L/day and in 1 day, the kidneys filter an amount of fluid equal to four times the total body water, 15 times extracellular fluid volume and 60 times the

plasma volume. Thus, 99% or more of the filtrate is normally reabsorbed (Barrett *et al.*, 2012).

Water and solutes are exchanged between fluid and plasma in the renal tubules to regulate the composition of plasma. Firstly, the filtration is initiated in the renal corpuscle which is the mass flow of protein free plasma from glomerular capillaries in the Bowman's capsule. On the other hand, reabsorption is done by the selective transport of glomerular filtrate from the lumen of the tubules to the interstitial cells which located outside the tubules. However, secretion is the reverse mechanism where molecules from the peritubular fluid are transported back into the lumen of the renal tubules. Generally, the tubular reabsorption is quantitatively more important than tubular secretion during the formation of urine. However, secretion plays a vital role in determining the amount of potassium and hydrogen ions and a few other metabolism end product substances such as urea, creatinine, uric acid and urates that are poorly reabsorbed and are therefore excreted in large amounts in urine. Certain foreign substances and drugs are also poorly reabsorbed but, in addition, are secreted from the blood into the tubules and hence their excretion rates are high. On the contrary, electrolytes such as sodium ions, chloride ions and bicarbonate ions are highly reabsorbed and therefore only small quantity will be found in the urine (Chia, 2013; Hall, 2015c).

GFR describes the amount of plasma ultrafiltrate flow rate each minute through the kidney. Under normal circumstances, the regulation of the GFR is governed by three intrinsic control mechanisms that consists of myogenic regulation of smooth muscle in the afferent arteriole, tubuloglomerular feedback and mesangial cell contraction. In