**Ministry of Higher Education**
**and Scientific Research**
**University of Misan**
**College of Science**
**Department of Biology**

# Relationship between Testosterone and Other Reproductive Hormones, Pro-inflammatory and Biochemical Parameters with Elderly Healthy and Hypertensive Men in Misan Province

A thesis

Submitted to Council of the College of Science / University of Misan in Partial Fulfillment of the Requirement for the Master Degree in Biology

By

**Athraa Sabeeh Abd Al-Najdi**

**B.Sc.Biology (2003)**

**Supervised by**

**Prof. Dr. Ahmed Aboud Khalifa**

**March , 2021 A.D** **Sha`ban,1442 A.H**

بسم الله الرحمن الرحيم

**(أَوَلَمْ يَرَ الْإِنسَانُ أَنَّا خَلَقْنَاهُ مِن نُّطْفَةٍ**

**فَإِذَا هُوَ خَصِيمٌ مُّبِينٌ)**

صدق الله العلي العظيم

سورة يس – الآية\77

## Supervisor's certificate

We certify that the thesis entitled " Relationship between Testosterone and Other Reproductive Hormones, Pro-inflammatory and Biochemical Parameters with Elderly Healthy and Hypertensive Men in Misan Province "

"Has been prepared under my supervision at the College of Science , University of Misan ; in partial fulfillment of the requirements for the degree of master of Biology "

**Signature**

**Prof. Dr. Ahmed Aboud khalifa**

**Department of Biology**

College of Science/ Misan University

Date : / /2021

## Recommendation of Head of Biology Department

In view of the available recommendations ; I forward this thesis to debate by the examining committee.

**Signature**

**Assist. prof. Dr. Maytham Abdul Kadhim Dragh**

**Head of Department of Biology**

College of Science / Misan University

Date : / /2021

## Dedication

I dedicate my work to the teacher of mankind , who has the greatest ethics , to whom every work that is small in the aspect of his sacrifices , to the master of beings , the Prophet of Allah Muhammad . And I dedicate my work to my family and my husband for their patient and support

.

## Acknowledgments

Praise and thanks to Allah for his grace and kindness during my life. Thanks and appreciation to my supervisor Prof. Dr. Ahmed Aboud khalifa , for his guidance , support and sincere efforts to accomplish this study.

I would like to express my thanks and gratitude to all the foculty of the Science College to give me the chance to get master degree , and for their moral support and scientific advice . Also I would like to thank Dr. Assad Yahya for his special work and data analysis. I also thank Mr. Salam, the worker at the Teaching Hospital in Basra for his spcial help , and Mrs. Jumana for her moral support.

I would like to thank the staff at Al-Sadr Hospital and the Heart Center for their help.

All thanks and appreciation for those who allowed me to take sample from them , patients and normotensive people.

Also , I thank my colleagues : ala' jamil , Zainab Jumaa , Nour Khudair , Nour Basil , Iman , Rabab , Hiba Naiem , Hawraa and Mysoon , for giving me the support to complete this work.

And all thanks for every one who has tried to help me to complete this work .

*2021*

## Summary

The current study aimed to investigate some hormonal , pro-inflammatory and biochemical parameters associated with progressive age and hypertension for men in Misan province . The sample of this study included 90 men aged (40-65) years who visited Al-Sadr Teaching Hospital and the Heart Center for the period from October 2019 till February 2020 . The sample has been divided into three main groups (30/group ) as follows : first group 40-45years , second groups 50-55 years , third group 60-65 years , and each of these three main groups was divided , also into two subgroups as follows : first subgroup ( 15 normotensive men ) ,second subgroup (15 hypertensive men ) .

The results revealed : testosterone (T) levels reduced significantly ($p \leq 0.05$) for all samples in different subgroups and groups during both the progressive age and hypertension . Both estradiol (E2 ) and prolactin (PRL) increased significantly ($p \leq 0.05$) for all samples in different subgroups and groups during both the progressive age and hypertension .

Tumor necrosis factor (TNF-α) , interleukin 6 (IL6 ) and C-reactive protein (CRP) levels increased significantly ($p \leq 0.05$) for all individuals in different subgroups and groups during both the progressive age and hypertension ( except the CRP levels between the normotensive groups) .

Nitric oxide ( NO ) decreased significantly ($p \leq 0.05$) (except third subgroup and between seconed and third hypertensive groups ) for all samples in different subgroups and groups during both the progressive age and hypertension .

Total cholesterol (TC) , triglyceride (TG ) , LDL cholesterol , VLDL cholesterol , increased significantly ( $P \leq 0.05$) and HDL cholesterol reduced significantly ($P \leq 0.05$) for all samples in different subgroups during both the progressive age and hypertension . TG raised significantly ($P \leq 0.05$) for all

samples in different groups during both the progressive age and hypertension , TC raised significantly ($P \leq 0.05$) for all sample in normotensive groups , HDL reduced significantly ($P \leq 0.05$) for all sample in normotensive groups during both the progressive age and hypertension . VLDL increased significantly ($P \leq 0.05$) for all sample in normotensive and hypertensive groups during both the progressive age and hypertension .

Total protein ( TP ) , albumin levels decreased significantly ($P \leq 0.05$ ) for all individual in different subgroups during both the progressive age and hypertension . Albumin declined significantly (probability $\leq 0.05$ ) for all samples in normotensive groups during the progressive age.

Besides that , Testosterone showed a significant ($P \leq 0.01$ and $P \leq 0.05$ ) negative or positive correlation with most of the parameters of the current study for normotensive and hypertensive men .

The physiological impacts of these findings were discussed , according to the influences of hypertension and progressive age , and the latest both two factors related with current hormonal , pro-inflammatory and biochemical findings .

Its concluded from the results of current study that Progressive age and hypertension considered as independent factor that influenced all the hormonal and biochemical parameter. Testosterone hormone playing a regulation role and has many impacts on the study parameters .

# Table of Contents

## List of Tables

## List of Figures

| List of Appendixes | |
|---|---|
| **Sequence** | **Title** |
| A | The Questionnaire paper used in this study . |
| B | Table of ELISA Kit contents . |
| B | Table of Interleukin 6 ELISA kit components |
| C | Table of Nitric oxide ELISA kit components . |
| D | Table of Electrophoresis protein (6) kit components. |

| List of abbreviations | |
|---|---|
| ADH | Antidiuretic Hormone |
| ADMA | Asymmetric dimethlarginine |
| ADP | Adenosine diphosphate |
| Ang II | Angiotensine II |
| ANP/ANF | Atrial natriuretic peptide |
| ART | Androgen replacement therapy |
| BH4 | Tetrahydrobiopterin |
| BP | Blood pressure |
| CAD | Coronary artery disease |
| cAMP | Cyclic adenosine monophosphate |
| cGMP | Cyclic Guanosine Mono Phosphate |
| CRP | C-reactive protein |
| D.W. | Distal Water |
| DBP | Diastolic blood pressure |
| DHEA | Dehydroepiandrosterone |
| DHT | Dihydrotestosterone |
| E2 | Estradiol |
| ED | Erectile dysfunction |
| EDRF | Endothelium derived relaxing factor |
| EH | Essential Hypertension |
| eNOs | Endothelial nitric oxide synthase |
| EPCs | Endothelial progenitor cells |
| ERβ | Estrogen receptor beta |
| ET-1 | Endothelin -1 |
| FAD | Flavin mononuclotide |
| GnRH | Gonadotropin-releasing hormone |
| HCG | Human chorionic gonadotropin |
| L1IRa | Interleukin 1 receptor antagonist |
| LDL | Low density lipoprotein |

| | |
|---|---|
| LH | Luteinizing Hormone |
| LPL | Lipoprotein lipase |
| LPS | Lipopolysaccharide |
| LV | Left ventricular |
| MCP-1 | Monocyte chemotactic protein 1 |
| NANC | Noradrenergic noncholinergic |
| NF-KB | Nuclear factor kappa B |
| nNOS | Neural nitric oxide synthase |
| NO | Nitric oxide |
| NOS | Nitric Oxide Synthase |
| ONOO- | Peroxynitrite |
| PAI 1 | Plasminogen activator inhibitor 1 |
| PDE5 | A phosphodiesterase type 5 |
| PRL | Prolactin |
| RAAS | Renin Angiotensin Aldosterone System |
| RCT | Reverse Cholestrol Transport |
| RNS | Reactive nitrogen species |
| ROS | Reactive oxygen species |
| SAA | Serum amyloid |
| SBP | Systolic blood pressure |
| SGC | Soluble guanylate cyclase |
| SHBG | Sex hormone bending globulin |
| sIL6r | Soluble IL-6 receptor |
| SIP | Sphingosine 1 Phosphate |
| STAR | Steroidgenic acute regulatory protein |
| T | Testosterone |
| TACE | TNF converting enzyme |
| TC | Total Cholesterol |
| TEMED | Tetra Methyl Ethylene Diamine |
| TG | Triglyceride |
| TNF α | Tumor necrosis factor-alpha |
| TNFR1α | Tumor necrosis factor-alpha receptor one |
| TNFβ | Beta Tumor Necrosis Factor |
| TP | Total protein |
| tPA | tissue Plasminogen Associated secretory phenotype |
| TRH | Thyrotropin-releasing hormone |
| VLDL | Very low density lipoprotein |
| VSM | Vascular smooth muscle |
| WHO | World Health Organization |
| ZIP14 | Zinc transport protein |

# Chapter One

# Introduction

## 1.Introduction :

"In human , aging is a continual and progressive process that results in decreased physiologic function across all organ systems" (Buford , 2016 ) .

Age advancement for along time counted as an independent harmful factor for a wide variety of disease states , such as cancer over all , Alzheimer's disease , diabetes , Parkinson disease (Belikov, 2019) , a heart and atherosclerosis , myocardial infarction , stroke and hypertension (Lakatta and Levy , 2003 ; North and Sinclair, 2012) . Furthermore , Lotti and Maggi (2018) , reported that the progressive age is associated with impaired male sexual and reproductive health . That means changes in the secretion of sex hormones (Van den Beld *et al.*, 2018) , especially testosterone hormone in men , which their levels are decreased with progressive age ( Harman *et al.*, 2001 ; Travison *et al.*, 2007 ) .

Androgen production begins to decrease around the age of 40 years , while a male at age 75 years has about half of the circulating free testosterone as a male does in his twenties (Bayer *et al.*, 2011) .

This deficiency is leading for some metabolic syndromes , such as diabetes type 2 , carotid intima media thickness , aortic and lower limb arterial disease , obesity , heart disease , chronic kidney disease , hypertension and erectile dysfunction (ED) ( Maranon and Reckelhoff , 2013 ; Hotta *et al.*, 2019) .

"Hypertension is a highly prevalent condition that dramatically rises in incidence with increasing age" ( Buford , 2016) , it represents the main hazard factor for myocardial infarction, stroke, and renal failure plus it is the head cause of unanticipated mortality everywhere in the world (Aflyatumova *et al.*, 2018) .

On the otherwise , androgen levels in hypertensive men be decreased and a close relationship between androgen levels and hypertension have been hypothesized in older men , therefore , low testosterone levels are associated with hypertension conditions during mid and old age ( Moretti *et al.,* 2017) .

Moreover , Hermann and his colleagues (2006) and Wu and his colleagues (2021) mentioned that hypertension is regulated by the nitric oxide (NO) molecule , and impaired NO bioactivity is an important component of hypertension .

Nitric oxide is a simple but pluripotent molecule, produced by nitric oxide synthase (NOS) in almost all kinds of mammalian cells, low-molecular-weight , highly water dissolve, free radical, it is remarkably reactive, easily creating nitrogen oxides, with a very brief half-life about five second and move only limited dimension were being oxidized (Levine *et al.,* 2012 ; Wu *et al.,* 2021) , predominantly manufactured in the vascular endothelium so it has long was recognized as an endothelium derived relaxing factor (EDRF) , a strong antioxidant , anti-inflammatory molecule , a vasodilator, effecting vascular tone blood pressure and hemodynamics , a character employed by nitrate donor treatment for angina , heart failure , pulmonary hypertension , ED (Levine *et al.,* 2012). NO production and NOS are regulate by testosterone levels and there are some reports showed that the low NO is associated with testosterone deficiency via altering the expression and the activity of NOS (Hotta *et al.,* 2019) .

Hotta and his colleagues (2019) and Mohamad and his coworkers (2019) considered that testosterone hormone has an anti-inflammatory action , and the low testosterone levels during hypertension condition were found to be associated with the increasing risk for systematic inflammation . Inflammation

occurs when there is an increase in the concentrations of inflammatory cytokines (Antonelli and kushners , 2017) .

These inflammatory cytokines included tumor necrosis factor (TNFα ) , interleukin 6 (IL6) and C-reactive protein (CRP) elevated in most , if not all inflammatory state (Bektas *et al.,* 2018) .

Bektas and his colleagues (2018) , mentioned that TNFα , IL6 and CRP increased with the progressive age (inflammaging ) , and with the decrease testosterone levels (Kupelian *et al.,* 2010) . Plasma proteins levels present reasonably expected changes in response to acute inflammation (O'Connell *et al.,* 2005) and these plasma protein levels be decreased with progressive age ( Tian *et al.,* 2014) .

**The aim of the study**

In view of this controversy this study aims to investigate the relationship between the testosterone hormone and nitric oxide , TNFα , interleukin 6 , C-reactive protein and other hormonal and biochemical parameters in elderly healthy and hypertensive men in Misan province associated with the progressive age and hypertensive men . This study will take into account the following parameters :

Hormonal parameters (Testosterone ( T) ,Estradiol ( E2) ,Prolactin (PRL)) , pro – inflammatory markers ( TNF-α, IL-6 , CRP) , biochemical parameters ( Nitric oxide, Lipid profile, Cholesterol ,Triglyceride , HDL-C, LDL-C, VLDL – C, total protein and its fractions albumin and globulins)

# Chapter Two

# Literature Review

# 2. Litrature Review :

## 2.1 : Testosterone : an overview

Arnold Berthold during the first experiment in the domain of scientific study of the behavioral endocrinology , in 1849 , was the first scientist who pointed that testes as the main secretion site for some chemicals that influencing the development of secondary sexual characteristics and aggressive behavior in male mammals ( Bird and Zilioli , 2017) .

Ernst Laqueur and his team in 1935, in Amsterdam isolated 10 mg of the chemical compound “testosterone” from 100 kg of bull testes , and at the same time , Butenandt and Hanisch (1935) , as well as Ruzicka and Wettstein (1935) , published the chemical structure of testosterone, this marked the beginning of recent clinical therapeutically and endocrine function of testosterone , and the physiology of male reproductive ( Nieschlag and Nieschlag , 2014) .

Testosterone is a C19 steroid hormone , that is produced mainly in the smooth endoplasmatic reticulum in leydig cells under the control of luteinizing hormone (LH) , and is also created in less amounts in the female gonad and the adrenal cortex (Handelsman , 2020) .

Gonadal signal testosterone production and release begins in the hypothalamus , specialized neurons produce and secrete gonadotropin releasing hormone (GnRH) , increases synthesis and release of the gonadotrophs , resulting in the creation of testosterone from its syntheses sources ( Vingren *et al.*, 2010 ) .

The system that connects signals from the hypothalamus to the pituitary gland and to the gonads is referred to as the hypothalamic-pituitary gonadal axis (HPG axis) , and the stimulation of this system depends on either a direct neurological effect by CNS or a reduction in the levels of Testosterone in the blood by negative feedback , testosterone will control GnRH secretion by

inhibiting the hypothalamus and the gonadotrophs in the anterior pituitary (Nassar and Leslie , 2018) .

The main substance in steroids building , including testosterone, is cholesterol , cholesterol side chain broken down by enzyme p450 in the inner membrane of mitochondria to produce pregnenolone , that metabolized by enzymes present in the inner endoplasmic reticulum into testosterone (Nieschlag *et al.* , 2012) .

Approximately 98% of the testosterone in plasma is bound to protein, with 65% being bound to a globulin called gonadal steroid–binding globulin (GBG) or sex steroid–binding globulin, and 33% to albumin ( Barrett *et al.*, 2010) , the free testosterone (FT) found in blood and the testosterone that binding to albumin is the bioactive fractions which is immediately ready for biological effect ( Kaufman and Vermeulen , 2005).

Many biological systems are linked to testosterone , including spermatogenesis, morphology, psychology, and behavior, all of which are vital for survival and reproduction (Bird and zilioli , 2017) , and its biological effects include promotion of secondary male-sex characteristics , in muscle , it induces protein synthesis (anabolic effect) and helps prevent protein degradation (anti-catabolic effect) ( Vingren *et al.*, 2010) , it has a potent on sexual potency, whether fertility, erection or libido , it also has an indirect effect on bone length through estradiol ( Belchetz *et al.*, 2010).

Testosterone is responsible for testicular descent , spermatogenesis, as well as other aspects of primary sexual , increases in hematocrit , causes voice change , additionally, 5-alpha-reductase can transform testosterone to dihydrotestosterone (DHT) , in contrast to testosterone, DHT is able to bind to and activate the same androgen receptors (ARs) inside cells, but DHT has a higher affinity and thus causes more male hair pattern (including facial, axial, and pubic hair), as well as increased sebaceous gland secretion , male pattern

balding , and acne , these both hormones induce puberty plus the male reproductive maintenance (Kalfa *et al.,* 2019).

The binding of testosterone to the intracellular androgen receptor (AR) causes it dimerization and bind latter to what it call hormone response element, a sequence in the DNA leading to expression of target genes (Chistiakov *et al.*, 2018) , this action is called the genomic or classic mode "Genomic Pathway" with its slow effect , where unbound testosterone can travel across the cell membrane, bind to the androgen receptor found in the cytoplasm of the cell, and latter migrate to the nucleus and activating or suppressing the gene , the other way is a non gemonic fast mode including the interaction between the hormone and membrane receptors , by these two ways androgens and even estrogens can use two different mechanisms to exert their effects (Michels and Hoppe , 2008 ; Bird and Zilioli , 2017 ).

In addition , in humans and other vertebrates , testosterone exerts its effects through a variety of pathways , including conversion to estradiol by the enzyme aromatase , and modulation of the estrogen receptors , or with the effect on its receptors as DHT (Bennett *et al.*, 2010).

Testosterone production levels be change by some factors , Huhtaniemi (2014) report Testosterone concentrations slowly decline in progressive age , which is called andropause or late-onset hypogonadism , beside that Vingren and his colleagues (2010) report that resistance training increases testosterone levels , while endurance training may end with a reduction in Testosteroene concentrations , fat cells synthesize the enzyme aromatase , which converts testosterone , into estradiol , so reduction in weight may lead to an increase in Testosterone concentration (Håkonsen *et al.*, 2011) . Furthermore Testosterone levels during rapid eye movement in sleeping increases (Andersen and Tufik , 2008) , and the victory in human competitive interactions stimulates intense testosterone-related feelings accompanied with its levels increase (Carré and

Putnam , 2010) , and natural man-made such as spearmint tea reduce testosterone levels (Akdoğan *et al.,* 2007).

In addition , it has been reported that the reduction in testosterone production in chemotherapy , hypothalamus-pituitary axis disorders , primary hypogonadism , cryptorchidism and orchitis , and Klinefelter and Kallmann syndrome (Bozzola *et al.,* 2018 ; Hauser *et al.,* 2018 ; Spaziani *et al.,* 2018) .

## 2.2 : The secretion of testosterone levels associated with progressive age:

The Fetal leydig cells (FLCs) which produce androgens by the sixth to seventh week of gestation , and reach their maximum maturition and develop around the 7 to 21 week of gestation (Svechnikov *et al.,* 2010) , these FLCs and endothelial cells travel to the gonads and produce testosterone , which supports the differentiation of the Wolffian duct into the male urogenital tract .

Testosterone be converted to (DHT) in the circulation and induces the formation of the prostate and penis , in addition, it is important to testicular descent , which occurs in the last two months of pregnancy (Nassar and Leslie, 2020).

In the age range of 7 to 10 years , boys and girls both have a steady increase in the levels of androgens and estrogens, which occurs before the sharp spike in these hormones at puberty , in addition , an increase in adrenal androgen secretion occurred in humans at the ages of 8–10 years in girls and 10–12 years in boys ( Barrett *et al.,* 2016).

Testosterone continues to influence the male traits, and production of sperm in men through the adulthood, in addition, it has other essential roles, such as erythropoiesis (production of red blood cells), stimulating growth factors IGF-1 and GH secretion, redistribution of body fat (Kelsey *et al.,* 2014; Ashton, 2018).

Testosterone's beneficial effects on libido, muscle increased mass , bone density, and a reduction in body fat have been proven with the opposed effected changes that happened with the progressive age as the concentration of this hormone decline (Snyder *et al.,* 2016 ; Wu *et al.,* 2010).

After middle age , there are gradual decreases in circulating testosterone as well as increases in gonadotropin and sex hormone–binding globulin (SHBG) levels (Matsumoto, 2002; Moreau *et al.,* 2020 ) , and these trends are clear in elderly men who , have many chronic illness , that impaired hypothalamic-pituitary testicular axis (HPA axis) function leading to reduce circulating testosterone levels during male aging ( MB , 2000).

Furthermore, the decline in testosterone levels with age reflects , in addition to a decrease in the number of testosterone-producing leydig cells, a decrease in the functional ability of the testes as well as hypothalamic control of gonadotropin production (Moreau *et al.,* 2020).

Nevertheless , many studies report unchanging in the testosterone levels associated with the progressive age (Atlantis *et al.,* 2009 ; Fukai *et al.,* 2010 ; Halmenschlager, *et al.,* 2011).

Many researchers mentioned the levels of testosterone during the different age in men . Moreau and his colleagues ( 2020) showed that (after the age of thirty) the amount of testosterone decreases by 1%, about 20% in men aged ≥ 60 year and 50% in men aged ≥ 80 year in comparison with young men . Erenpreiss and his colleagues (2019) has reported that (after age of 40 years) , men testosterone concentration decreases about 1% -1.6% by a year, SHBG levels increase by 1.3% by a year, and free testosterone (FT) levels decrease by 2.8% by a year .

## 2.3 : Hypertension as a risk factor for the levels of testosterone hormone:

" Blood pressure is the amount of force exerted by the blood on the inside of the arteries as the blood is pumped throughout the circulatory system , each time the heart muscle contracts , blood is pressed against the walls of the arteries and is measured as systolic , the top number in blood pressure reading , when the heart relaxes between beats , the pressure on the artery wall eases , measured as diastolic blood pressure "(Casey *et al.,* 2006) .

There are several mechanisms through which the body regulates the blood pressure : Baroreceptor which are sensitive to the vessel expansion , Antidiuretic Hormone (ADH) , Angiotensin II , and Renin-Angiotensin-Aldosterone System (RAAS) , arterial pressure regulation to keep enough perfusion pressure for the body's tissues and organs , but not so high enough pressure to harm them , where body may enter a state of chronic hypertension (Oparil *et al.,* 2003) .

Hypertension is considerd when the systolic blood pressure (SBP) is $\geq$ 140 mm/Hg and diastolic blood pressure (DBP) $\geq$ 90 mm / Hg according to the seventh report of the Joint National Committee (JNC7) guideline and WHO organization (Chobanian *et al.,* 2003 ; WHO , 2013) . Cardio vascular disease is the world's most significant avoidable cause of early death , and hypertension is one of them (WHO, 2009).

Ischemic heart disease , strokes , peripheral artery disease , and other cardiovascular disorders , such as heart failure , diffuse atherosclerosis , chronic kidney disease , atrial fibrillation , and pulmonary embolism , cognitive dysfunction and dementia are companion with increasing the blood pressure ( Lewington , 2002 ; Lau *et al.,* 2017 ; Sierra , 2020).

The essential hypertension comprises more than 95% of instances of hypertension , many pathophysiologic factors have been implicated in the genesis of essential hypertension : increased sympathetic nervous system activity , in response to psychosocial stress , overproduction of sodium-retaining hormones and vasoconstrictors , long-term high sodium intake , inadequate dietary intake of potassium and calcium , increased or inappropriate renin secretion with resultant increased production of angiotensin II and aldosterone .

More than that deficiencies of vasodilators , such as prostacyclin , nitric oxide , and the natriuretic peptides , alterations in expression of the kallikrein–kinin , abnormalities of resistance vessels , including selective lesions in the renal microvasculature , diabetes mellitus , insulin resistance , obesity , increased activity of vascular growth factors , alterations in adrenergic receptors that influence heart rate, inotropic properties of the heart , vascular tone , and altered cellular ion transport (oparil *et al.*, 2003). (Figure : 2.1)

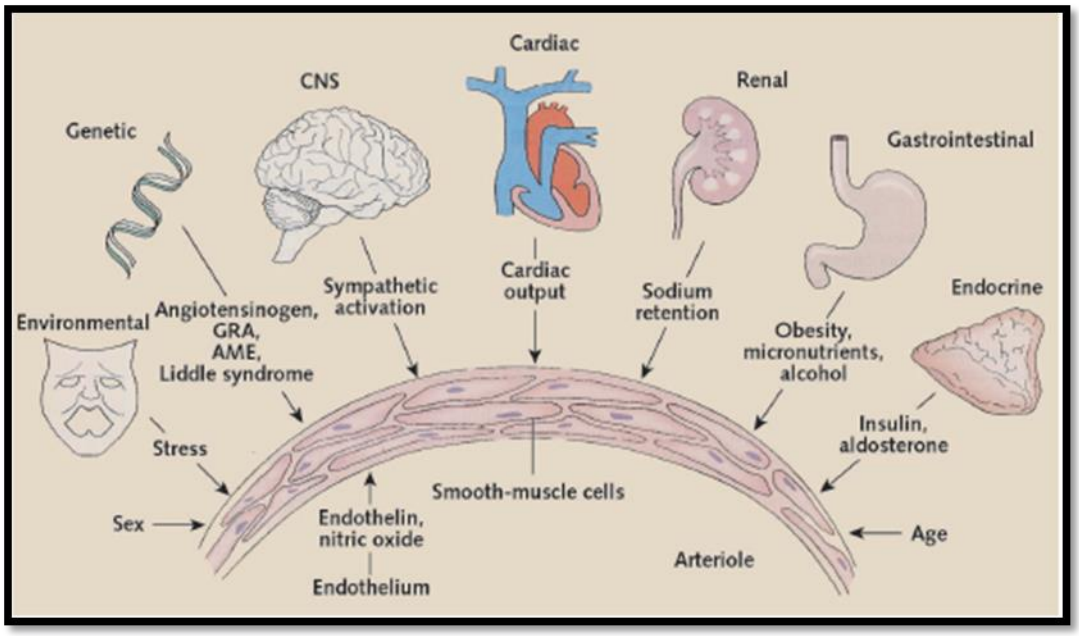



Figure (2.1) : Pathophysiologic mechanism of hypertension .

**(Oparil *et al.* , 2003)**

Researchers believe that the idea that structural and functional abnormalities in the vasculature precede hypertension and help to drive its development has gained traction (oparil *et al.*, 2003) .

Erectile dysfunction (ED) is common in hypertensive men , and the prevalence of both arterial hypertension and erectile dysfunction rises with age , available evidence suggests that blood pressure regulation improves erectile function (Doumas *et al.,* 2016 ) .

Erectile dysfunction has long been thought to be an early indicator of cardiovascular risk , signaling the existence of vascular disease before typical clinical symptoms of atherosclerosis (Javaroni and neves , 2012).

Contraction of endothelial smooth muscle depends on hormones, neurotransmitters, and endothelial productions in the circulation and in the penis , and disturbing these factors may lead to contracting in vascular smooth muscle (VSM) leading to high blood pressure and / or ED , hypertension may also cause erectile dysfunction as a result of the treatment that is used to reduce high blood pressure , even so an increased study reported that erectile dysfunction precedes high blood pressure , and that some of the treatments used for erectile dysfunction can enhance the blood pressure , (Nunes *et al.,* 2012) . Both ED and low testosterone (hypogonadism) increase with age (Mulligan *et al.,* 2006).

Erectile dysfunction , low testosterone concentration and metabolic and cardiovascular diseases are now recognized to be related to each other and to patient sickness and death (Corona and Maggi, 2010).

In the elderly, total testosteroen concentration are negatively linked to systolic pressure and raise morbidity within the next twenty years, regardless of health status (Moretti *et al.,* 2017) .

Many studies mentioned that the decrease in testosterone is associated with hypertension ( Torkler *et al.*, 2011; Firtser *et al.*, 2012 ; Moon *et al.*, 2017) , Laughlin and his colleagues (2008) mentioned eight percent of testosterone to be reduced in its levels in hypertensive men , furthermore , Torkler and his colleagues (2011) show the benefit of testosterone demonstrated in reducing cardiovascular events , by contrast , other studies found no association between testosterone and high blood pressure (Zmuda *et al.*, 1997 ; Blaya *et al.*,2016).

From other side , lower levels of testosterone were seen as having their own cardiac danger ( Jones , 2010 ) , while Araujo and his colleagues (2011) , suggested that the general health status of a person with cardiovascular disease leads to a decrease in their testosterone level , but this does not negate the beneficial effect of testosterone on the risk factors associated with CV disease, such as increased inflammation , obesity and insulin resistance , which can lead to a greater decrease in the amount of testosterone later (Kelly and Jones , 2013) and this may lead to the assumption that the lack of testosterone is the cause of disease generation and not vice versa (Maranon and Reckelhoff , 2013).

The mechanism that explains the importance of sex hormones in regulating blood pressure is not well known (Moretti *et al.*, 2017).

The vasodilation effects for testosterone on the smooth muscle are most likely mediated by inhibition of dihydropyridine channels (Hall *et al.*, 2006) . Furthermore , various cell types, including endothelial cells and vasculature myocytes, have been shown to have sex steroid receptors (Orshal and Khalil , 2004) , an intracoronary injection of Testostoerne lead to coronary artery dilation ( Wu and Eckardstein , 2003) , besides that , Zitzmann and Nieschlag (2007) , show that testosterone used as a therapy had a good effects on blood pressure in human . Androgen suppressive therapy increased the blood pressure

but not significantly (Braga-Basaria *et al.*, 2006) .The radial artery found to be vasodilat in responding to testosterone at physiological levels in patients had by pass surgery (Kelly and Jones, 2013) . Testosterone may be have antihypertensive action by triggering the dilation of aorta in rat plus rabbit animals ( Perusquía *et al.*, 2017).

## 2.4 : Relationship between testosterone , inflammation and nitric oxide

It is crucial to understand the relationship between testosterone and inflammation since inflammation is a significant pathogeneses of several illnesses ( Mohamad *et al.*, 2018) ( Figure 2.2).

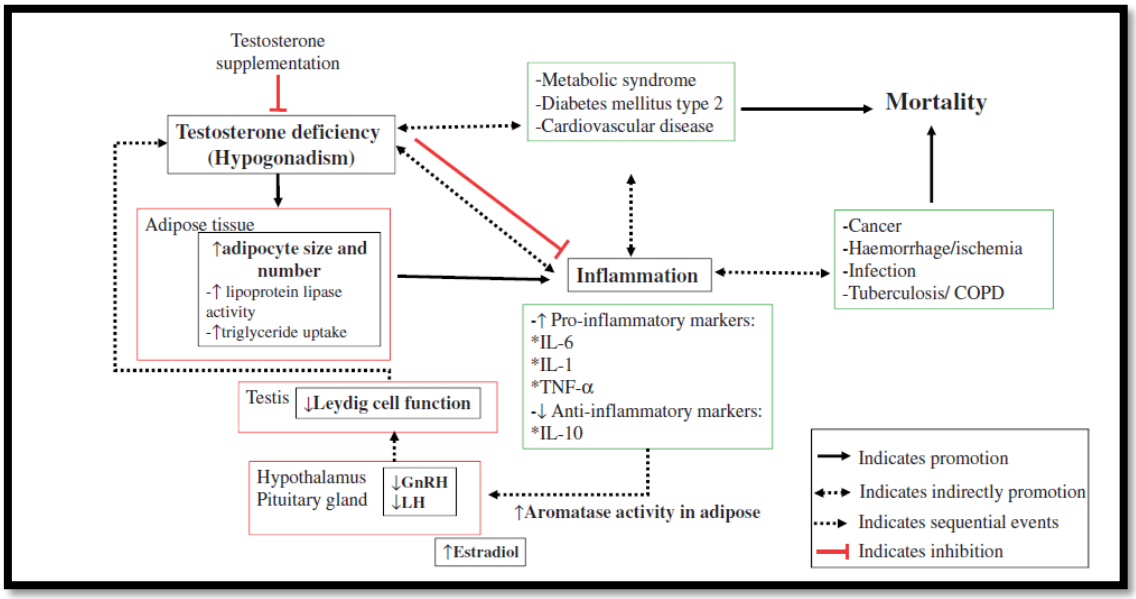



Figure (2.2) : Interaction between testosterone and inflammation

(**Mohamed *et al.*, 2018**)

In men , many studies refers to the importance of testosterone in modulating the inflammation (Mohamad *et al.*, 2018 ) , and many research investigations have demonstrated that a modest pro-inflammatory state is found

in older individual due to lower levels of circulating androgens such Dehydroepiandrosterone (DHEA) (Maggio *et al.,* 2006).

" Low Testosterone levels in men were significantly associated with high level of inflammatory markers in different clinical conditions such as obesity, metabolic syndrome, heart failure , healthy elderly population, carotid atherosclerosis, hypogonadism , type 2 diabetes " (Bianchi, 2019).

Kupelian and his colleagues (2010) , found a strong inverse relationship between testosterone and CRP levels, with these results, it adds to the previous research which has proven that androgen can impact cardio metabolic hazards and related illnesses including metabolic syndrome, diabetes, and CVDs via regulating inflammatory processes .

A study conducted on men with metabolic syndrome confirmed the inverse relationship between testosterone and CRP ( Laaksonen *et al.,* 2003 ; Kupelian *et al.,* 2010) .

It has been refered to the beneficial effects of testosterone treatment in reducing in the concentration of inflammatory cytokines in men suffer of testosterone deficiency, in addition , visceral fat also contributes to this decrease by increasing the process of aromatization and the production of this fat for inflammatory cytokines , which may affect the production of SHBG , luteinizing hormone and testosterone (Moretti *et al.,* 2017) .

Furthermore , a few studies found a negative correlation between the reduction in testosterone concentration and IL6 (Maggio *et al.,* 2006 ; Tremellen *et al.,* 2017) and TNFα (Bobjer *et al.,* 2013).

There are studies also showed that cytokines , such as (IL6 ), (TNFα) could suppressive testosterone secretion by effecting on the HPG-axis pathway (Malkin *et al.,* 2004 ; Norata *et al.,* 2006). Nevertheless , the processes underlying the links between steroid sex hormones and inflammatory

biomarkers are not well known , however there is proof that androgens have an immunosuppressive impact as autoimmune disorder is more common in androgen-deficient men (Tengstrand *et al.*, 2002 ). It has been suggested that the mechanisms for the immunosuppressive effect of androgens could be either a direct effect on the expression of inflammatory genes , or an indirect effect through inhibition of NF-kB activation (Tsilidis *et al.*, 2013) .

In both women and men , elevated in androgen level reduces adipose tissue accumulation and improves insulin cell sensitive and glucose tolerance , then in the reduction of fat mass, which responsible of many inflammatory factors production , the testosterone administration has the primary anti-inflammatory benefit(Bianchi,2019)(Figure2.3).

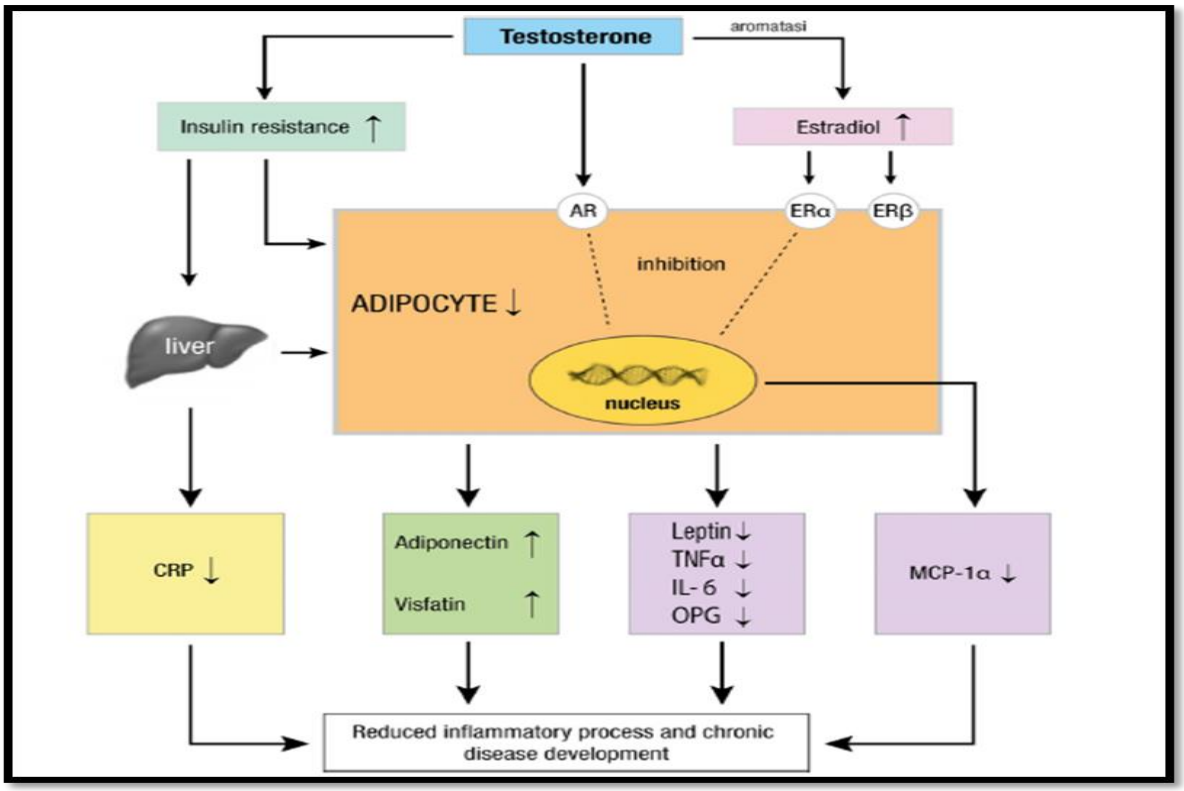



Figure (2.3) : Testosterone exerts its anti-inflammatory activity through different mechanisms ( **Bianchi , 2019**)

More ever , even in studies that have conducted on men with stable coronary artery vascular disease (CAD) have noticed an opposite relationship

between testosterone blood concentration and inflammatory markers, in young men , and older men (Mohamad *et al.,* 2018).

In contrast , the relationship between testosterone level and inflamatory markers results have not been consistent , Nakhai-Pour and his colleages (2007) show a negative relationship of total testosterone with Soluble Interleukin-6 Receptor (sIL6r) but not with IL6, TNFα, and other studies have found that treatment with testosterone has reduced the levels of TNFα and IL1β while leaving CRP unchanged ( Malkin *et al.,* 2004 ; Nakhai-Pour *et al.,* 2007; Kupelian, *et al.,* 2010).

In other hand , many studies have been done to find the relationship between testosterone and endothelial function , Moreau and his colleagues (2020) mentioned that low serum testosterone associated with both reduced , and higher macro- and microvascular endothelial function in men of various ages , and also, show that improvements in endothelial function with testosterone treatment were conducted in middle and older aged men , whereas in younger hypogonadal males, studies that documented endothelial function impairments after testosterone therapy were performed (Zitzmann *et al.,* 2002 ; Sader *et al.,* 2003 ; Bernini *et al.,* 2006 ) .

It was proven in clinical and fundamental investigations that endothelial progenitor cells EPCs play a significant part in the endothelium overhaul system, and it was established that testosterone levels are correlated with EPC levels (Hotta *et al.,* 2019) .

Accordingly , Hotta and his colleagues (2019) found that testosterone regulated the production of nitric oxide synthase (NOS) and NO . Testosterone improve endothelial NO synthesis across various pathways and also increases NO activity directly in endothelial cells while plasma concentrations are at physiological amounts (Li *et al.,* 2019) .

Human endothelial cells cultured in vitro exhibit increased nitric oxide (NO) synthesis in response to physiological concentrations of testosterone through activation of rapid signaling pathways which not depend on nucleus and $Ca^{+2}$ influx, which induces NO release and this the last factor is induced vasodilation for that it is considered as therapeutically targeted in hypertension and angina (Kelly and jones , 2013).

Androgen deficiency results in a decrease in neuronal NOS expression, which is attributed to a reduce in intracevernosal pressure in penile arteries during erection, but after administration of testosterone the production of NO are increasing ( Kelly and Jones , 2013).

In addition to direct effects on NOS expression , Testosterone may also affect A phosphodiesterase type 5 (PDE5) production , a vasodilator preventing enzyme controlling the degradation of cGMP (Morelli *et al.,* 2004) . Inhibiting the PDE5 is one of the most important ways to treatment erectile dysfunction (Andersson, 2018) .

While low levels of testosterone are associated with erectile dysfunction and Cardiovascular diseases (CVDs ), it has been found that a high concentrations of estradiol causes erectile dysfunction and testosterone replacement has no effect with this high levels (Hotta *et al.,* 2019).

In patients with ED, there is a positive correlation between free testosterone (FT) levels and the compliance of cavernous arteries of the penis, suggesting an involvement of circulating androgens in the regulation of intrapenile vasodilatation , and important effect of high enough concentration of Testosterone is by NOS expression to produce NO in the penis (Aversa *et al.,* 2000; Kelly and Jones , 2013).

Park and his colleagues (1999) reported that in rat penile tissue, castration reduced nNOS and eNOS expression , resulting in ED. In men , testosterone

lowering levels reduced endothelial NOS production in the penis , according to some studies , Skogastierna and his colleagues (2014) cleared that low testosterone levels decreased eNOS mRNA expression and NO excretion levels in urine, meaning that testosterone reduction possibly decreased NO activity by effecting the action of NOS (Hotta *et al.*, 2019).

Sphingolipids might have revealed one of the mechanisms that explained the testosterone control eNOS expression beside its activation , as this relationship between testosterone concentration and VSM dilation via sphingolipids was reported by Yin and his colleagues (2018) on his trail concerning the castration in rats found first that the reduction in Sphingosine 1 Phosphate 1 (S1P1) which modulate the relaxation of Smooth muscle cells (SMCs) of blood vessels by activating the releasing of NO from endothelial cells , and second increased S1P2 and S1P3 which related to the SMCs contraction wher it found in the pains , associated with smooth muscle contraction receptor mRNA exhibit levels in the rats corpus cavernosum (Yin *et al.*, 2018 ; Hotta et al, 2019) .

Furthermore, Wang and his colleagues (2014) showed that S1P2 and S1P3 levels increased in the corpus cavernous for rats with hypertension , whereas endothelial NOS (eNOS) have been reduced . Finally, testosterone probably involved in regulating S1P levels in endothelial cells in the corpus cavernous and its decreasing in amount might make endothelial dysfunction effecting the action of eNOS and S1P ( Hotta et al, 2019) (Figure 2.4).

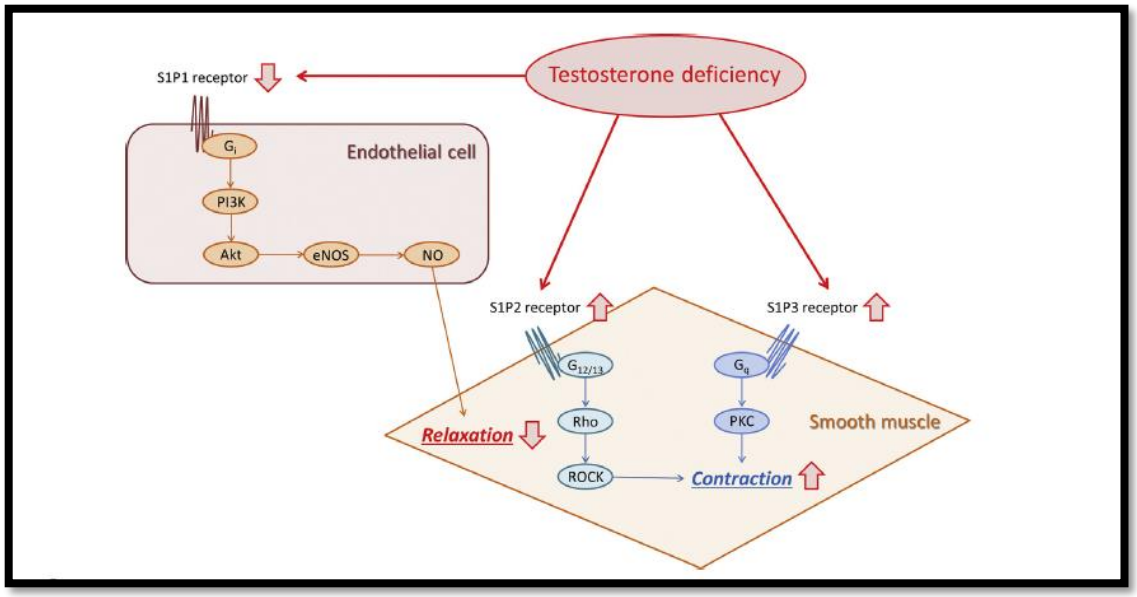



Figure (2.4) : The association between testosterone deficiency and smooth muscle relaxation via sphingolipids

**( Hotta et al., 2019**)

## 2.5: **Inflammation : an overview and some of their markers (TNFα , IL6 , CRP)**

Inflammation is a restrict to a localized response to tissue injury , or infection which help to tissue healing and /or the removal of the harmful agent , acute inflammation , which is traditionally marked by " pain , burning , redness , swelling , and loss of function" , is normally be clear in a short period of time to allow tissue function regeneration (Buford, 2016).

Excessive inflammation plays a major role in the development and/or initiation of stress-related diseases, as it were identified to be in many chronic illnesses, including cardiology and metabolism disease , psychotic neurological disease, and cancer, as an molecular origin that is critical in their development (Liu *et al.*, 2017).

It is considered to be inflamed when there is an increase in the quantities of mediator of the innate immune response components such as inflammatory

cytokines, or the transcription factor NF -KB , in addition , it is now widely accepted that even mild increases in CRP levels indicate inflammation ( Antonelli and Kushner, 2017).

"Cytokines are small secreted proteins released by cells have a specific effect on the interactions and communications between cells" (Zhang and An , 2007) , they are cell-signaling group with low weight for its muscular and its extracellular polypeptides / glycoproteins , produced by different cells of immunity such as T cells, neutrophils and macrophages , have an ability to stimulate and regulate immune response (Ferreira *et al.,* 2018).

Depending on their actions , the cytokines can be classified as pro-inflammatory cytokines, produce inflammation, endogenous pyrogenic, and chemoattractant functions, as well as being able to influence the inflammatory reaction , and the cytokines which are classified as anti-inflammatory , can inhibit the pro-inflammatory effects , thus , reducing the inflammation (Tsounis *et al.,* 2014). The equilibrium of pro – inflammatory with anti-inflammatory cytokines is thought to be more important than the concentrations of pro-inflammatory cytokines alone (Peeters *et al.,* 2001).

Pro and anti-inflammatory cytokines are released at the site of inflammation and promote the influx of lymphocytes , neutrophils , monocytes , and other cells that help with antigen removing and healing , this known as the acute-phase reaction , is a feature of the site inflammatory reaction and companied with systemic response (Brüünsgaard and Pedersen , 2003).

Cytokines are redundant in their actions, meaning similar functions can be triggered by different cytokines , they are often produced in a cascade , in which one cytokine trigger cells to manufacture more cytokines , cytokines can also act synergistically or antagonistically (Zhang and An , 2007) .

Cytokines superfamily include : "chemokines, interferons (IFN) , interleukins (IL), lymphokines and tumor necrosis factor (TNF)" (Zhang and An , 2007).

The abbreviation TNF-alpha stands for tumor necrosis factor, commonly known as cachexin or cachectin , its activity was observed to be increased following the action of immune system cells, and this was used to establish the superfamily of TNFα (commonly named as TNF) and its receptors , TNF superfamily comprises over 40 members, including a lot of cytokines and membrane proteins , the binding of this family of cytokines with their receptors initiates especially inflammatory action ( Ferreira *et al.*, 2018).

Tumor necrosis factor is a type II trans-membrane protein with a molecular weight of 27 KD (233 amino acids) that is cleaved by the metalloprotease TNF-converting enzyme (TACE) into a homotrimer with a soluble pyramid shaped structure (17 kD,157 amino acids) , with the remaining 76 amino acids acting as an anchor for binding the precursor protein to the membrane(Schultz ,2018 ).

Tumor necrosis factor alpha , which was thought to be a circulatory factor that may cause tumor necrosis, has subsequently been discovered to be a critical regulator of the inflammatory response, cell death, survival, differentiation, proliferation, and migration (Minciullo *et al.*, 2016) . TNF α and IL1 , both are first cytokines which trigger to produce of the rest mediator of acute-phase response in the experimental studies ( Brüünsgaard and Pedersen, 2003) , for that they are referred to as pro-inflammatory cytokines , and they are triggering the release of IL 6 (Ferreira *et al.*, 2018).

Tumor necrosis factor alpha is formed by a number of cells , the principal synthesizers being monocytic cells including " macrophages, astroglia, microglia, langerhans cells , Kupffer cells, and alveolar macrophages, as well as neutrophils, mast cells, eosinophils, and NK cells, T-lymphocytes, dendritic cells, endothelial and epithelial cells" ( Schultz , 2018 ) .

Tumor necrosis factor alpha works by two transmembrane receptors: TNF receptor one (TNFR1/p55 or p60) , and TNF receptor two (TNFR2/ p75 or p80) (Schultz , 2018) . Parameswaran and Patial (2010) reported the TNFR1 expressed in most kinds of cells , whereas TNFR 2 is typically expressed in the cells of the immune system, and TNFα binds to both with high affinity , however, both TNFR1 and TNFR2 can be separated from the cell membrane by the metalloproteinase enzymes as a reflection to inflammatory signal.

Tumor necrosis factor alpha is a potent pro-inflammatory cytokine that controls several aspects of macrophage activity , it is rapidly released after trauma , infection , or exposure to bacterial-derived lipopolysaccharide (LPS) and has been shown to be one of the most abundant early mediators in inflamed tissue, and it has been consider the master of regulation for the other cytokines that are involved in inflammation condition (Parameswaran and Patial, 2010 ).

Moreover , Schultz (2018) pointed that excess of TNFα release causes inflammation , locally or systemically , and there are signs and symptoms including : " fever , redness, and swelling , painful , and It is capable of causing death to a variety of cells ranging from normal to cancer cells , thus TNFα can play a part in cell proliferation , apoptosis, lipid metabolism and coagulation (formation) of blood clots".

Tumor necrosis factor alpha also stimulates vascular endothelial cells to display adhesion mulculars for the purpose of recognizing immune cells (Sedger and Mcdermott , 2014).

Interlukines (ILs) are a large group of immunomodulatory proteins that induce a broad range of responses in cells and tissues , these cytokines , which include a large number of recognized immunological ‘second messenger' molecules in mammals , modulate development , differentiation , and activation during an immune response , and contain cytokine groups and their closest homologues (Brocker *et al.,* 2010).

Interleukin 6 is a glycosylated protein of 21–28 kDa and has the typical four-helix bundle structure characteristic for all IL6 type cytokines (Scheller *et al.*, 2011) , a multifunctional cytokine , that regulate the responsiveness of acute phase and immunity , hematopoiesis , working as pro-and anti-inflammatory cytokines , releasing by endothelial cells, fibroblasts, monocytes, and macrophages as a consequence to many motives in systemic inflammation (Minciullo *et al.*, 2016).

Interleukin 6 is secreted from white adipose mass (about 1/3), and secreted also by muscle and liver (Bianchi, 2019).

After IL6 is produced in a local lesion during the early stages of inflammation , it travels across the bloodstream to the liver , where it causes a rapid activation of a wide variety of acute phase proteins such as C-reactive protein (CRP) , serum amyloid A (SAA) , as well as a decrease in the synthesis of fibronectin , albumin , and transferrin ( Tanaka *et al.*, 2014 ).

When IL 6 reaches the bone marrow, it stimulate megakaryocyte maturation, causing the release of platelets (Senchenkova *et al.*, 2013).

"In acute inflammation, IL 6 promote the expansion and activation of T cells and the differentiation of B cells , fever , activation of the hypothalamic-pituitary-adrenal axis, anorexia, and lethargy" (Maggio *et al.*, 2006).

The anti-inflammatory action of IL6 limits the inflammatory response by suppressing TNF alpha and IL1 beta action and improving the synthesis of IL 1 receptor opponent (IL 1 Ra) and soluble TNF receptor p55, and IL6 limits neutrophil uses, favoring their place taken by mononuclear cells (Maggio *et al.*, 2006).

C-reactive protein is, a 115-KD a pentamer protein , produced by liver synthesized under the control of IL 6 in hepatocytes (Nehring *et al.*, 2020 ), although it could further be provided in other places such in the kidney and

adipose tissue, where there is an innate, non-specific immune response to several disease markes conditions such as inflammation, infection, apoptosis, ( Tsounis *et al.,* 2014), the forming of CRP extrahepatic tissues has been described in neurons, atherosclerotic plaques, monocytes, and lymphocytes (Black *et al.,* 2004).

It is the usual steady inflammatory marker which has no change in its concentration between day and night, making it the usually marker that has been studied in cardiovascular condition, it also has several influences on the vascular endothelium , while it minimizes the creation of nitric oxide (NO), prostacyclin and plasminogen activator (Tsounis *et al.,* 2014).

C-reactive protein has both pro-inflammatory and anti-inflammatory properties, it aids in the identification and elimination of external pathogens and damage infected cells , it can stimulate the classic complement pathway plus stimulates phagocytic cells to facilitate this elimination , it can further make the tissue more worse damage in some circumstances ( Nehring *et al.*, 2020).

C-reactive proteinis involved in the induction of anti-inflammatory cytokines in circulating monocytes and the suppression of pro-inflammatory cytokine production by tissue macrophages (Brüünsgaard and Pedersen, 2003).

There are numerous causes of an increased C-reactive protein, include acute and chronic diseases, infectious and non-infectious causes, infectious are the common ones for elevated CRP levels (Vanderschueren *et al.,* 2006). Elevate CRP levels can be caused by trauma, but perhaps more modest peaks are associated with a broader range of pathophysiology, ranging from poor sleep to periodontal disease (Nehring *et al.,* 2020) .

In human, plasma levels of CRP can increase very fast and markedly after an acute inflammatory stimulus, largely reflecting the increased expression of

its gene in hepatocytes, plus production of many plasma proteins are increasing, whereas albumin is decreased (Black *et al.,* 2004).

## 2.6 : The relationship between inflammation , aging and hypertension

" The aging process is driven by interrelated mechanisms that lead to the emergence of characteristic phenotypes that include changes in body composition, energy production and utilization imbalance, homeostatic dysregulation, and neurodegeneration and loss of neuroplasticity" (Bektas *et al.,* 2018 ) .

Chronic inflammation, which occurs during aging, shows in a low-grade manner for an extended time, is causally linked with alterations in the cellular redox state and apoptosis pathways, where cytokines and chemokines responsible for the principal role in the rise of chronic inflammation and the process of senescence for the immunity (Woods *et al.,* 2012) .

Currently, there are two major hypotheses about age-related inflammation: molecular inflammation (Chung *et al.,* 2006), and inflammaging (Franceschi, 2007) .

Molecular inflammation was first proposed in 2002, based on molecular alterations in inflammation-related transcription protein and the representation levels of their genes, the vulnerability of the transcriptional factor NF-kB to oxidative stress plus alterations in redox equilibrium gives conform to this idea ( Chung *et al.,* 2019) .

Inflammaging means the association between the increase in cytokines responsible for inflammation and aging, it was first termed by Franceschi and his group at the beginning of the twenty-first century ( Franceschi and Campisi, 2014). This concept pronounces that activation of the aged natural immune system leads to a dysregulation in inflammation that reduces the capacity to

induce a potent innate and adaptive immune program when effected by antigens or environmental motives ( Baylis *et al.*,2013).

Inflammaging leading to tissue degeneration, and serves as a static hazard agent causing morbidity and death in the aging (Sanada *et al.,* 2018 ) .

Brüünsgaard and Pedersen ( 2003), have proposed that the raised in the production of cytokines both in healthy and older individuals result from obesity and insulin resistance, and changes in circulating sex hormone concentrations, aging, genetic programming, environmental plus lifestyle factors.

"The number of senescent cells in various organs increases with age, and these cells secrete several inflammatory cytokines, resulting in low-grade inflammation, this phenotype of senescent cells is known as the senescence-associated secretory phenotype or SASP, and it has been suggested as the key source of inflammaging in both aging and age-related diseases like atherosclerosis, cancer, and diabetes" ( Sanada *et al.*, 2018) .

The inflammatory markers that are more persisting to related with aging "inflammaging" are the raised circulating levels of IL 6, CRP, and TNFα (Howcroft *et al.,* 2013; Morrisette-Thomas *et al.*, 2014; Buford, 2016 ; Bektas *et al.,* 2018) .

Even in the absence of chronic disease, (2-4) fold increases in levels of pro-inflammatory markers like IL6, TNF, acute phase proteins such as CRP and (SAA) are common in the old when matched to the young ( Woods *et al.*, 2012) , which may induce muscle atrophy and cancer through DNA damage, visceral fat tissue from obese individuals can also produce both IL 6 and TNF α, affecting systemic metabolism (Sanada *et al.*, 2018 ) .

With progressive age, the capacity to overcome inflammation going to be reduced, resulting in persistent tissue leaking out of leukocytes and the

continuous release of pro-inflammatory cytokines and chemokines (Buford, 2016).

High levels of TNF have been related to frailty, a severe loss of muscle strength, the hazard of cerebrovascular plus cardiovascular diseases, and a higher rate of cognitive impairment in old people (Minciullo *et al.*, 2016).

Interleukin 6 expression is typically low in the absence of inflammation, and serum levels are generally undetectable; but, as people grow older, serum levels become detectable, and there is clear data that IL 6 serum concentration rises by aging, which may suggest a problem in the regulation of gene expression for this particle (Palmeri *et al.*, 2012) .

Interleukin 6 was proposed as a strong marker for function impairment, as well as a predictor of morbidity and fatality in the aging (Giovannini *et al.*, 2011), and IL 6 concentration are related to the origin of frailty, poor physical achievement, lack of muscle control, cognitive impairment, cardiological, neurological, and vascular events, as well as the genesis of cancers, with the cardiac remodeling in heart failure, and with the risk of community-acquired pneumonia requiring hospitalization (Minciullo *et al.*, 2016 ).

Studies indicate that a defect in the production or disposal of oxygen free radicals is what contributes to the increased production of IL6 (Maggio *et al.*, 2006).

Inflammation dysregulations with progressive age are present in more if not all living organisms, however, the exact causes of this dysregulation remain unclear, in normal circumstances, inflammation is beneficial to health since it aids organisms in battling microbes and plays important roles in organ repair and maintenance, as it rises when required and fades when not, however, when the inflammation lasts for a long time for some reason represented by a defect in the immune system or the inability to get rid of inflammation, then a disease

is generated as a result of the accumulation of damage such as obesity, cardiovascular disease, and neurodegenerative diseases (Sanada *et al.,* 2018) .

While several studies have shown that inflammatory biomarkers are increased in elderly individuals in the absence of clear disease, other studies have not demonstrated any changes in inflammatory markers in healthy older individuals, this difference may be attributed to disparities in the subjects' actual health status in these studies, but taken together, these findings demonstrate the dynamic relationship between aging, inflammation, and chronic diseases ( Woods *et al.,* 2012) .

Finley Brüünsgaard and Pedersen (2003) concluded that increased low-grade inflammatory activity in older people may either cause or indicate age-related disease, allowing it to be used as a disease marker .

Increasing evidence indicates that hypertension is a chronic inflammatory state; however, whether inflammation triggers hypertension or hypertension causes systemic inflammation is uncertain (Tanase *et al.,* 2019).

Hermann and his colleagues (2006) and Chuang and his coworkers (2013), concluded that inflammation may increase the hazard of diseases in particular hypertension, and to be the inflammation as a useful marker for identifying groups at high risk of hypertension .

The inflammatory process during high blood pressure is a complicated immune response including interactions between macrophages and T lymphocytes, which appears in the developed production of adhesion molecules, cytokines, matrix metalloproteinases, and growth factors (Quiroz *et al.,* 2012 ; Schiffrin, 2013) . Besides that, many researchers believe that hypertension causes inflammation by increasing the expression of inflammatory mediators such as endothelin-1 and angiotensin II, apart from its function in vasoconstriction and salt and fluid homeostasis, angiotensin II also

plays a role in vascular inflammation and oxidative stress (Wadley *et al.,* 2013; Rubio-Ruiz *et al.,* 2014) .

Angiotensin II raises NF-KB dependent gene creation and arouses the expression of many inflammatory molecules such as chemokines, cytokines, plus adhesion molecules and by initiating NADPH oxidase, increases the formation of ROS (Tsounis *et al.,* 2014). Angiotensin-converting enzyme inhibitors lower the expression of IL 6 , adhesion molecules, and selectins in sufferers of high blood pressure, enhance the endothelial role , and suppress plasma aldosterone production (Nakamura *et al.,* 2009 ) .

Dinh and his colleagues (2014) , reported that the strongest link between "inflammation / oxidative stress and hypertension" look like to be vascular dysfunction .

Since inflammation is one of the main mechanisms underlying endothelial dysfunction , its has major role be in atherosclerosis and other CVDs including hypertension ( Guarner and Rubio-Ruiz, 2015) .

The endothelial layer of the vessels produces nitric oxide in its natural state to prevent leukocyte adhesion, and at the same time, some research found that the released NO induces smooth muscle rest and vasodilation (Buford, 2016) .

Otherwise , complex immune reactions are caused by metabolic/chemical, mechanical, or infectious endothelial aggressions, leading to a pro-inflammatory state , which causes endothelial dysfunction and atherosclerosis through ROS, a downstream product of cellular and soluble immune factors (Agita and Alsagaff, 2017) , accordingly , ROS caused pro-inflammatory cytokine secretion, raised IL6 expression and decreasing NO availability, IL6 trigger high blood response to angiotensin II infusion even in normotensive individuals (Tanase *et al.,* 2019) .

Owing to an imbalance between vasodilatory and vasoconstrictor agents, endothelial dysfunction leads to resistance of systemic vascular, and thereby raises blood pressure (Chrissobolis *et al.*, 2011), yet, endothelial dysfunction provides to further worsening inflammation and oxidative stress, producing an even larger bad cycle (Buford, 2016) .

In addition , as a consequence of aging and hypertension, the endothelium releases other vasoactive elements , all of which offer to weakened endothelium-dependent vasodilation, plus, NO leads to reacting with oxidants, especially $O^{-}_{2}$ , to form the powerful free-radical peroxynitrite which removes Nitric-Oxide from the endothelial- layer and so reducing the vasodilatory function of the vessel (Rubio-Ruiz *et al.*, 2014). Ang II, TNF-$\alpha$, ET-1, IL-1$\beta$, and tissue hypoxia/ischemia stimulate IL 6 secretion with increased ROS production, Several studies showed that angiotensin II infusion caused both blood pressure and IL 6 serum levels to increase in hypertensive patients (Tanase *et al.*, 2019) .

Pro-inflammatory cytokines secretion, insulin resistance, and increased atherogenic lipoprotein levels were all linked to histological arterial wall inflammation in hypertensive patients (Tanase *et al.*, 2019) .

Many studies referred to an association between inflammatory markers and hypertension , some studies manifest higher plasma concentration of TNFα, IL 6, CRP (Stuveling *et al.*, 2004; Yu *et al.*, 2010; Mattace-Raso *et al.*, 2010; Lakoski *et al.*, 2011) in hypertensive patients when checking with normotensive persons (Naya et al, 2007), and in the animals (Chou *et al.*, 1998; lee *et al.*, 2006) .

However, no association between baseline CRP and the development of hypertension was observed in the Strong Heart Study (Glasser *et al.*, 2009), and there appears to be no association between CRP and hypertension in

individuals with Type 1 diabetes mellitus (Sahakyan *et al.,* 2010) , or in postmenopausal women ( Wang *et al.,* 2011) .

## 2.7: Nitric oxide: an overview and its relationship with aging and hypertension

"Nitric oxide (NO) is a gaseous signaling molecule that regulates various physiological and pathophysiological responses in the human body, NO is a lipophilic free radical which reacts with various molecules to cause pleiotropic effects", it's also termed endothelium-derived relaxing factor (EDRF) (Danylovych *et al.,* 2018).

Nitric oxide is structurally simple, low-molecular-weight, is extremely reactive, readily forming other nitrogen oxides, it has a very short half-life up to 5 sec depending on microenvironment, and can travel only limited distances before being oxidized ( Levine *et al.,* 2012).

It can diffuse into the cell and is synthesized through enzymatic and nonenzymatic pathways, this diffusible gas is produced by the conversion of L-arginine to L-citrulline by three distinct isoforms of nitric oxide synthase (NOS): neuronal synthase (nNOS), endothelial synthase (eNOS) and inducible synthase (iNOS) (Florentino *et al.,* 2017) . There is a mitochondrial variant of NOS that contributes to the regulation of NO-related mitochondrial activities in addition to cytosolic NOS (Keshet and Erez, 2018).

These three NOS isoforms (eNOS, nNOS, iNOS) have similar enzymatic mechanisms that involve electron transfer for oxidation of the terminal guanidine nitrogen of L-arginine, and all enzymes require several cofactors for proper function, including tetrahydrobiopterin (BH4), nicotinamide adenine-dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and haem ( Toma *et al.,* 2014).

Because NO has been shown to affect nearly every organ system in the body, so many of the diseases, conditions, and multi-systemic symptoms could

be caused or exacerbated by the body's dysregulation of NO production ( Torregrossa *et al.,* 2011) .

Nitric oxide deficiency is declared in many states characterized by cell senescence, oxidative stress, inflammation, endothelial dysfunction, vascular disease, insulin resistance, and type 2 diabetes mellitus, obesity (Levine *et al.*, 2012).

Nitric oxide appears to have two roles in the development of an erection: calcium-dependent activation of nNOS initiates the erectile process, whereas PI3K/Akt-dependent phosphorylation of eNOS results in sustained NO production and thereby enables full erection attainment, eractil dysfunction, then depend on NO reducing release (Davies, 2015).

Furthermore, Studies in the human being and experimental models reveal that constitutive production of nitric oxide (NO) is reduced with progressive age (Torregrossa et al, 2011).

The term "vascular aging" refers to all of the structural and functional changes in vessels of the blood that occur as people age (Herrera *et al.,* 2010). Increased intimal thickening and arterial stiffness, vascular tone abnormalities, left ventricular (LV) hypertrophy, the reduced threshold for cell calcium overload, reduced CV reserve, reduced heart rate variability, and reduced myocardial contractility are all age-related changes, many of these pathophysiological processes are modulated by the nitric oxide (NO) system, especially arterial stiffness, vascular tone, platelet function, myocardial hypertrophy, and contractility (Sverdlov *et al.,* 2014).

The mechanisms explain the vascular aging are complex and recruitment many pathways and factors ( Seals *et al.,* 2011) , vascular function is dependent on the releasing of nitric-oxide, which is produce by normal endothelial nitric-oxide synthase (eNOS) activity , however , excessive NO generated by iNOS

caused vascular dysfunction , and reduced NO availability while raises RNS which linked to aging- vascular dysfunction, found to be as a result of unusually expression and activities of NOS isoforms ( Cau *et al.*, 2012).

The eNOs availability in the plasma membrane during advancing age is reduced due to the increment linkage of eNOs with caveolin-1, Hsp90 expression and binding to eNOS be declining also in old endothelial cells, together, these may explain some of the decrease of eNOS activity and the reduction of relaxation in the walls of vascular blood depending on NO with the progressive age ( Cau *et al.,* 2012).

These data, provide further support to the role eNOS that produced NO in along with age in vascular dysfunction, and eNOS look like to playing a deleterious role in aging , via their uncoupled rote and be a source for $O_2^{\circ-}$ in the vasculature meaning a reduction in NO availability (Yang *et al.,* 2009).

Nevertheless, the effect of aging on eNOS production is often debatable, vascular eNOS production has been shown to remain stable as people age (Sun *et al.,* 2004; Rodríguez-Mañas *et al.,* 2009; Yang *et al.,* 2009; Donato *et al.,* 2009), or increased (Matz *et al.,* 2000; Goettsch *et al.,* 2001). The role of NOs in endothelial function and aging-associated endothelial dysfunction show in (Figure: 2.5) .

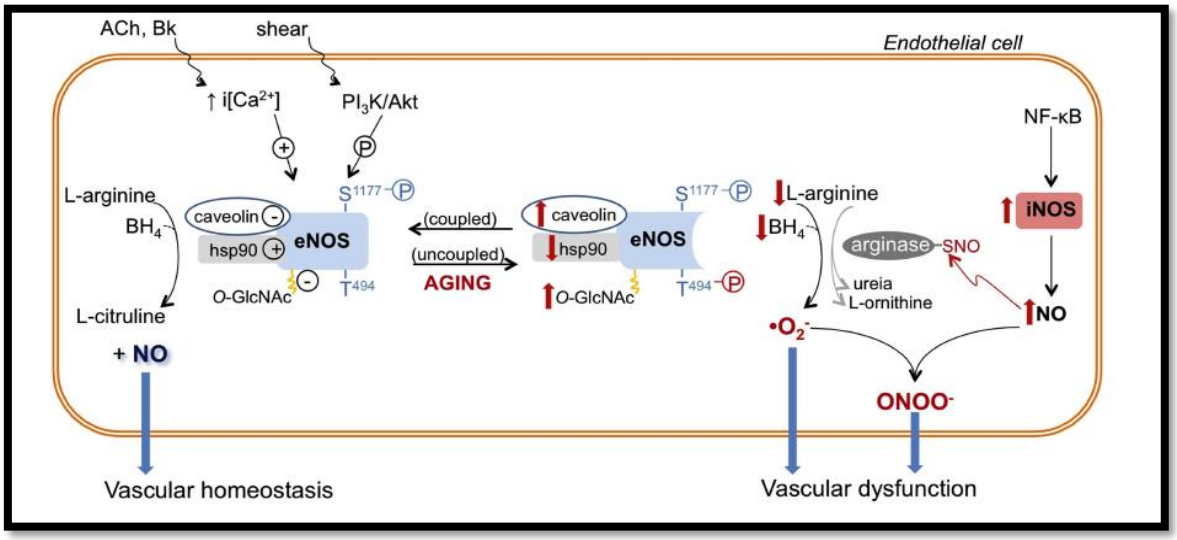



Figure (2.5): Role of nitric oxide synthase (NOS) enzyme in endothelial function and aging associated endothelial dysfunction.

(**Cau *et al.*, 2012**)

In the other hand , Wu and his coworkers ( 2021 ) concluded that nitric oxide playing an important role in keeping up the vascular health as well as for the controlled blood pressure . NO is well known as the strongest vasodilator substance , which effect vascular resistance and heart function , thus , controlling blood pressure ( Kunes *et al.*, 2004).

More NO molecules are inactive associated with hypertension or its bioavailability and decreased as an outcome of weakened synthesis by eNOS (Pinheiro *et al.*, 2017). The rise in superoxide formation by dysfunctional NOS as a result of NO reduction contributes to the clinical course of cardiometabolic disease (Aflyatumova *et al.*, 2018). Animal and human researches have proven that antioxidants such as superoxide dismutase and vitamin C, which guard against the harmful effects of free radicals, can help improve endothelium-dependent vasodilation with hypertension ( Toma *et al.*, 2014; Chuaiphichai *et al.*, 2017; Keshet and Erez, 2018) .

By nature of its ability to produce NO, the endothelium is a major regulator of vascular tone and blood pressure, a hallmark of endothelial dysfunction is

the loss of NOS protective effects, NO bioavailability, due to a reduction in its synthesis by eNOS and an increase in scavenging by ROS (Wu *et al.,* 2021) . Endothelial dysfunction is linked to high blood pressure and other CV hazard factors such as diabetes , smoking, and aging, and it is an important independent hazard factor for CV events in the individual with high blood pressure , with impaired NO bioactivity playing the major role ( Hermann *et al.,* 2006) .

Reduced NO bioavailability is implicated in various experimental models of hypertension as well as in human primary and secondary hypertension (Pechanova *et al.,* 2015) . After one week of therapy, newly diagnosed mild hypertensive patients who were given L-arginine (2 g three times a day) had lower blood pressure and improved vascular function (Miller, 2006) .

Patients with essential hypertension have lower NO levels in their blood, and endothelium-dependent vasodilation is impaired, the importance of eNOS and nitric oxide in the prevention of hypertension is confirmed by the fact that mice lacking a functional eNOS gene develop hypertension (Ahmad *et al.,* 2018 ) . Serum NO levels were higher in pre-hypertensive children than in hypertensive children (Aflyatumova *et al.,* 2018). Houston and Hays (2014) found in hypertensive patients, a single administration of oral NO supplementation lowers blood pressure and increases endothelial function.

Since NO is needed for renal perfusion and glomerular filtration, increased oxidative stress caused by angiotensin II reduces NO found in the renal micro-vasculature, ending in raised afferent arteriolar tone and high blood pressure (Pinheiro *et al.,* 2017) .

## 2.8 : Estradiol

Estrogens ($C_{18}H_{24}O_2$ ) are steroid hormones including four different kinds of hormones : estrone (E1) , estradiol (E2) , estriol (E3) , and estetrol (E4) ( Thomas and potter , 2013).

In women, estradiol is primarily synthesized by the granulosa cells of the ovary during the aromatization of androstenedione (produced in the theca cells) to estrone, and finally to estradiol, in addition, estradiol is produced by the corpus luteum, adrenal glands, liver, and mammary glands, however, the quantity is not significant (Lee et al. 2012) .

It supports the lining of the endometrium, cervix, uterine tubes, and vagina, as well as the development and maturation of oocytes and the orchestration of folliculogenesis, throughout the menstrual cycle , estradiol activates hypothalamic-pituitary events through a feedback mechanism to induce ovulation, besides participating with progesterone in the preparation of the endometrium for blastocyst implantation (Chuffa *et al.*, 2013).

Estradiol is produced primarily in the Leydig cells of the testis in human males, and it is responsible for not only the differentiation and function of the testes, but also for the epididymis, efferent ductules, prostate, seminal vesicles, and even the penis, however, excessive estrogen exposure has been postulated to be the main cause of low sperm counts which can lead to subfertility or infertility-related disorders (Chuffa *et al.*, 2013).

Testes released only about 20% of circulating estrogens in men, with the rest coming from adipose, brain, skin, and bone, which convert testosterone to estrogen through aromatase action (Cooke *et al.*, 2017) . In the testis, both Leydig cells and germ cells can synthesize estradiol (Hess, 2000) .

Estradiol controls prostate growth during life via indirect mechanisms such as suppressing androgen production through the hypothalamic-pituitary-

gonadal axis, through prolactin, and even directly in the Leydig cells of the testis, or through direct mechanisms such as local aromatization of testosterone into E2 (Härkönen and Mäkelä, 2004).

Estradiol can protect against brain injury, neurodegeneration, and cognitive decline, and may act as a "recovery agent" in some central nervous system disorders (Palmeri and Grimaudo,2013). Estradiol production increased in areas of the brain linked to sexual arousal, and estrogen receptors are located in the corpus cavernosum, with a high concentration around neurovascular bundles in the penis, however, both low testosterone and elevated estrogen increased the frequency of erectile dysfunction separately ( Schulster *et al.,* 2016).

Moreover, Schuster and his colleagues (2016) mentioned that estrogen modulates spermatogenesis, and the modulation of these testicular cells by estradiol reveals both inhibitory and stimulatory influences, thus, estradiol in men is essential for modulating libido, erectile function , and spermatogenesis .

The aromatase activity increases with age and obesity (Vermeulen *et al.,* 2002) . Eractil dysfunction and sexual desire have been associated with increased estradiol levels in elderly men (Kataoka and Kimura, 2018) .

Nevertheless, data about age-related concerning the estradiol levels are conflicting , Haring and his colleagues (2012) mentioned no changes in estradiol levels during the progressive age while Jasuja and his colleagues (2013) and Lewerin and his colleagues (2014) found that estradiol levels decreased with the progressive age.

On the other hand, Estradiol is an antioxidant that protects against oxidative stress, a causative factor in endothelial dysfunction associated with hypertension ( Reckelhoff, 2005).

## 2.9 : Prolactin

Prolactin (PRL), Lactotropin is a polypeptide hormone synthesized and secreted from lactotroph cells of the anterior pituitary gland, best known for allowing female mammals (and birds) to produce milk, its chemical composition is identical to that of growth hormone and placental lactogen hormone ( Fitzgerald and Dinan, 2008) . Raut and his colleagues (2019) summarized that prolactin plays a role in osmoregulation, growth and development, endocrine functions, metabolism, neurobiological functions, immunomodulation as well as their men role in reproduction, prolactin composed of 199 amino acids ( 23 KD), mature form is produced after the proteolytic cleavage of the signal peptide of the prolactin precursor and modification of post-translational happened.

The anterior pituitary is the primary site for prolactin production, but also by mammary gland, prostate, skin, decidua, brain, some immune cells and adipocytes (Bernichtein *et al.*, 2010).

Many substances controlling the releasing of PRL, both stimulating and inhibiting ones, some work upon the lactotrophs while others affect the PRL releasing via the hypothalamus dopamine neurons, the mediators that stimulated prolactin secretion such as angiotensin II, estradiol, serotonin (Ignacak *et al.*, 2012 ) .

Elevated levels of serum prolactin have a detrimental effect on male reproduction through inhibition of the pulsatile release of gonadotrophins from the anterior pituitary gland, and a direct effect on spermatogenesis (Dabbous, and Atkin, 2018).

Prolactin has been studied as a metabolic hormone, and some studies have found that men with higher body weight have macroprolactinomas and that dopamine agonists or a reduction in prolactin levels reduced body weight,

prolactin can also affect lipoprotein metabolism and insulin sensitivity, prolactinoma patients have been shown to have elevated levels of atherogenic lipoproteins and insulin resistance, in addition, hyperprolactinemia has been associated with low-grade inflammation and a higher level of C-reactive protein levels ( Pala *et al.,* 2015).

Serum prolactin slightly raised with increasing age in men, and PRL signaling may be important in retinal degeneration as people get older (Shen *et al.,* 2007).

Li and his colleagues (2019) found that prolactin correlated positively with hypertension during progressive ages in women. Prolactin influences the arterial wall through several tools, including nitric oxide synthase modulation, smooth muscle cell reproduction , low-grade inflammation , and inflammatory cell adhesion to endothelium, every of which is changing vascular function and building , according to succeeding investigation ( Gonzalez, 2004; Molinari *et al.,* 2007) , on the additional hand, Insulin resistance, gain weight, homocysteine concentration, and low-grade inflammation signs are all existing in sufferers with prolactinomas, which has an unwanted cardiovascular hazard profile , therefore, hyperprolactinemia will be linked with endothelial dysfunction (Stamatelopoulos *et al.,* 2011).

It was discovered that approximately half of the patients with essential hypertension had elevated prolactin levels, and that serum prolactin was higher in patients with essential hypertension who had organ damage due to arterial hypertension, and there was no connection between the drug's hypotensive effect and its inhibitory effect on prolactin, suggesting that prolactin is unlikely to have a role in the development of essential hypertension, rather, the increase in prolactin levels could be a late event in the natural history of essential hypertension (Hauger-Klevene *et al.,* 1981) . Previous experimental studies have indicated that PRL may play a role in the pathogenesis of hypertension, in

decerebrate rabbits, intravenous PRL infusion induces widespread vasoconstriction and increased arterial pressure, and in hyperprolactinemic humans showed an increased pressor response to angiotensin-II, possibly due to prolactin-induced up-regulation of adrenal and vascular angiotensin-II receptors, PRL promotes the production of intracellular adhesion molecule-1 and is linked to the aggregation of monocytes plus macrophages in the local area, which might induce hypertension (Zhang *et al.*, 2010) .

Excessive prolactin levels interfere with the function of the testicles, the production of testosterone, and spermatogenesis, affect the erection make it difficult, causes breast enlargement ( Snyder, 2019).

However, low prolactin levels in older men have been linked to a variety of negative health effects, including metabolic syndrome, depressive symptoms, reduced physical activity, and a general sense of being unwell, as well as subcomponents of sexual appetite, sexual activity, erection, morning erection, and orgasm (Corona *et al.*, 2014).

# Chapter Three

# Materals and Methods

# 3. MATERIALS AND METHODS :

## 3.1. Materials

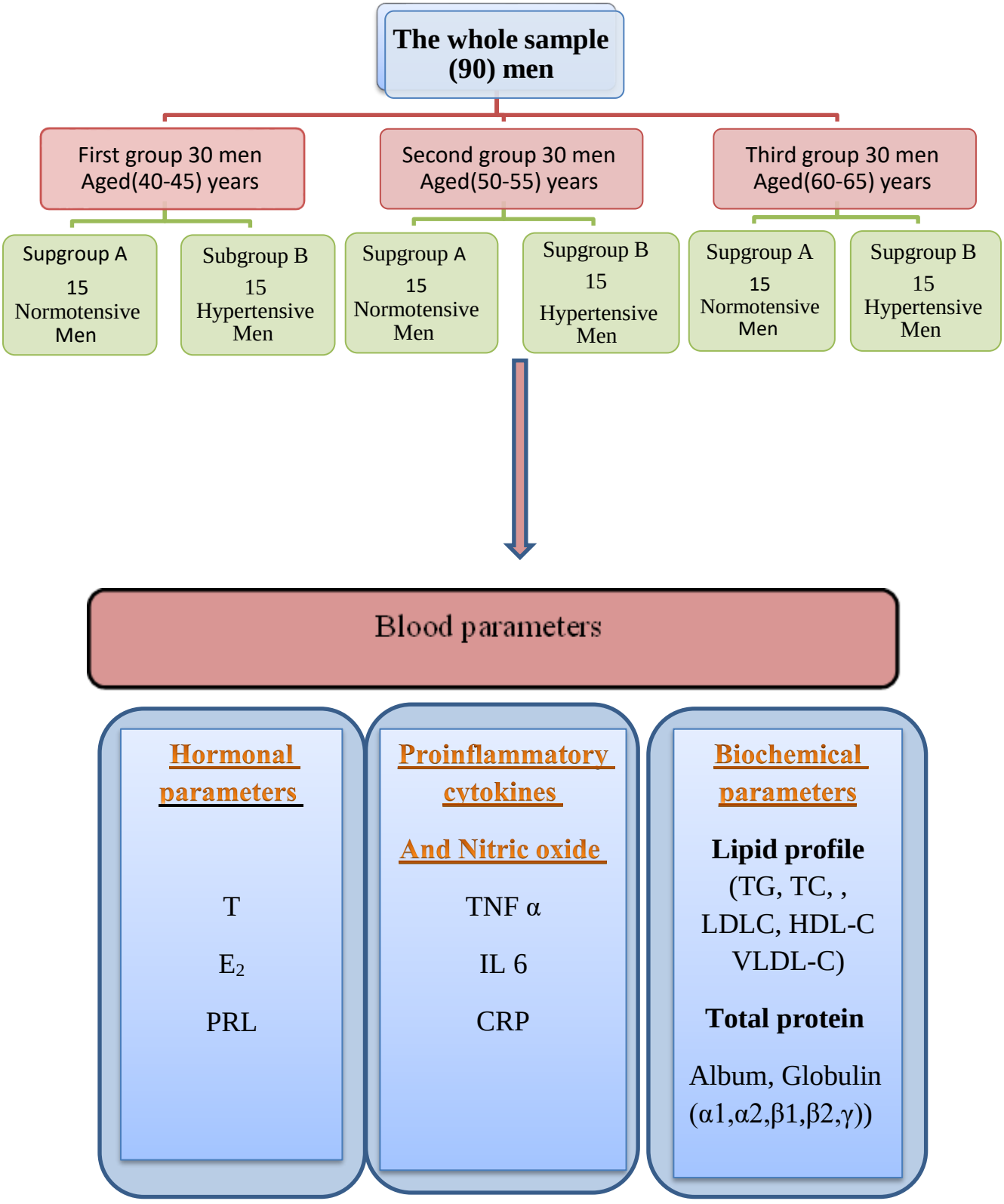
### 3.1.1. Subjects of the study :

The current study was carried out in Internal Consultation of Al Sadder Hospital , Heart Center in Misan province , from October 2019 till February 2020 , including 90 men divided ( according to their ages) into three groups ( 30 men / group ) as the follows :

- First group : normotensive and hypertensive men aged between 40-45 years and contain :
  - ❖ subgroup a (15 normotensive men) .
  - ❖ subgroup b (15 hypertensive men ) .
- Second group : normotensive and hypertensive men aged between 50-55 years and contain :
  - ❖ subgroup a (15 normotensive men) .
  - ❖ subgroup b ( 15 hypertensive men ) .
- Third group : normotensive and hypertensive men aged between 60-65 years and contain :
  - ❖ Subgroup a (15 normotensive men) .
  - ❖ Subgroup b ( 15 hypertensive men ) .

Men with hypertension have been checked medically by a specialist physician and have been diagnozed with hypertension and some of these men were excluded due to their attack by diabetes , thyroid disease , pituitary tumors , and others taking hormonal drugs . Aquestionnaire has been designed to obtain the actual information about the sample .

## 3.1.2. Experimental design



### 3.1.3 Instruments and Equipments

The instruments and equipments used in this study with their companies and countries of origin are listed in table (3.1) .

Table (3.1) : The instruments and equipment used in this study.

| NO | INSTRUMENT | COMPANY/ORIGIN |
|---|---|---|
| 1 | 100 ml and 500 ml graduated cylinders. | China |
| 2 | Can tube | China |
| 3 | Centrifuge | Germany |
| 4 | COBAS e411 | Roche / Germany |
| 5 | Cotton | Turkey |
| 6 | Disposable syringe 10 ml | Trogector3 /Germany |
| 7 | Elisa reader | Bio –tek /Germany |
| 8 | Elisa washer | Bio – tek /Germany |
| 9 | Eppendorf tubes(1.5 ml) | M.O.H/ China |
| 10 | Gel tube | Sun /Jordan |
| 11 | Gloves | Turkey |
| 12 | Horizontal microtiterplate shaker | Germany |
| 13 | Incubator | Heraeus /Germany |
| 14 | Magnetic stirrer | Germany |
| 15 | Pipettes for delivery of : 2 µl,10 µl, 100 and 200 µl, 1000ml | Dragon LAP. CO. |
| 16 | Refrigerator | Concord /Lebanon |
| 17 | Sebia Minicap electrophoresis | U.S.A |
| 18 | sphygmomanometer | Alpk2 / Japan |
| 19 | Test tubes for dilution | China |
| 20 | Tips (10ml,20ml,100ml and 200 ml) | China |
| 21 | Vortex mixer | Germany |

### 3.1.4. Laboratory kits

The laboratory kits used in this study are shown in table (3.2) .

**Table (3.2) : kits and their suppliers .**

| No | Kits | Company / Origin |
|---|---|---|
| 1 | C reactive protein | ROCH / GERMAMY |
| 2 | Estradiol | ROCH / GERMAMY |
| 3 | Interleukin 6 | CUSABIO/CHINA |
| 4 | Lipid profile | ROCH / GERMAMY |
| 5 | Nitric oxide | SHANGHAI /CHINA |
| 6 | Prolactin | ROCH / GERMAMY |
| 7 | Protein fraction | Sebia / U.S.A |
| 8 | Total protein | ROCH / GERMAMY |
| 9 | Total testosterone | ROCH / GERMAMY |
| 10 | Tumor necrosis factor alpha | DIMEDITEC/GERMANY |

## 3.1.5. Diagnostic kit found in the appendix

## 3.2.Methods

## 3.2.1 Blood samples collection

Venous blood Sample (8-10 mL) was drown from each healthy and hypertensive men at 8-10 AM in gel tubes . The blood samples were left for 15 minutes to clot at room temperature , to get the serum which separated by centrifugation at 3000 (rpm) for 15 (min) , to measure all the parameters for the current study . Serum was transferred into labeled plain tube and stored at -20 Ċ° until used for evaluation of hormones and the other parameters .

## 3.2.2 Determination of reproductive hormones assay

### 3.2.2.1 Determination of total testosterone ( TT)

The Elecsys testosterone II assay is based on a competitive test principle using a high affinity monoclonal antibody (sheep) specifically directed against testosterone (Wheeler, 1995).

**The Procedure**

▪ Twenty µL of serum was incubated with a biotin labeled antibody.

▪ Streptavidin-coated microparticles was added and a labeled testosterone derivate with a ruthenium complex , the reaction of biotin and streptavidin maked the complex attach to the solid phase .

▪ The complex converted into the measuring cell to magnet microparticles on electrode . Remove any unbound substances by washing . A voltage to the electrode of lead was applied to produce emission which estimated by a photomultiplier .

- Calibration curve was used which is instrumentspecifically generated by 2-point calibration and a master curves provided via the reagent barcode or e-barcode to get the results .

**The Ranges**

Measuring range 0.025-15.0 ng/mL or 0.087-52.0 nmol/L .

## 3.2.2.2. Determination of estradiol hormone ($E_2$)

The Elecsys Estradiol III assay employs a competitive test principle using two monoclonal antibodies specifically directed against 17β-estradiol. Endogenous estradiol released from the sample by mesterolone competes with the added estradiol derivative labeled with a ruthenium complex for the binding sites on the biotinylated antibody ( Thienpont *et al.*, 1988) .

**The Principle**

- Twenty five µL of serum was incubated with two biotin labeled antibodies .

- Streptavidin-coated microparticles and an labeled estradiol derivative with a tris bi pyridine was added , the reaction of biotin and streptavidin maked the complex attach to the solid phase .

- The complex was converted into the measuring cell to magnet microparticles on electrode . Remove any unbound substances by washing . of A voltage to the electrode lead was applied to produce emission which estimated by a photomultiplier .

- Calibration curve was used and a master curve provided via the reagent barcode or e-barcode to get the results

**The Ranges**

Measuring range 18.4-11010 pmol/L (5-3000 pg/mL) .

### 3.2.2.3 Determination of prolactin (PRL)

The Elecsys Prolactin II assay uses a sandwich two monoclonal antibodies specifically directed against human prolactin ( Fahie-Wilson and Smith, 2013) .

**The Principle**

- Incubated 10 µL of serum and a biotin labeled antibody will form a first complex .
- Second labeled antibody was added with a ruthenium complex plus streptavidin-coated microparticles , the reaction of biotin and streptavidin make the complex attach to the solid phase .
- Converted the complex into the measuring cell to magnet microparticles on electrode . Remove any unbound substances by washing . Avoltage was applied to the electrode lead to produce emission which estimated by a photomultiplier .
- calibration curve was used and a master curve provided via the reagent barcode or e-barcode to get the results .

**The Ranges**

Measuring range 1.00-10000 µIU/mL or 0.0470-470 ng/mL .

## 3.2.3. Determination of inflammatory markers

### 3.2.3.1. Determination of tumor necrosis factor (TNF α)

The TNF α was evaluated by using enzyme-linked immunosorbent assay (ELISA) system , with human TNF α kit (Aukrust *et al.,* 1994) .

## The Principle

Direct sandwich solid phase immunoassay depend on chromogenic reaction consist of monoclonal antibody one and monoclonal antibody two and the last was labeled with HPR which is working on TMB.

## Reagent Preparation :

**A**. The Calibrators : fill the zero calibrator to the volume specified on the QC data sheet with D.W. and the rest calibrators with two ml D.W .

**B**. The Controls : the control vial was filled with two ml of D.W .

**C.** For the 96 wells the 600 µl of the conjugate solution was diluted with its buffer 6000 µl so that the working volume become 6600 µl .

TABLE CONJUGATE DILUTION

| Number of wells | Concentrated conjugate | Conjugate buffer | Working volume |
|---|---|---|---|
| 8 | 50 µl | 500 µl | 550 µl |
| 16 | 100 µl | 1000 µl | 1100 µl |
| 24 | 150 µl | 1500 µl | 1650 µl |
| 32 | 200 µl | 2000 µl | 2200 µl |
| 48 | 300 µl | 3000 µl | 3300 µl |
| 96 | 600 µl | 6000 µl | 6600 µl |

**D.** A 199 volume of D.W. was added , one volume of washing solution 200x homogenized it by using a magnetic stirrer .

## The Procedure

1. fifty µl of incubation buffer was added to the wells .

2. 200 µl of each Calibrator, Control and Sample was added into the appropriate wells.

3. The incubation was done two h at 18 - 25°C with using a horizontal shaker

( 700 rpm ± 100 rpm) .

4. The liquid was remonved from each well .

6. 0.4 ml of washing solution was used for each well and remove this liquid , repeat the washing three times .

7. 100 µl of zero calibrator was added to the wells .

8. 50 µl of HRP conjugate was added into all the wells.

9. Incubated as the thired point.

10. The liquid from the wells was removed by tabbing the plat .

11. Washing as in the sixth point .

12. 100 µl of the TMP solution was added into each after 15 minute of washing .

13. Incubation for 15 minutes in( 18 - 25°C ) on a horizontal shaker ( 700 rpm± 100 rpm) , a way of direct sunlight .

14. 100 µl Stop solution was added to the well .

15. 450 nm and 490 nm was used (reference filter 630 nm or 650 nm) for reading the absorbance in 30 minutes .

16. Find the O.D. for each well using microplate reader .

## Calculation

1. The plate was read at 450 nm against a reference filter set at 650 nm (or 630 nm).

2. The mean of duplicate determinations was Calculated.

3. On semi-logarithmic or linear graph paper the OD values (ordinate) was plotted for each calibrator against the corresponding concentration of TNF α (abscissa) and a calibration curve was draw through the calibrator points by connecting the plotted points with straight lines .

4. The concentration for each control and sample was read by interpolation on the calibration curve .

5. Computer assisted data reduction will simplify these calculations. If automatic result processing is used , a 4 parameter logistic function curve fitting is recommended .

### 3.2.3.2. Determination of interleukin 6 (IL6)

The IL 6 was evaluated by using enzyme-linked immunosorbent assay (ELISA) system , with human IL 6 kit (Bowcock *et al.*, 1988) .

### The Principle

This assay employs the sandwich ELZA , the well pre coated with anti IL6 antibodies , when add the samples which have IL6 as antigen it will binds to Ab to get a complex immune . Any un bound substance removed by washing to add later a vidin conjugated HPR , washing again and adding later substance cause the color production coordinate with the concentration of IL6 after its reaction is stopped , its density are calculated after .

### Reagent Preparation

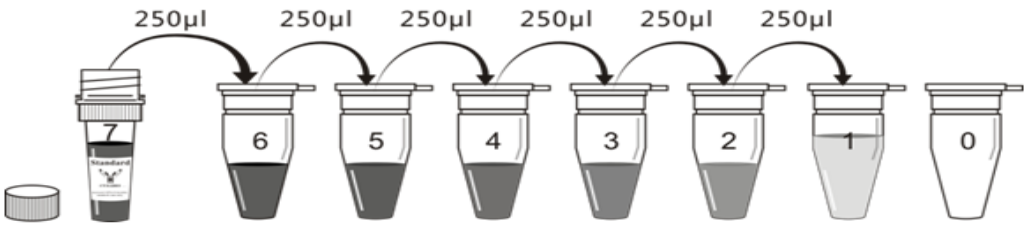
1- Biotin-antibody vial was centrifuged and diluted the labeled antibody with biotin 100 fold by adding 10 µl of it to 990 µl of Biotin-antibody Diluent .

2- The HRP-avidin vial was centrifuged and 10 µl of HRP-avidin was added to 990 µl of HRP-avidin Diluent .

3-Wash Buffer (1x) – was removing any formed crystals by het it to room temperature and mix . later Diluted 20 ml of it (25 x) into D.W . to prepare 500 ml.

## Standard

- Centrifuging at 6000-10000 rpm for 30s at first .
- 1.0 ml of Sample Diluent was added to get 500 pg/ml of stock solution which was leaved for 15 minutes with gentle agitation prior to making dilutions .
- 250 µl was added to six tubes a sample Diluent diluated the stock soluation by adding 250 ul of it to the first tube and serially for other each tubes as in the figure below :




| Tube | S7 | S6 | S5 | S4 | S3 | S2 | S1 | S0 |
|---|---|---|---|---|---|---|---|---|
| pg/ml | 500 | 250 | 125 | 62.5 | 31.2 | 15.6 | 7.8 | 0 |

### The Procedure

1. At room temperature all the sample was centrifuged before use . its recommended that all samples and standards be assessed in duplicate .
2. All solutions as indicated beforewas prepare .
3. 96 well in this assay was used .
4. 100 ul of standard and samples was added for each well after covered , incubation performed for two hours at 37 c .
5. The liquid was decanted.
6. Incubation after adding 100 ul of biotin antibody 1x for one hour at 37 c.
7. Two to three times it was washed after its filled with 200 ul of washing solution , between one washing and the next there is two minute , ending with decantation and using a clean paper towels.

8. Incubation for one hour at 37 c of the well after 100µl of HRP-avidin (1x) was added .
9. As indicated in point 6 it was washed for five times .
10. Incubation of the plat for 15-30 minutes at 37°C after the 90µl of TMB Substrate was added to all wells

11- The reaction was stopped by added 50µl of Stop Solution .

12-The O.D. was fonded by using micro plate reader (450- 540 -570) nm substract the results of 540 -570 from 450 one to get an accurate reading.

**Calculation of Results**

excel program was used to find the average of duplicate reading for all wells and subtract from it the zero O.D. standard . aprism program was used to create the curve (for parameter logistic 4PL) curve fit , x axis presented the O.D , while y axis presented the concentration , the OD was plotted to find the sample concentration.

**Detection Range**

7.8pg/ml-500pg/ml.

## 3.2.3.3.Determination of c-reactive protein (CRP) .

C-Reactive Protein ( CRP ) was evaluated by particle enhanced immunoturbidimetric assay, with human CRP kit (Price *et al.*, 1987) .

**Principle of CRP Procedure assay**

Human CRP bind tightly with latex particles coated with monoclonal anti-CRP antibodies. The aggregates are determined turbidimetrically .

**The Measuring Range**

0.3-350 mg/L (2.9-3333 nmol/L).

### 3.2.4 Determination of nitric oxide :

The NO was determined by (ELISA) system , with human NO kit ( Hou, *et al.*, 1999) .

| | | |
|---|---|---|
| 320µmol/L | Standard No.5 | 120µl Original Standard + 120µl Standard diluents |
| 160µmol/L | Standard No.4 | 120µl Standard No.5 + 120µl Standard diluents |
| 80µmol/L | Standard No.3 | 120µl Standard No.4 + 120µl Standard diluent |
| 40µmol/L | Standard No.2 | 120µl Standard No.3 + 120µl Standard diluent |
| 20µmol/L | Standard No.1 | 120µl Standard No.2 + 120µl Standard diluent |

**The Principle**

The wells coated with antibodies for nitric oxide, incubated with samples , then the second anti body that labeled with biotin was added and streptavidin HRP to form a complex was added .Washing after incubation and A and B reagents was added to give the color and then the acidic stop solution . The density of color depend on the concentration of the nitric oxide .

## Assay Procedure

A) The 640 u mol /L of standard solution diluted with standard diluent and as the figure below :

B) Washing solution was diluated by 30 x .

C) The 96 wells was used .

D ) 50 ul of standard dilated solution was added for each standard well.

E) 40 ul of sample , 10 ul of nitric oxide antibody plus 50 ul of Streptavidin HRP was added for sample well.

F) seal membrane was used for the incubation in 37 c for one hour

G) Washing afer decanted for five times .

H) 50 ul of a substance was added and then 50 ul of B substance color change to blue indicating the present of nitric oxide after incubation away from light .

I) To stop the reaction 50 ul of stop solution was used .

J) After 10 minute by using a 450 um to find O.D. of each well.

K) regression equation are used to find the standard curve and corresponding concentration of the sample founded by prism program .

## The Measuring Range :

2μmol/L→600μmol/L.

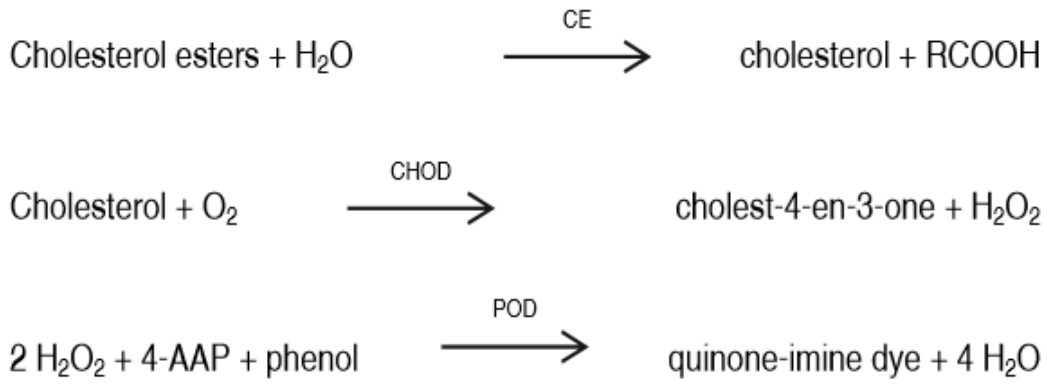
## 3.2.5 Determination of lipid profile

### 3.2.5.1 Determination of total cholesterol (TC)

TC was evaluated by using Enzymatic colorimetric test , with human cholesterol kit (Tarbutton and Gunter, 1974) .

**Principle of Assay**

Cholesterol esterase cleave the cholesterol esterse to get cholesterol and RCOOH . The cholesterol then oxide by Cholesterol oxidase to cholestenone and $H_2O_2$ . $H_2O_2$ plus phenol and 4AAP by peroxidase produce a red dye .




The dye color density is proportional to the cholesterol concentration . It is determined by photometric .

**Measuring range**

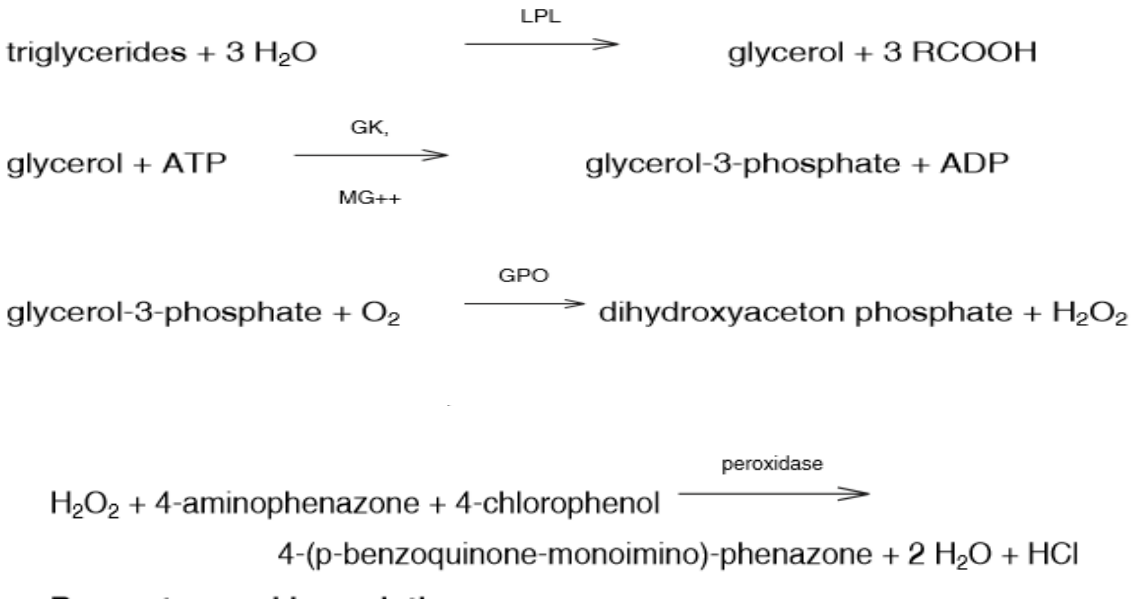
0.25-20.7 mmol/L (9.7-800 mg/dL) .

### 3.2.5.2 Determination of triglycerides (TG)

TG was evaluated by using enzymatic colorimetric test , with human TG kit (Siedel *et al.*, 1993) .

## The Principle

TG was hydrolaysis by LPL to get glycerine plus fatty acids , glycerin phosphated to get glycerol -3- phosphate which oxidiesed to get the red dye .



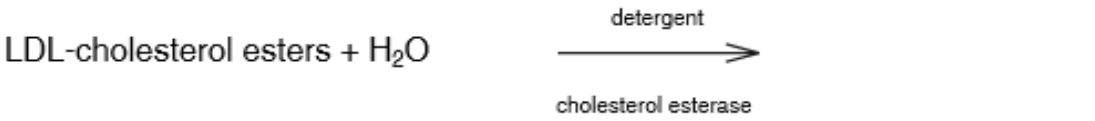

The density of this dye is propotional to concentration of TG .

## Measuring Range

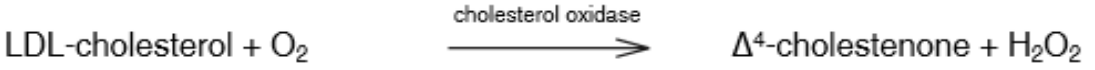
0.1-10 mmol/L (8.85-885 mg/dL) .

## 3.2.5.3. Determination of serum low density lipoprotein cholesterol (LDL-C )

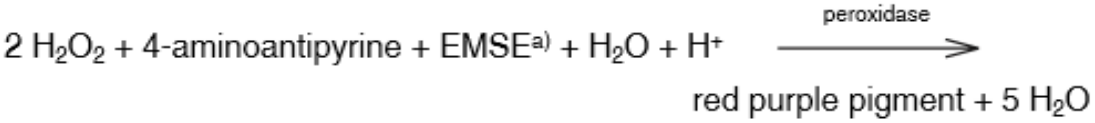
LDL-C was evaluated by using Homogeneous enzymatic colorimetric assay , with human LDL- C kit (Bachorik, 2000) .




Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.




In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to $\Delta^4$-cholestenone and hydrogen peroxide.




**The Principle**

Selectively LDL chosen by surfactant compound plus sugar inhibited for other lipoproteins .The Cholesterol esterase work on LDL- cholesterol esters to get Cholesterol which Oxidases to cholestenone and $H_2O_2$ . H2O2 by the enzyme peroxidase and EMSE produce a red purple dye . The density of dye are the reflection of the LDL concentration .

**Measuring Range**

0.10-14.2 mmol/L (3.87-549 mg/dL) .

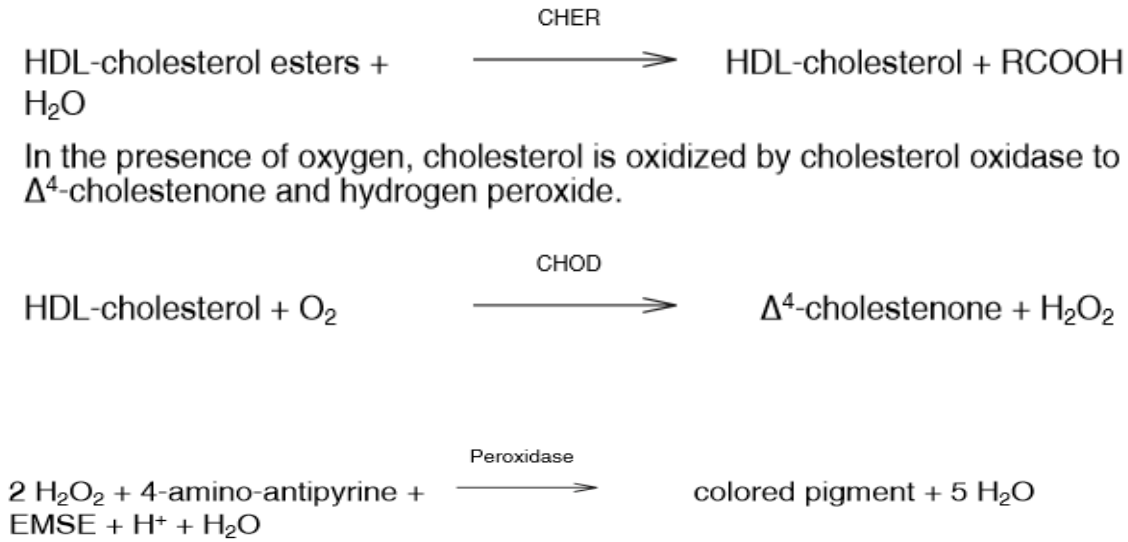
## 3.2.5.4 Determination of high density lipoprotein (HDL-C)

HDL-C was evaluated by using Homogeneous enzymatic colorimetric test , with human HDL- C kit (Katayama, *et al.*, 2009 ).

**The Principle**

Other lipoprotein reactions was prevented except HDL after the treat with polyanions and a detergent . The Cholesterol esterase worked on HDL-

cholesterol esters to get Cholesterol which Oxidased to cholestenone and H2O2 . H2O2 by the enzyme peroxidase and EMSE produced a red purple dye . The density of dye are the reflection of the HDL concentration and is measured photometrically .




**Measuring Range**

0.08-3.88 mmol/L (3.09-150 mg/dL) .

## 3.2.5.5 Determination of serum very low density lipoprotein cholesterol (VLDL-C)

Concentration of VLDL-C estimated by dividing TG by five (Friedewald *et al.*,1972) .

## 3.2.6 Determination of total protein (TP)

Tp was evaluated by using Colorimetric assay , with human TP kit (Weichselbaum , 1946 ) .

## Principle

Divalent copper reacted in alkaline solution with protein peptide bonds to form the characteristic purple-colored biuret complex. Sodium potassium tartrate prevented the precipitation of copper hydroxide and potassium iodide prevented autoreduction of copper .

$$\text{Protein} + Cu^{2+} \xrightarrow{\text{alkaline solution}} \text{Cu-protein complex}$$

The color intensity is directly proportional to the protein concentration which can be determined photometrically .

## Measuring Range

2.0-120 g/L (0.2-12 g/dL) .

## 3.2.7.Determination of blood proteins fractions :

## The Principle

The Minicap Protein (E) 6 assay is based on the principle of capillary electrophoresis in a free solution . Serum proteins are separated in silica capillaries by their electrophoretic movement and electroosmotic flow at high voltage in an alkaline buffer. Proteins are directly detected during migration by UV absorbance .This technique allows charged molecules to be separated on the basis of their electrophoretic mobility in an alkaline buffer with a specific pH of (9.9). Separation also occurs according to the electrolyte pH and osmotic flow. Normal serum proteins separated into six major fractions (Jellum *et al.*, 1997).

**Reagents**

- Protein 6 Buffer damping solution

The buffer is ready for use. Contains alkaline buffer pH 9.9; additives essential for optimal performance Ready to use for protein analysis in capillary electrophoresis.

- Wash Solution :Vial of stock wash solution was diluted up to 250 mL with distilled or deionized water. Contains sodium hydroxide

**Procedure**

1-A sample dilution with buffer is prepared and injected by aspiration at the anodic end of the capillary.

2- A high voltage protein separation is then performed and direct detection of the proteins is made at 200 nm at the cathodic end of the capillary.

3- Then the capillaries are washed with the Wash solution and prepared for the next analysis with buffer.

## 3.3 Statistical Analysis

The results are expressed as Mean ± Standard Division (SD) , Statistical analysis was performed by IBM SPSS statistics , version 26 (IBM Co., Armonk , NY, USA) . The statistical analysis was performed by one-way Analysis Of Variance (ANOVA) , followed by t test used to compartion between subgroups and Duncan's test to comparted between groups at ($p \leq 0.05$) value is a significant level .

# Chapter Four

# Results

# 4 . Results :

## 4.1 The levels of hormonal and biochemical parameters in normotensive and hypertensive in different subgroups of age.

### 4.1.1 Hormonal parameters

#### 4.1.1.1 Testosterone

**First subgroup** : The results revealed that the testosterone levels in hypertensive men (3.21 ± 0.68 ng/ml) decreased significantly ($P \leq 0.05$) in comparison with the normotensive men (4.56 ± 0.59 ng/ml) as shown in (Table 4.1; Figure 4.1) .

**Second subgroup :** The results revealed that the testosterone levels in hypertensive men (2.66 ± 0.68 ng/mL) decreased significantly ($P \leq 0.05$) in comparison with the normotensive men (3.80 ± 0.66 ng/mL) as shown in (Table 4.1; Figure 4.1).

**Third subgroup :** The results revealed that the testosterone levels in hypertensive men (2.08 ± 0.52 ng/ml) decreased significantly ($P \leq 0.05$) in comparison with the normotensive one (2.64 ± 0.55 ng/ml) as shown in (Table 4.1; Figure 4.1) .

### 4.1.2 Estradiol (E2)

**First subgroup :**The Levels of estradiol are increased significantly ($P \leq 0.05$) in hypertensive men (36.29 ± 0.78 pg/ml) in comparison with normotensive men (32.84±1.54 pg/ml) as shown in (Table 4.1; Figure 4.2) .

**Second subgroup :** The Levels of estradiol are increased significantly ($P \leq 0.05$) in hypertensive men (38.18±0.85 pg/mL) in comparison with the normotensive men (35.26 ± 1.43 pg/mL ) as shown in (Table 4.1; Figure 4.2) .

**Third subgroup :** The Levels of estradiol are increased significantly ($P \leq 0.05$) in hypertensive men (39.29 ± 0.85 pg/ml) in comparison with the normotensive men (36.44 ± 1.45 pg/ml) as shown in (Table 4.1; Figure 4.2) .

### 4.1.1.3 Prolactin (PRL)

**First subgroup :** The prolactin levels (6.21 ± 0.44 ng/ml ) increased significantly ($P \leq 0.05$) in hypertensive men in comparison with the normotensive men (5.60 ± 0.99 ng/ml) as shown in (Table 4.1; Figure 4.3 ) .

**Second subgroup** : The prolactin levels (6.97 ± 0.94 ng/mL) increased significantly ($P \leq 0.05$) in hypertensive men in comparison with the normotensive men (6.23 ±0.79 ng/mL) as shown in (Table 4.1; Figure4.3) .

**Third subgroup** : The prolactin levels increased significantly ($P \leq 0.05$) in hypertensive men (8.51 ±1.04 ng/ml) in comparison with the normotensive men (6.86 ± 0.51 ng/ml ) as shown in (Table 4.1; Figure 4.3) .

**Table (4.1) : Hormonal parameters ( Testosterone , Estradiol and Prolactin) within subgroups for different groups .**

| Subgroups \ Parameters | | Testosterone ng/ml | Estradiol Pg/ml | Prolactin ng/ml |
|---|---|---|---|---|
| Subgroup 1 (40-45) yre | Normotensive | 4.56 ± 0.59 [a] | 32.84 ± 1.54 [a] | 5.60 ± 0.99 [a] |
| | Hypertensive | 3.21 ± 0.68 [b] | 36.29 ± 0.78 [b] | 6.21 ± 0.44 [b] |
| Subgroup2 (50-55) yre | Normotensive | 3.80 ± 0.66 [a] | 35.26 ± 1.43 [a] | 6.23 ± 0.79 [a] |
| | Hypertensive | 2.66 ± 0.68 [b] | 38.18 ± 0.85 [b] | 6.97 ± 0.94[b] |
| Subgroup 3 (60-65)yre | Normotensive | 2.64 ± 0.55 [a] | 36.44 ± 1.45 [a] | 6.86 ± 0.51 [a] |
| | Hypertensive | 2.08 ± 0.52 [b] | 39.29 ± 0.85 [b] | 8.51 ± 1.04 [b] |

***The values represent Mean ± SD .**

***Similar small letters represent no significant difference between subgroups . * Different small letters represent a significant different at (P ≤ 0.05) between subgroup .**

## 4.1.2 Inflammatory markers

### 4.1.2.1 The tumor necrosis factor alpha (TNFα)

**First subgroup :** The TNFα levels increased significantly (4.64 ± 0.76 pg/ml ) (P ≤ 0.05) in hypertensive men in comparison with the normotensive men (4.06 ±0.12 pg/ml) (Table 4.2; Figure 4.4) .

**Second subgroup :** The TNFα levels increased significantly (6.05 ± 0.77 pg/ml ) ($P \leq 0.05$) in hypertensive men in comparison with the normotensive men (4.65 ± 0.39 pg/ml) (Table 4.2; Figure 4.4) .

**Third subgroup :** The TNFα levels increased significantly (8.309 ±0.610pg/ml ) ($P \leq 0.05$) in hypertensive men in comparison with the normotensive men (5.96 ± 0.60 pg/ml) . (Table 4.2; Figure 4.4).

### 4.1.2.2 The interleukin 6 (IL6) .

**First subgroup :** The interleukin 6 levels increased significantly (1.158± 0.525pg/ml ) ($P \leq 0.05$) in hypertensive men in comparison with the normotensive men (0.59 ± 0.240 pg/ml) **,** (Table 4.2; Figure 4.5) .

**Second subgroup :** The interleukin6 levels raised significantly (1.735± 0.789pg/ml) ($P \leq 0.05$) in hypertensive men when compared with the normotensive men (0.82 ± 0.373 pg/ml) **,** (Table 4.2; Figure 4.5) .

**Third subgroup :** The interleukin6 levels raised significantly (2.42 ± 0.7425 pg/ml) ($P \leq 0.05$) in hypertensive men in comparison with the normotensive men (0.89 ± 0.42 pg/ml) **,** (Table 4.2; Figure 4.5) .

### 4.1.2.3 The C- reactive protein (CRP)

**First subgroup :** The levels of CRP raised significantly (3.499± 0.831 mg/dl) ($P \leq 0.05$) in hypertensive men when compared with the normotensive men (1.917± 0.475 mg/dl) , (Table 4.2; Figure 4.6) .

**Second subgroup:** The levels of CRP raised significantly (4.181± 0.984mg/dl) ($P \leq 0.05$) in hypertensive men if checked with the normotensive men (2.029± 0.794mg/dl) , (Table 4.2; Figure 4.6).

**Third subgroup :** The levels of CRP raised significantly (5.45 ± 0.67 mg/dl) ($P \leq 0.05$) in hypertensive men if checked with the normotensive men (2.10 ± 0.61 mg/dl) , (Table 4.2; Figure 4.6) .

### 4.1.3. The nitric oxide (NO)

**First subgroup :** Levels of nitric oxide had decreased significantly (0.44±0.02µmol/L) ($P \leq 0.05$) in hypertensive men if checked with normotensive men. (0.47±0.031µmol/L ) , ( Table 4.2; Figure 4.7) .

**Second subgroup :** Levels of nitric oxide had decreased significantly (0.42 ± 0.02µmol/L) ($P \leq 0.05$) in hypertensive men if checked with normotensive . (0.44 ± 0.01µmol/L ) , ( Table 4.2; Figure 4.7) .

**Third subgroup :** Levels of nitric oxide had decreased significantly (0.41 ± 0.012 µmol/L) ($P \leq 0.05$) in hypertensive men if checked with normotensive men (0.42 ± 0.02µmol/L) , (Table,4.2; Figure,4.7).

## Table (4.2): Inflammation parameters ( TNFα , IL6 , CRP) and Nitric oxide within subgroups for different ages .

| Subgroups \ Parameter | | TNF α pg/ml | IL6 pg/ml | CRP mg/dl | NO µmol/L |
|---|---|---|---|---|---|
| Subgroup 3 (40-45)yre | Normotensive | 4.06± 0.123 [a] | 0.593± 0.240 [a] | 1.917± 0.475 [a] | 0.465± 0.031 [a] |
| | Hypertensive | 4.639± 0.760 [b] | 1.158± 0.525 [b] | 3.499± 0.831 [b] | 0.435± 0.022 [b] |
| Subgroup 2 (50-55)yre | Normotensive | 4.649± 0.391 [a] | 0.824± 0.373 [a] | 2.029± 0.794 [a] | 0.443± 0.011 [a] |
| | Hypertensive | 6.047± 0.770 [b] | 1.735± 0.789 [b] | 4.181± 0.984 [b] | 0.415± 0.02 [b] |
| Subgroup 3 (60-65)yre | Normotensive | 5.962± 0.604 [a] | 0.891± 0.417 [a] | 2.095± 0.610 [a] | 0.416± 0.019 [a] |
| | Hypertensive | 8.309± 0.610 [b] | 2.415± 0.743 [b] | 5.447± 0.672 [b] | 0.406± 0.012 [a] |

*** The values represent mean ± SD .**

*** Similar small letters represent no significant difference between subgroups .**

*** Different small letters represent a significant different at ($P \leq 0.05$) between subgroup.**

### 4.1.4 The lipid profile

#### 4.1.4.1 The total cholesterol (TC)

**First subgroup :** Results revealed that the TC level in hypertensive men (190.67 ± 8.252 mg/dl) increased significantly ($P \leq 0.05$) if checked with the normotensive men (164.27 ± 8.430mg/dl) , ( Table 4.3; Figure 4.8) .

**Second subgroup :** Results revealed that the TC level in hypertensive men is (198.4 ± 8.95 mg/dl) had significantly increased ($P \leq 0.05$) if checked with the normotensive men (171.8 ± 8.265 mg/dl) , ( Table 4.3; Figure 4.8) .

**Third subgroup :** The results revealed that the TC level in hypertensive men (203.93 ± 8.87 mg/dl) increased significantly ($P \leq 0.05$) if checked with the normotensive men (180.87 ± 8.97 mg/dl). ) , ( Table 4.3; Figure 4.8) .

#### 4.1.4.2 The triglyceride (TG)

**First subgroup :** The TG level increased significantly (234 ± 4.33 mg/dl) in hypertensive men if checked with the normotensive men (181.8 ±7.54 mg/dl) , ( Table 4.3; Figure 4.9) .

**Second subgroup :** The TG level rasid significantly (240.6 ± 5.18 mg/dl) in hypertensive men if checked with normotensive men (195.07 ± 8.82 mg/dl) , (Table 4.3; Figure 4.9) .

**Third subgroup :** The TG level increased significantly (248.73 ± 6.80mg/dl) in hypertensive men if checked with the normotensive men (205 ± 7.40 mg/dl) , (Table 4.3; Figure 4.9) .

### 4.1.4.3 The low density lipoprotein (LDL)

**A .First subgroup :** The levels of LDL increased significantly (94.93 ± 5.82 mg/dl) in hypertensive men if checked with the normotensive men (89.73 ± 6.41 mg/dl), (Table 4.3; Figure 4.10).

**Second subgroup :** The levels of LDL increased significantly ( 96.87 ± 7.44 mg/dl) in hypertensive men if checked with the normotensive men (88.27±6.75 mg/dl). (Table 4.3; Figure 4.10).

**Third subgroup :** The levels of LDL increased significantly (112.93 ±5.89 mg/dl) in hypertensive men if checked with the normotensive men (95.8 ± 6.66 mg/dl) , (Table 4.3; Figure 4.10) .

### 4.1.4.4 The high density lipoprotein (HDL)

**First subgroup :** The results revealed that the HDL levels in hypertensive men is (29.2±2.04 mg/dl) decreased significantly ($P \leq 0.05$) if checked with the normotensive men (42.33±2.26 mg/dl) , (Table 4.3; Figure 4.11) .

**Second subgroup :** The results revealed that the HDL levels hypertensive men is (27.07 ± 2.96 mg/dl) decreased significantly ($P \leq 0.05$) in comparison with the normotensive men (40 ± 2.07mg/dl ) , (Table 4.3; Figure 4.11).

**Third subgroup :** The results revealed that the HDL levels in hypertensive men (26.4 ± 2.56 mg/dl) decreased significantly ($P \leq 0.05$) if checked with the normotensive men (38.2 ± 2.21 mg/dl) , (Table 4.3), (Figure 4.11) .

### 4.1.4.5 The very low density lipoprotein (VLDL-C)

**First subgroup :** The results revealed that the VLDL level in hypertensive men (46.8 ± 0.87 mg/dl ) increased significantly ($P \leq 0.05$) if checked with the normotensive men (36.36 ±1.508 mg/dl) , (Table 4.3; Figure 4.12) .

**Second group :** The results revealed that the VLDL level in hypertensive men (48.12 ±1.04 mg/dl) increased significantly ($P \leq 0.05$) if checked with the normotensive men (39 ± 1.76 mg/dl). ) , ( Table 4.3; Figure 4.12) .

**Third group :** The VLDL level in hypertensive men (49.75 ±1.36 mg/dl) increased significantly ($P \leq 0.05$) if checked with the normotensive men (41±1.48 mg/dl) , (Table 4.3; Figure 4.12) .

**Table ( 4.3) :lipid profile levels within the different subgroups .**

| Subgroups \ Parameters | | TC mg/dl | TG mg/dl | LDL mg/dl | HDL mg/dl | VLDL mg/dl |
|---|---|---|---|---|---|---|
| Subgroup 1 (60-65)yre | Normotensive | 164.27 ±8.43 [a] | 181.8± 7.54 [a] | 89.73± 6.41 [a] | 42.33± 2.26[a] | 36.36± 1.60 [a] |
| | Hypertensive | 190.67± 8.25 [b] | 234± 4.33 [b] | 94.93± 5.82 [b] | 29.2± 2.04[b] | 46.8± 0.87[b] |
| Subgroup 2 (50-55)yre | Normotensive | 171.8± 8.27 [a] | 195.07± 8.82 [a] | 88.27± 6.75 [a] | 40± 2.07[a] | 39± 1.76 [a] |
| | Hypertensive | 198.4 ± 8.95 [b] | 240.6± 5.18 [b] | 96.87± 7.44 [b] | 27.07± 2.96[b] | 48.12± 1.04[b] |
| Subgroup 3 (60-65)yre | Normotensive | 180.87 ± 8.97 [a] | 205 ± 7.40 [a] | 95.8± 6.66 [a] | 38.2 ± 2.21[a] | 41± 1.48 [a] |
| | Hypertensive | 203.93± 8.89 [b] | 248.73± 6.80 [b] | 112.93± 5.89 [b] | 26.4± 2.56[b] | 49.75± 1.36[b] |

***The values represent mean ± SD .**

***Similar small letters represent no significant difference between groups**

*** Different small letters represent a significant different at (P ≤ 0.05) between subgroup.**

### 4.1.5 The total protein and its fractions

### 4.1.5.1 Total protein (TP)

**First subgroup :** The results revealed that the TP levels in hypertensive men is ( 6.95 ± 0.26 g/dl) , decreased significantly (P ≤ 0.05) if checked with the normotensive men (7.43 ± 0.22 g/dl) , (Table 4.4; Figure 4.13).

**Second subgroup :** The results revealed that the TP levels in hypertensive men (6.493 ± 0.597 ng/ml) decreased significantly ( $P \leq 0.05$) if checked with the normotensive one without hypertension (7.21 ± 0.20 ng/ml) , (Table 4.4; Figure 4.13) .

**Third subgroup:** The results revealed that the TP levels in hypertensive men ( 6.5 ± 0.39 g/dl) decreased significantly ($P \leq 0.05$) if checked with the normotensive men (6.90 ± 0.24 g/dl) , (Table 4.4; Figure 4.13) .

### 4.1.5.2. The Albumin

**First subgroup :** Albumin levels ( 4.16 ± 0.29 g/dl) decreased in hypertensive men significantly ($P \leq 0.05$) when compared with the normotensive men ( 4.68 ± 0.25 g/dl) , (Table 4.4) , (Figure 4.14) .

**Second subgroup :** Albumin levels ( 3.61 ± 0.39 g/ml) decreased in hypertensive men significantly ($P \leq 0.05$) if checked with the normotensive one without hypertension (4.41 ± 0.17 ng/ml) . (Table 4.4) , (Figure 4.14).

**Third subgroup :** Albumin levels (3.54 ±0.31 g/dl) in hypertensive men decreased had asignificantly ($P \leq 0.05$) in comparison with the normotensive men (4.02 ±0.190 g/dl) , (Table 4.4; Figure 4.14).

### 4.1.5.3 The alpha one (α1) globulin

**First subgroup :** The levels of α 1 (0.35 ± 0.06 g/dl) in hypertensive men had no significantly ($P \leq 0.05$) difference in comparison with the normotensive men (0.35 ± 0.06 g/dl) , (Table 4.4; Figure 4.14) .

**Second group :** The levels of α 1 (0.37 ± 0.04 g/dl) in hypertensive men had no significantly ($P \leq 0.05$) difference in comparison with the normotensive men (0.36 ± 0.04 g/dl) , (Table 4.4; Figure 4.14) .

**Third group :** The levels of α 1 (0.39 ± 0.09 g/dl) in hypertensive men had no significantly ($P \leq 0.05$) difference if checked with the normotensive men (0.37 ± 0.03 g/dl) , (Table 4.4; Figure 4.14) .

### 4.1.5.4 The alpha two (α2) globulin

**First subgroup :** The levels of α 2 (0.46 ± 0.13 g/dl) in hypertensive men had no significantly ($P \leq 0.05$) difference if checked with the normotensive men (0.45 ± 0.05 g/dl) , (Table 4.4; Figure 4.14) .

**Second subgroup :** The levels of α 2 ( 0.49 ± 0.07 g/dl) in hypertensive men had no significantly ($P \leq 0.05$) difference if checked with the normotensive men (0.47 ± 0.091g/dl) , (Table 4.4; Figure 4.14) .

**Third subgroup :** The levels of α 2 (0.50 ± 0.08 g/dl) in hypertensive men had no significantly ($P \leq 0.05$) difference if checked with the normotensive men (0.48±0.15 g/dl) , (Table 4.4; Figure 4.14) .

### 4.1.5.5 The beta One (β1) globulin

**First subgroup :** The levels of β 1 (0.46 ± 0.09g/dl) in hypertensive men had no significantly ($P \leq 0.05$) difference in comparison with the normotensive men (0.44 ± 0.09 g/dl) . (Table 4.4; Figure 4.14) .

**Second group :** The levels of β 1 (0.46 ± 0.10 g/dl) in hypertensive men had no significantly ($P \leq 0.05$) difference in comparison with the normotensive men (0.45 ± 0.09 g/dl) . (Table 4.4; Figure 4.14) .

**Third subgroup :** The levels of β 1 (0.47 ± 0.10 g/dl) in hypertensive men had no significantly ($P \leq 0.05$) difference in comparison with the normotensive men (0.46 ± 0.08 g/dl) , (Table 4.4; Figure 4.14) .

### 4.1.5.6. The beta two (β2) globulin

**First subgroup :** The levels of β 2 (0.43 ±0.04 g/dl) in hypertensive men had no significantly ($P \leq 0.05$) difference if checked with the normotensive men (0.44± 0.05 g/dl) , (Table 4.4 ; Figure 4.14) .

**Second group :** The levels of β 2 (0.43 ± 0.05 g/dl) in hypertensive men had no significantly ($P \leq 0.05$) difference in comparison with the normotensive men (0.44 ± 0.09 g/dl) , (Table 4.4; Figure 4.14) .

**Third subgroup :** The levels of β 2 (0.43 ± 0.05 g/dl) in hypertensive men had no significantly ($P \leq 0.05$) difference in comparison with the normotensive men (0.44 ± 0.07 g/dl) , (Table 4.4; Figure 4.14) .

### 4.1.5.7 The gamma globulin

**First subgroup :** The levels of gamma (1.10 ± 0.08 g/dl) in hypertensive men had no significantly ($P \leq 0.05$) difference in comparison with the normotensive men ((1.08 ± 0.06 g/dl) , (Table 4.4; Figure 4.14).

**Second group :** The levels of gamma (1.14 ± 0.11g/dl) in hypertensive men had no significantly ($P \leq 0.05$) difference in comparison with the normotensive men (1.09 ± 0.05 g/dl) , (Table 4.4; Figure 4.14).

**Third group :** The levels of gamma (1.18 ± 0.07 g/dl) in hypertensive men had no significantly ($P \leq 0.05$) differences in comparison with the normotensive men (1.13 ± 0.09g/dl) , (Table 4.4; Figure 4.14) .

**Table ( 4.4) : Total protein levels within the different subgroups .**

| Subgroups \ Parameters | | TP g/dl | ALBUMIN g/dl | α 1 g/dl | α 2 g/dl | β 1 g/dl | B2 g/dl | GAMMA |
|---|---|---|---|---|---|---|---|---|
| **Subgroup 1 (40-45)yre** | **Normotensive** | 7.43± 0.22$^{a}$ | 4.68± 0.25$^{a}$ | 0.35± 0.06$^{a}$ | 0.45± 0.05$^{a}$ | 0.4± 0.10$^{a}$ | 0.435± 0.05$^{a}$ | 1.08± 0.06$^{a}$ |
| | **Hypertensive** | 6.95± 0.26$^{b}$ | 4.16± 0.258 | 0.35± 0.09$^{a}$ | 0.46± 0.13$^{a}$ | 0.46± 0.10$^{a}$ | 0.431± 0.04$^{a}$ | 1.09± 0.08$^{a}$ |
| **Subgroup 2 (50-55)yre** | **Normotensive** | 7.21± 0.20$^{a}$ | 4.41± 0.17$^{a}$ | 0.36± 0.04$^{a}$ | 0.46± 0.09$^{a}$ | 0.45± 0.09$^{a}$ | 0.44±0 .09$^{a}$ | 1.08± 0.05$^{a}$ |
| | **Hypertensive** | 6.49± 0.60$^{b}$ | 3.60± 0.39$^{b}$ | 0.36± 0.04$^{a}$ | 0.48± 0.07$^{a}$ | 0.46± 0.10$^{a}$ | 0.43± 0.05$^{a}$ | 1.14± 0.11$^{a}$ |
| **Subgroup 3 (60-65)yre** | **Normotensive** | 6.89± 0.24$^{a}$ | 4.02± 0.19$^{a}$ | 0.37± 0.03$^{a}$ | 0.47± 0.15$^{a}$ | 0.46± 0.08$^{a}$ | 0.44±0 .07$^{a}$ | 1.13± 0.09$^{a}$ |
| | **Hypertensive** | 6.5± 0.39$^{b}$ | 3.54± 0.31$^{b}$ | 0.38± 0.09$^{a}$ | 0.5±0 .08$^{a}$ | 0.47± 0.10$^{a}$ | 0.43±0 .05$^{a}$ | 1.18± 0.07$^{a}$ |

***The values represent mean ± SD .**

***Similar small letters represent no significant difference between subgroups . *Different small letters represent a significant difference ($P \leq 0.05$) between subgroups .**

## 4.2 The Effect of Age On The Paramters of the Normotensive Men

### 4.2.1. Hormonal Parameters

The results revealed that the T levels in the third group (2.64 ± 0.55 ng/ml) decreased significantly ($P \leq 0.05$) when compared with the second group ( 3.80 ± 0.66 ng/ml) and with the first group (4.56 ± 0.59 ng/dl) . The T levels in the second group (3.80 ± 0.66 ng/ml) decreased significantly ($P \leq 0.05$) when compared with the first group (4.56 ± 0.59) , (Table 4.5; Figure 4.20).

The results revealed that the $E_2$ levels in the third group (36.44 ± 1.45 pg/ml) increased significantly ($P \leq 0.05$) when compared with the second group (35.26±1.47 pg/ml) and with the first group(32.84 ± 1.54 pg/ml) . the $E_2$ levels in the second group (35.26 ± 1.43 pg/ml) increased significantly ($P \leq 0.05$) when compared with the first group (32.84 ± 1.54 pg/ml) , (Table 4.5; Figure 4.20) .

The results revealed that the PRL levels in the third group (6.86 ± 0.51 ng/ml) increased significantly ($P \leq 0.05$) when compared with the second group (6.23 ± 0.79 ng/ml) and with the first group (5.60 ±0.99 ng/ml) . The PRL levels in the second group (6.23 ± 0.79 ng/ml) increased significantly ($P \leq 0.05$) when compared with the first group (5.60 ± 0.99 ng/ml) , (Table 4.5; Figure 4.20) .

**Table (4.5 ) :The Hormonal parameters ( Testosterone , Estradiol and Prolactin ) in normotensive men between different groups .**

| Parameter / Groups | Testosterone ng/dl | Estradiol pg/ml | Prolactin ng/ml |
|---|---|---|---|
| First group(40-45)yre | 4.563 ± 0.593 [a] | 32.84±1.54 [a] | 5.60±0.99 [a] |
| Second group(50-55)yre | 3.80 ± 0.66 [b] | 35.26±1.426 [b] | 6.23±0.79 [b] |
| Third group(60-65)yre | 2.64 ± 0.55 [c] | 36.44±1.45[c] | 6.86±0.51 [c] |

***The values represent mean ± SD .**

***Similar small letters represent no significant difference between groups.**

*** Different small letters represent a significant different at (P ≤ 0.05) between subgroup.**

## 4.2.2 Inflammatory markers

The results revealed that the TNFα levels in the third group (5.96 ± 0.60 pg/ml) had significant difference ($P \leq 0.05$) when compared with the second group (4.65 ± 0.39 pg/ml) and with the first group (4.06 ± 0.12 pg/ml), the TNFα levels in the second group (4.65 ± 0.39 pg/ml) had significantly difference ($P \leq 0.05$) if checked with the first group (4.06 ± 0.12 pg/ml). (Table 4.6; Figure 4.21).

The results revealed that the In6 levels in the third group ( 0.89 ± 0.41 pg/ml) had no significant difference ($P \leq 0.05$) when compared with the second group (0.82 ± 0.37 pg/ml) and had no significant difference with the first group (0.59± 0.24 pg/ml) . The In6 levels in the second group (0.82 ± 0.37 pg/ml ) had no significant difference ($P \leq 0.05$) when compared with the first group (0.59 ± 0.24 pg/ml) . (Table ,4.6; Figure 4.21).

The results revealed that the CRP levels in the third group (2.10 ± 0.61 mg/dl) had no significant difference ($P \leq 0.05$) when compared with the second group (2.03 ± 0.79 mg/dl) and with the first group (1.917 ± 0.48 mg/dl) . The CRP levels in the second group ( 2.03 ± 0.79 mg/dl) had no significant difference ($P \leq 0.05$) when compard with the first group (1.92 ± 0.48 mg/dl) . (Table 4.6; Figure 4.21) .

### 4.2.3. Nitric Oxide

The results revealed that the nitric Oxide levels in the third group (0.42 ±0.02 µmol/L) decreased significantly ($P \leq 0.05$) in comparison with the second group (0.44 ± 0.01 µmol/L) and with the first group (0.47 ± 0.03 µmol/L) . The nitric Oxide levels in the second group (0.44 ±0.01 µmol/L) decreased significantly ($P \leq 0.05$) in comparison with the first group (0.47 ± 0.03 µmol/L) . (Table 4.6; Figure 4.21) .

**Table (4.6 ):The levels of (TNFα , IL6 , PRL and NO) in normotensive men between different groups .**

| Parameters / Groups | TNF α pg/ml | IL6 pg/ml | CRP mg/dl | NO µmol/L |
|---|---|---|---|---|
| First group (40-45)yre | 4.06 ± 0.12[a] | 0.59 ±0.24[a] | 1.92 ± 0.48[a] | 0.47 ± 0.03[a] |
| Second group (50-55)yre | 4.65 ± 0.39[b] | 0.82 ±0.37[a] | 2.03 ± 0.79[a] | 0.44 ± 0.01[b] |
| Third group (60-65)yre | 5.96 ± 0.60[c] | 0.89 ±0.42[a] | 2.10 ± 0.61[a] | 0.42 ± 0.02[c] |

***The values represent mean ± SD .**

***Similar small letters represent no significant difference between groups**

*** Different small letters represent a significant different at (P ≤ 0.05) between subgroup.**

## 4.2.4 Lipid profile parameters

The results revealed that the TC levels in the third group (180.87 ± 8.97 mg/ml) increased significantly (P ≤ 0.05) when compared with the second group (171.8 ± 8.27 mg/dl) and with the first group(164.27 ±8.43 mg/dl) . The TC levels in the second group (171.8 ± 8.27 mg/dl ) increased significantly (P

≤ 0.05) when compared with the first group (164.27 ± 8.43 mg/dl) . (Table 4.7; Figure 4.22).

The results revealed that the TG levels in the third group (205±7.40 mg/dl) increased significantly (P ≤ 0.05) when compared with the second group (195.07 ± 8.82 mg/dl) and with the first group (181.8±7.542 mg/dl) . The TG levels in the second group (195.07 ± 8.82 mg/dl ) increased significantly (P ≤ 0.05) when compared with the first group (181.8 ± 7.54 mg/dl) . (Table 4.7; Figure 4.22) .

The results revealed that the LDL levels in the third group (95.8 ± 6.66g/dl) decreased significantly (P ≤ 0.05) when compared with the second group (88.27 ± 6.75 g/dl) and with the first group (89.73 ± 6.41 g/dl) . The LDL levels in the second group (88.27 ± 6.75 g/dl ) had no significant difference (P ≤ 0.05) when compared with the first group (89.73 ± 6.41 g/dl) . (Table 4.7; Figure 4.22) .

The results revealed that the HDL levels in the third group (38.2 ± 2.21 mg/dl) decreased significantly (P ≤ 0.05) when compared with the second group (40 ± 2.07 mg/dl) and with the first group (42.33 ± 2.26 mg/dl) . The HDL levels in the second group (40 ± 2.07 mg/dl) decreased significantly (P ≤ 0.05) when compared with the first group (42.33 ± 2.26 mg/dl) . (Table 4.7; Figure 4.22) .

The results revealed that the VLDL levels in the third group (41±1.48 mg/ml) had a significant difference (P ≤ 0.05) if checked with the second group (39 ± 1.76 mg/dl) and with the first group (36.36 ±1.51 mg/dl) , The VLDL levels in the second group (39 ±1.76 mg/dl) had a significant difference (P ≤ 0.05) if checked with the first group (36.36 ± 1.51 mg/dl) . (Table 4.7; Figure 4.22) .

**Table (4.7) Lipid profile levels in normotensive men for different groups.**

| parameters / Groups | TC mg/dl | TG mg/dl | LDL mg/dl | HDL mg/dl | VLDL mg/dl |
|---|---|---|---|---|---|
| First group (40-45)yre | 164.27± 8.43 [a] | 181.8± 7.54 [a] | 89.73± 6.41 [a] | 42.33± 2.26[a] | 36.36± 1.51[a] |
| Second group (50-55)yre | 171.8± 8.27 [b] | 195.07± 8.82 [b] | 88.27± 6.75 [a] | 40± 2.07[b] | 39 ± 1.76 [b] |
| Third group (60-65)yre | 180.87± 8.96[c] | 205 ± 7.40 [c] | 95.8± 6.66 [b] | 38.2± 2.21[c] | 41 ± 1.48[c] |

***The values represent mean ± SD .**

***Similar small letters represent no significant difference between groups.**

***Different small The letters indicate a statistical difference (P value ≤ 0.05) between groups .**

## 4.2.5. Total protein and its fractions

The results revealed that the TP levels in the third group (6.89 ±0.24 g/dl) had a significant difference (P ≤ 0.05) when compared with the second group (7.21 ± 0.20 g/dl) and with the first group (7.43 ± 0.22 g/dl) , The TP levels in the second group (7.21 ± 0.20 g/dl ) had no significant difference (P value ≤ 0.05) when compared with the first group (7.43 ± 0.22 g/dl) . (Table 4.8; Figure 4.2)) .

The results revealed that the albumin levels in the third group (4.02 ± 0.19 g/dl) had a significant difference (P ≤ 0.05) when compared with the second group (4.41 ± 0.17 g/dl) and with the first group(4.68 ± 0.25 g/dl) . The albumin levels in the second group (4.41 ± 0.17 g/dl ) had a significant difference (P ≤ 0.05) if checked with the first group (4.68 ± 0.25 g/dl) . (Table 4.8; Figure 4.23) .

The results revealed that the α 1 levels in the third group (0.37 ± 0.03 g/dl) had no significantly difference (P ≤ 0.05) if checked with the second group (0.36 ± 0.04 g/dl) and with the first group (0.35 ± 0.06 g/dl) . The α 1 levels in the second group (0.36 ± 0.04 g/dl) had no significant difference (P ≤ 0.05) if checked with the first group (0.35 ± 0.06 g/dl) . (Table 4.8; Figure 4.23) .

The results revealed that α 2 levels in the third group (0.48 ± 0.15 g/dl) had no significantly difference (P ≤ 0.05) if checked with the second group (0.47 ± 0.09 g/dl) and with the first group (0.45 ± 0.05 g/dl) , The α 2 levels in the second group (0.47 ± 0.09 g/dl) had no significantly difference (P ≤ 0.05) if checked with the first group (0.45 ± 0.05 g/dl) . (Table 4.8 ; Figure 4.23) .

The results revealed that the β 1 levels in the third group (0.46 ± 0.08 g/dl) had no significantly difference (P ≤ 0.05) if checked with the second group (0.45 ± 0.09 g/dl) and with the first group (0.44 ± 0.09 g/dl) . The β 1 levels in the second group (0.45 ± 0.09 g/dl ) had no significantly difference (P ≤ 0.05) if checked with the first group (0.44 ± 0.09 g/dl) . (Table 4.8; Figure 4.23) .

The results revealed that the β 2 levels in the third group (0.44 ± 0.07 g/dl) had no significantly difference (P ≤ 0.05) if checked with the second group (0.44 ± 0.09 g/dl) and with the first group (0.44 ± 0.05 g/dl) . The β 2 levels in the second group (0.44 ± 0.09 g/dl ) had no significantly difference (P ≤ 0.05) if checked with the first group (0.44 ± 0.05 g/dl) .

The results revealed that the gamma levels in the third group (1.13 ± 0.09 g/dl) had no significantly difference ($P \leq 0.05$) if checked with the second group (1.086 ± 0.053 g/dl) and with the first group (1.08 ± 0.06 g/dl) . The gamma levels in the second group (1.09 ± 0.05mg/ml ) had no significantly difference ($P \leq 0.05$) in differentiate with the first group (1.08 ± 0.06 g/dl) . (Table 4.8; Figure 4.23) .

**Table (4.8) : Total Protein levels in normotensive men for different groups .**

| Parameters / Groups | TP g/dl | Albumin g/dl | Alpha1 g/dl | Alpha 2 g/dl | Beta 1 g/dl | Beta 2 g/dl | Gamma g/dl |
|---|---|---|---|---|---|---|---|
| Group 1 (40-45)yre | 7.43± 0.22$^{a}$ | 4.68± 0.25$^{a}$ | 0.35± 0.06$^{a}$ | 0.45± 0.05$^{a}$ | 0.44± 0.09$^{a}$ | 0.44± 0.05$^{a}$ | 1.08± 0.06$^{a}$ |
| Group 2 (50-55)yre | 7.21± 0.20$^{a}$ | 4.41± 0.17$^{b}$ | 0.36± 0.04$^{a}$ | 0.47± 0.09$^{a}$ | 0.45± 0.09$^{a}$ | 0.44± 0.09$^{a}$ | 1.09± 0.05$^{a}$ |
| Group 3 (60-65)yre | 6.89± 0.24$^{b}$ | 4.02± 0.19$^{c}$ | 0.37± 0.03$^{a}$ | 0.48± 0.15$^{a}$ | 0.46± 0.08$^{a}$ | 0.44± 0.07$^{a}$ | 1.13± 0.09$^{a}$ |

***The values represent mean ± SD .**

***Similar small letters represent no significant difference between groups .**

*** Different small letters represent a significant different at ($P \leq 0.05$) between subgroup.**

## 4.3 The Effect of Age on the Parameters of the Hypertensive Men

### 4.3.1. Hormonal parameters

The results revealed that the testosterone levels in the third group (2.08 ± 0.52 ng/ml) decreased statically ($P \leq 0.05$) if checked with the second group (2.661 ± 0.682 ng/ml) and with the first group (3.21 ± 0.68 ng/ml) . The testosterone levels in the second group (2.66 ± 0.68 ng/ml) decreased significantly ($P \leq 0.05$) if checked with the first group (3.21 ± 0.68 ng/ml) . (Table 4.9; Figure 4.24)).

The results revealed that the $E_2$ levels in the third group (39.29 ± 0.85 pg/ml) increased significantly ($P \leq 0.05$) if checked with the second group (38.18 ± 0.85 pg/ml) and with the first group (36.29 ± 0.78 pg/ml) , the $E_2$ levels in the second group (38.18 ± 0.85 pg/ml) increased significantly (P value ≤ 0.05) in differentiate with the first group (36.29 ± 0.78 pg/ml). (Table 4.9; Figure 4.24).

The results revealed that the PRL levels in the third group (8.51 ±1.04 ng/ml) increased significantly ($P \leq 0.05$) if checked with the second group (6.97 ±0.94 ng/ml) and with the first group (6.21 ± 0.44 ng/dl) . The PRL levels in the second group (6.97 ± 0.94 ng/ml) increased significantly ($P \leq 0.05$) in differentiate with the first group (6.21 ± 0.44 ng/dl). (Table 4.9; Figure 4.24).

**Table(4.9): Hormonal parameters ( T , E2 and PRL ) in hypertensive men between different groups .**

| Parameters / Groups | Testosterone ng/ml | Estradiol pg/ml | Prolactin ng/dl |
|---|---|---|---|
| First group(40-45)yre | 3.21 ± 0.68 [a] | 36.29 ± 0.78 [a] | 6.21 ± 0.44 [a] |
| Second group(50-55)yre | 2.66 ± 0.68 [b] | 38.18 ± 0.85 [b] | 6.97 ± 0.94 [b] |
| Third group(60-65)yre | 2.079 ± 0.52 [c] | 39.29 ± 0.85 [c] | 8.51 ± 1.04 [c] |

***The values represent mean ± SD .*Similar small letters represent no significant difference between groups * Different small letters represent a significant different at (P ≤ 0.05) between subgroup.**

### 4.3.2 Inflammatory markers

The results revealed that the TNFα levels in the third group (8.31 ± 0.61 pg/ml) increased significantly (P ≤ 0.05) in differentiated with the second group (6.05 ± 0.77 pg/ml) and with the first group (4.64 ± 0.76 pg/ml) . The TNFα levels in the second group (6.05 ± 0.77 pg/ml) had significant difference (P ≤ 0.05) if checked with the first group (4.64 ± 0.76 pg/ml). (Table 4.10; Figure 4.25).

The results revealed that the In6 levels in the third group (2.42 ± 0.74 pg/ml) increased significantly (P ≤ 0.05) if checked with the second group (1.74 ± 0.79 pg/ml) and with the first group (1.16 ± 0.53 pg/ml) . The In6 levels in the

second group (1.74 ± 0.79 pg/ml) increased statically ($P \leq 0.05$) in differentiated with the first group (1.16 ± 0.53 pg/ml) . (Table 4.10; Figure 4.25).

The results revealed that the CRP levels in the third group (5.45 ± 0.67 mg/dl) increased significantly ($P \leq 0.05$) in different with the second group (4.18 ± 0.98 mg/dl) and with the first group (3.50 ± 0.83 mg/dl) . The CRP levels in the second group (4.18 ± 0.98 mg/dl ) increased significantly ($P \leq 0.05$) if checked with the first group ( 3.50 ± 0.83 mg/dl) . (Table 4.10;Figure 4.25).

### 4.3.3. Nitric oxide

The results revealed that the NO levels in the first group (0.44 ± 0.02 µmol/L) increased significantly ($P \leq 0.05$) in differentiated with the second group (0.42 ± 0.02 µmol/L ) and with the third group (0.41 ± 0.01 µmol/L) . The NO levels in the second group (0.42 ± 0.02 µmol/L ) had no significantly differences ($P \leq 0.05$) if checked with the third group (0.41 ± 0.01 µmol/L ) . (Table 4.10; Figure 4.25).

**Table (4.10): The levels of TNFα, IL6, CRP and NO in the hypertensive men for different groups .**

| Parameter / Groups | TNF α pg/ml | IL6 pg/ml | CRP mg/dl | NO µmol/L |
|---|---|---|---|---|
| First group(40-45)yre | 4.64± 0.76 [a] | 1.16±0.53 [a] | 3.50±0.83[a] | 0.44±002[a] |
| Second group(50-55)yre | 6.05±0.77[b] | 1.74±0.79 [b] | 4.18±0.98[b] | 0.42±0.02[b] |
| Third group(60-65)yre | 8.31± 0.61 [c] | 2.42±0.74[c] | 5.45±0.67[c] | 0.41±0.01[b] |

***The values represent mean ± SD .**

***Similar small letters represent no significant difference between groups**

*** Different small letters represent a significant different at (P ≤ 0.05) between subgroup.**

### 4.3.4. Lipid profile parameters

The results revealed that the TC levels in the third group (203.93 ± 8.89 mg/dl) increased significantly (P ≤ 0.05) if checked with the first group(190.67 ± 8.25 mg/dl) but had no significantly difference with the second group (198.4±8.95 mg/dl). The TC levels in the second group (198.4 ± 8.95 mg/dl) had increased significantly (P ≤ 0.05) in comparison with the first group (190.67 ± 8.25 mg/dl) . (Table 4.11; Figure 4.26).

The results revealed that the TG levels in the third group (248.73 ± 6.80 mg/dl) increased significantly (P ≤ 0.05) when compared with the second group (240.6 ± 5.18 mg/dl) and with the first group (234 ±4.33 mg/dl) , the TG levels in

the second group (240.6 ± 5.18 mg/dl ) increased significantly ($P \leq 0.05$) when compared with the first group (234 ± 4.33 mg/dl). (Table 4.11; Figure 4.26).

The results revealed that LDL levels in the third group (112.93 ± 5.89 b mg/dl) increased significantly ($P \leq 0.05$) if checked with when compared with the first group (94.93 ± 5.82 mg/dl) and the second group (96.87 ±7.44 mg/dl) .The LDL levels in the second group (96.87 ± 7.44 mg/dl ) had no significant difference ($P \leq 0.05$) when compared with the first group (94.93 ± 5.82 mg/dl). (Table 4.11; Figure 4.26).

The results revealed that the HDL levels in the first group (29.2 ± 2.04 mg/dl) increased significantly ($P \leq 0.05$) when compared with the second group (27.07 ± 2.96 mg/dl) and with the third group (26.4 ± 2.56 mg/dl) . The HDL levels in the second group (27.07 ± 2.96 mg/dl) had no significantly differences ($P \leq 0.05$) when compared with the third group (26.4 ±2.56 mg/dl) . (Table 4.11; Figure 4.26).

The results revealed that the VLDL levels in the third group (49.75± 1.36 mg/dl) decreased significantly ($P \leq 0.05$) in comparison with the second group (48.12 ± 1.04 mg/dl) and with the first group (46.80 ± 0.87 mg/dl) . The VLDL levels in the second group (48.12 ± 1.04 mg/dl) had a significantly differences ($P \leq 0.05$) in comparison with the first group (46.80 ± 0.87 mg/dl) . (Table 4.11; Figure 4.26).

**Table( 4.11) : The levels of lipid profile in the Hypertension men during progressive age in the different groups .**

| parameters / Groups | TC mg/dl | TG mg/dl | LDL mg/dl | HDL mg/dl | VLDL mg/dl |
|---|---|---|---|---|---|
| First group (40-45)yre | 190.67± 8.25[a] | 234± 4.33[a] | 94.93± 5.82[a] | 29.2± 2.04[a] | 46.8± 0.87[a] |
| Second group (50-55)yre | 198.4± 8.95[b] | 240.6± 5.18[b] | 96.87± 7.44[a] | 27.07± 2.96[b] | 48.12± 1.04[b] |
| Third group (60-65)yre | 203.93± 8.87[b] | 248.73± 6.80[c] | 112.93± 5.89[b] | 26.4± 2.558[b] | 49.75± 1.34[c] |

***The values represent mean ± SD .**

***Similar small letters represent no significant difference between groups .**

*** Different small letters represent a significant different at (P ≤ 0.05) between subgroup.**

## 4.4.5. Total protein and its fractions

The results revealed that the TP levels in the third group (6.5±0.39 g/dl) had no significant difference (P ≤ 0.05) when compared with the second group (6.49 ± 0.60 g/dl) and had a significant difference with the first group (6.95 ± 0.26 g/dl) . The TP levels in the second group (6.49 ± 0.60 g/dl) had a significantly differences (P ≤ 0.05) when compared with the first group (6.95 ±0.26 g/dl) . (Table 4.12; Figure 4.27).

The results revealed that the albumin levels in the third group (3.59 ±0.31 g/dl) had no significant difference (P ≤ 0.05) when compared with the second

group (3.61 ± 0.39 g/dl) but had a significant difference with the first group (4.16 ± 0.26 g/dl) . The albumin levels in the second group (3.61 ± 0.39 g/dl) a significantly defrences ($P \leq 0.05$) when compared with the first group (4.16 ± 0.26 g/dl) . (Table 4.12; Figure 4.27).

The results revealed that the α 1 levels in the third group (0.39 ± 0.09 g/dl) had no significant difference ($P \leq 0.05$) when compared with the second group (0.37 ± 0.04 g/dl) and with the first group (0.35 ± 0.06 g/dl) . The α 1 levels in the second group (0.37 ± 0.04 g/dl) had no significant difference ($P \leq 0.05$) when compared with the first group (0.35 ± 0.09 g/dl) . (Table 4.12; Figure 4.27).

Results revealed that the α 2 levels in the third group (0.50 ± 0.08 g/dl) had no significant difference ($P \leq 0.05$) when compared with the second group (0.49 ± 0.07 g/dl) and with the first group(0.46 ± 0.13 g/dl) . The α 2 levels in the second group (0.49 ± 0.07 g/dl)had no significant difference ($P \leq 0.05$) when compared with the first group (0.46 ±0.13 g/dl) . (Table 4.12; Figure 4.27).

The results revealed that the β 1 levels in the third group (0.47 ± 0.10 g/dl) had no significant difference ($P \leq 0.05$) when compared with the second group (0.46 ± 0.10 g/dl) and with the first group(0.46 ±0.09 g/dl) . The β 1 levels in the second group (0.46 ± 0.10 g/dl) had no significant difference ($P \leq 0.05$) when compared with the first group (0.46 ± 0.09 g/dl) . (Table 4.12; Figure 4.27) .

The results revealed that the β2 levels in the third group (0.43 ±0.05 g/dl) had no significant difference ($P \leq 0.05$) in comparison with the second group (0.43 ± 0.05 g/dl) and with the first group (0.43 ± 0.04 g/dl) . The β2 levels in the second group (0.43 ± 0.05 g/dl) had no significant difference ($P \leq 0.05$) if checked with the first group (0.43± 0.036 g/dl) . (Table 4.12; Figure 4.27) .

The results revealed that the γ levels in the third group (1.18 ± 0.07 g/dl) had no significant difference ($P \leq 0.05$) when compared with the second group (1.14 ± 0.11g/dl) but had a significant difference with the first group (1.10 ± 0.08 g/dl) . The γ levels in the second group (1.14 ± 0.11 g/dl) had no significant difference ($P \leq 0.05$) when compared with the first group (1.10 ± 0.08 g/dl) . (Table 4.12; Figure 4.27).

**Table (4.12) The levels of total protein in the Hypertensive men during progressive age in the different groups**

| Parameters / Groups | TP g/dl | Albumin g/dl | α 1 g/dl | α 2 g/dl | β 1 g/dl | β 2 g/dl | γ g/dl |
|---|---|---|---|---|---|---|---|
| Group1 (40-45)yre | 6.95± 0.26$^{a}$ | 4.16± 0.26$^{a}$ | 0.35± 0.06$^{a}$ | 0.46± 0.13$^{a}$ | 0.46± 0.09$^{a}$ | 0.43± 0.04$^{a}$ | 1.10± 0.08$^{a}$ |
| Group 2 (50-55)yre | 6.49± 0.60$^{b}$ | 3.61± 0.39$^{b}$ | 0.37± 0.04$^{a}$ | 0.49± 0.07$^{a}$ | 0.46± 0.10$^{a}$ | 0.43± 0.05$^{a}$ | 1.14± 0.11$^{ab}$ |
| Group 3 (60-65)yre | 6.5± 0.39$^{b}$ | 3.54± 0.31$^{b}$ | 0.39± 0.09$^{a}$ | 0.5± 0.08$^{a}$ | 0.47± 0.10$^{a}$ | 0.43± 0.06$^{a}$ | 1.18± 0.07$^{b}$ |

***The values represent mean ± SD .**

***Similar small letters represent no significant difference between groups .**

***Different small letters represent a significant difference ($p \leq 0.05$) between groups .**

## 4.4 Correlation between testosterone levels and other parameters in normotensive and hypertensive men during progressive age :

The results reveled that testosterone hormone correlate significantly and non-significantly with the all findings of this study as the following :

### 4.4.1 - Normotensive men

A- A significant ( $p \leq 0.01$) positive correlation with nitric Oxide (0.535) , HDL (0.496) , TP (0.477) and albumin (0.617).

B- A significant ($p \leq 0.01$) negative correlation with estradiol (-0.450) , prolactin (-0.507 ) , TNF α (-0.736) , IL6 (-0.438) , TC (-0.528) , TG (-0.624 ) , VLDL (-0.624) and for (p ) with , LDL (-0.337) and gamma (-0.359) .

C- Not a significant positive correlation with beta two (0.019) and un significant negative correlation with CRP (-0.185 ), alpha one (-0.067), alpha two (-0.134 ), and Beta one (-0.264 ).

### 4.4.2 Hypertensive men

A- A significant ( $p \leq 0.05$) positive correlation with TP ( 0.329) and Albumin ( 0.357) .

B- A significant ($p \leq 0.01$) negative correlation with estradiol (-(0.416) , prolactin ( -0.395 ) , TNF α (-0.583 ) , TG ( -0.530) , VLDL (-0.530 ) and for ($p \leq 0.05$ ) with IL6 ( -0.322) , LDL ( -0.316) , TC( -0.335 ) .

C- Not a significant positive correlation with nitric oxide (0.294) , HDL ( 0.064) , alpha two (-0.015 ) , beta one (0.124) and beta two (0.235) and negative

correlation with CRP (-0.203 ) , alpha one (-0.092) , and gamma ( -0.161) .Table (4.13) .

**Table (4.13) : Correlation between testosterone levels and other parameters in normotensive and hypertensive men during progressive age.**

| | Normotensive | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variables** | **Estradiol** | **Prolactin** | **TNF** | **IL6** | **CRP** | **NO** | **TC** | **TG** | **LDL** | **HDL** | **VLDL** | **TP** | **Albumin** | **Alpha1** | **Alpha2** | **Beta1** | **Beta2** | **Gamma** |
| **Testosterone** | -.450** | -.507** | -.736** | -.438** | -0.185 | .535** | -.528** | -.624** | -.337* | .496** | -.624** | .477** | .617** | -0.067 | -0.134 | -0.264 | 0.019 | -.359* |
| | **Hypertensive** | | | | | | | | | | | | | | | | | |
| | -.416** | -.395** | -.583** | -.322* | -0.203 | 0.294 | -.335* | -.530** | -.316* | 0.064 | -.530** | .329* | .357* | -0.029 | 0.015 | 0.124 | 0.235 | -0.161 |

* Significant at the level of 0.05 (2-tailed) , ** Significant at the level of 0.01 (2-tailed) .

# Chapter Five

# Discussion

# 5. Discussion

## 5.1 Hormonal parameters

### 5.1.1Testosterone

The current results demonstrated that testosterone levels reduced significantly during both the progressive age and hypertension for the all samples for different groups and subgroups (Table 4.1 , Table 4.5 and Table 4.9 ).

This reduction in testosterone levels associated with the progressive ages may be attributed to dysfunction of hypothalamic - pituitary- gonads axis , unresponsiveness of leydig cells for LH , continual decrease in age Leydig cells number and / or the increment releases of ROS.

Xia and his colleagues (2017) reported that the progressive age caused a reduction in the testosterone levels may be via : the reduction in hypothalamic GnRH outflow , decreased testicular responsiveness to hCG / LH and attenuated androgenic negative feedback , moreover they also been found that ROS derived from the mitochondrial electron transport chain , steroidogenesis , and / or macrophages during aging affect cAMP production and cholesterol transport into the mitochondria by altering the redox environment of the age Leydig cells , therefore a reduction in testosterone levels . Furthrmore , many studies agreed with these finding due to a reduced steroidogenic enzymic activity , and attenuation of the testicular response to LH and the inhibiter role of ROS in testosterone production by the Leydig cells via dissipating mitochondrial membrane potential , therefore , reducing the expression and activity of testicular steroidogenic enzymes ( Syntin *et al.*, 2001; Wang and Stocco, 2005 ; Duan *et al.*, 2016).

these findings related with reduction levels of testosterone that associated with the hypertension may be attributed to dysfunction in the endothelial layer , and this dysfunction leading to many aspect problems including the low blood flow, may resulted in decrease LH level or change the receptor levels and reducing the stimulation of the testes resulting in the decline of testosterone production .

Fogari and his colleagues (2005) agreed with the conclusion of current study that the hypertensive persons had lower levels of testosterone , and that might be refer to either testosterone influences on blood pressure (BP) regulation or / and elevated BP negatively affects steroidogenesis or clearance and / or there are genes involved in the regulation of BP that also affect steroidogenesis , this latter possibility is supported by data demonstrating that men with a family history of hypertension have been shown to have a lower levels of testosterone than normal men .

Several studies have demonstrated that the vasodilation effects of testosterone on smooth muscle could be by dihydropyridine channel (Orshal and Khalil , 2004 ; Hall *et al.*, 2006 ) , therefore , testosterone replacement therapy had beneficial effects on blood pressure in human , that means the androgen suppressive therapy increased the blood pressure (Zitzmann and Nieschlag , 2007) , and there is a vasodilator response due to testosterone treatment at physiological concentrations in radial artery patients undergoing bypass surgery (Kelly and Jones, 2013) , Furthmore , testosterone may exert antihypertensive effects by inducing the relaxation of aorta in rat and rabbit animals ( Perusquía *et al.*, 2017).

In addition , Colli and his colleagues (2019) showed in rat , that the hypertension is a main cause for the testicular damage associated to alterations

in the local microcirculation , increased ROS activity , increased expression of testicular hypoxia-induced proteins , impaired mitochondrial activity .

Kapasi and his colleagues (1996) , showed that in hypertensive rats the isolated leydig cells secreted more testosterone than the isolated Leydig cells in normal rats due to the more ANP stimulation in hypertensive rats . ANP / ANF are Atrial natriuretic peptides / factors to produce testosterone from testes and LH from anterior pituitary gland (Pandey, 2014).

Nevertheless , many studies pointed that no changes occurred in the testosterone levels related with both the progressive age (Fukai *et al.*, 2010 ; Halmenschlager *et al.*, 2011 ; Sartorius *et al.*, 2012 ) , and hypertension ( Khaw *et al.*, 2007 ; Blaya *et al.*, 2016).

### 5.1.2 Estradiol

The current results demonstrated that estradiol levels increased significantly during both the progressive age and hypertension for the all samples (Table 4.1 , Table 4.5 and Table 4.9 ).

These increased levels of estradiol associated with the progressive ages may be attributed to the ageing influencing on the aromatization process , which the intensity of this process be increased related with the progressive age .

The present result agreed with many studies concerning the relationship between estradiol and progressive age (Akishita *et al.*, 2010 ; Jasuja *et al.*, 2013 ; Yeap *et al.*, 2014 ). Estradiol in circulation is produced in males from the testes ~20% and the remainder from the skin , adipose , bone and brain by the aromatization process of testosterone (Cooke *et al.*, 2017). Most circulating 17β-estradiol in men is produced from aromatization of testosterone , predominantly in adipose tissue (Finkelstein *et al.*, 2013).

Cohen and his colleagues (2001) and Vermeulen and his colleagues (2002) mentioned that the aromatase activity increases with age and this high activity of aromatase enzyme related with age and age-associated with fat tissue increase even without weight gain.

However , Muller and his team (2003) , they found no changeable level in estradiol hormone in elderly people , while Lewerin and his colleagues (2014) they pointed a decreasable change in estradiol levels with the progressive age .

In the other hand , the present study revealed that hypertension has a significant effect on the levels of estradiol hormone in different groups and subgroups , these findings are related with the increasing levels of estradiol and decreasing levels of testosterone associated with the progressive age respectively , and might be caused due to the high activity of aromatization process companioned to age , this process leading to these both changeable of hormones levels beside other probable dysfunction that an elderly men expose.

Shimodaira and his colleagues (2008) , found that estrogens and the cytochrome P450 19 (CYP19) gene (aromatase) are thought to be susceptibility factors for essential hypertension , also Spratt and his colleagues (2006) , concluded that the primary cause of increased estrogen levels in acute illness is increased aromatase P450 gene expression, resulting in enhanced aromatization of androgens to estrogens, a previously un described endocrine response to acute illness .

It has been found that ERβ and E2 exposure increased atherosclerosis in coronary arteries harvested from men , suggesting a role for E2 in early coronary atherosclerosis (Stanhewicz *et al.,* 2018). Circulating levels of cortisol , tumor necrosis factor-α , IL-1 , IL-6, and IL-10 are all elevated in acute

illness and have been demonstrated to increase aromatase activity in vitro (Simpson *et al.*, 2002)

### 5.1.3 Prolactin

The current results demonstrated that prolactin levels increased significantly during both the progressive age and hypertension for the all samples in different groups and subgroups (Table 4.1 , Table 4.5 and Table 4.9 ).

To discuss these significant high increase in prolactin levels it must be linked to some studied parameters such as estradiol increment and testosterone reduction during both advancing age and hypertension , that they reflect the picture of prolactin hormone during these studies circumstances .

Serum prolactin rose slightly with increasing age in men , and still un changeable after estrogen treatment (sawin *et al.*, 1989) . Roelfsema and his colleagues (2012) , found that prolactin has a significant association with BMI and rather exhibited a slight 18% increase with age .

Normal prolactin levels correlated with parameters associated with hypertension , while high or very high levels of prolactin (above reference values) might adversely affect endothelial function and perhaps other markers of atheromatosis (Clapp *et al.*, 1994) , prolactin receptors were discovered in atherosclerotic lesions of the coronary arteries (Roselli , *et al.*, 2008) , which further indicates the probable role of prolactin in atherosclerosis . The association of daily fluctuations of circulating prolactin with decreased endothelial function in men with arterial hypertension was also described by Stamatelopoulos et al.,( 2011).

Estradiol is one of the mediators that stimulated prolactin secretion (Ignacak *et al.*, 2012) , and this mediation may be enhancing the effect of TRH

and inhibiting that of dopamine (Levine and Muneyyirci-Delale , 2018 ). Prolactin levels tended to be slightly higher in hypertensive men with lower testosterone , with substantial correlation of prolactin level with parameters of arterial stiffness , the correlation of prolactin with testosterone was insignificant (Grabowska-Markowska *et al.,* 2019). Hyper secretion of prolactin in men has been associated with decreased sexual desire , infertility , reduction of testosterone and erectile dysfunction (Maria , 2016) . New fathers had lower levels of testosterone but higher levels of prolactin than new paired males ( Wang *et al.*, 2018) .

Physiologic levels of prolactin in males enhance LH-receptors in Leydig cells , resulting in testosterone secretion plus spermatogenesis ( Hair *et al.*, 2002 ). Hyperprolactinemia induces hypogonadism by interfering with the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus , and in turn decreases the secretion of FSH and LH from the anterior pituitary , the resulting decrease in serum testosterone ( Zeitlin and Rajfe , 2000 ).

Estrogens are known to promote the proliferation of lactotropic cells as well as the synthesis and lactotropes secretion of PRL , which may explain why hyperprolactinaemia is characterized by a femal preponderance (Colao *et al.*, 2003) . The number of lactotropes in emasculate and testosterone-replaced emasculate male rats did not vary , the sensitivity of androgen to aromatization was discovered to influence the intensity of lactotroph feature (Krysiak *et al.*, 2020) . Unlike non-aromatizable androgens , aromatizable androgens stimulated PRL release (Barrado *et al.,* 2014) .

## 5.2 Pro-inflammatory markers

The present study revealed that TNF α , IL6 , CRP increase significantly in both progressive age and hypertension in all different subgroups and groups ( except the CRP concentration between the normotensive groups ) (Table 4.2 , Table 4.6, Table 4.10).

In order to discuss the impact and the influences of progressive aging and hypertension on the presented studied parameters (TNFα , IL6, CRP ) , the aging and hypertension considered as a phenomenon of a progressive increase in pro-inflammatory status , and a chronic inflammatory state , respectively , both of them leading to this significant increase in current pro-inflammatory markers findings.

In addition , testosterone recognized as anti-inflammatory agent that have a suppressive role of pro-inflammatory markers , therefore , the present finding of results showed of reduction in testosterone hormone levels associated with the progressive age and hypertension (Table 4.1 , Table 4.5 and Table 4.9 ) explained additional evidence to the high concentration of pro-inflammatory markers TNF, IL6, CRP .

Chung and his colleagues (2019) considered that Age as a chronic inflammation and described it as multivariable low-grade. Inflammaging , a new term that mix between inflammation and aging including the following disorders : accumulated of damaged macromolecules and debris of cells with age , the product of microbial , cellular senescence , coagulation system activation , immunosenescence and inappropriate regulation of the complement pathway (Franceschi and Campisi , 2014).

In elderly men , chronic low-grade inflammation is a risk factor for the development of aging-related diseases and frailty (Puzianowska-Kuźnicka *et*

*al.*, 2016) , and Chronic inflammation is necessary for tissue degeneration and reconstruction at the same time ( Franceschi and Campisi , 2014).

The inflammatory biomarkers most consistently associated with aging are elevated circulating concentrations of IL-6 , CRP , and TNF-α (Ferrucci *et al*., 2005 ; Singh and Newman , 2011; Howcroft *et al*., 2013; Morrisette-Thomas *et al*., 2014 ) . Many investigators have reported that aging is associated with increased levels of pro-inflammatory cytokines , including IL-6 , TNFα , CRP, and TNF-R1 (Singh and Newman 2011; Varadhan *et al*. 2014) . Wyczalkowska-Tomasik and his coworkers (2016) detected slight increases of CRP,IL 6 with age , and no significantly significant difference was found in TNF levels , but there was a clear trend of increase with age .

"Hypertension is a complex immune system response that involves interactions between inflammatory cells such as macrophages and T lymphocytes , resulting in increased expression of adhesion molecules , cytokines , matrix metalloproteinases , and growth factors "(Quiroz *et al.*, 2012 ; Schiffrin , 2013) , besides that , many researchers suggested that hypertension exerts pro-inflammatory actions through increased expression of several inflammatory mediators including endothelin-1 and angiotensin , apart from angiotensin II association with vasoconstriction and salt and fluid homeostasis, its role is equally important in the vascular inflammation and oxidative stress ( Tsounis *et al.*, 2014) .

Metabolic / chemical , mechanical , or infectious endothelial aggressions cause complex immune reactions , leading to a pro-inflammatory state , and inflammation in turn promotes endothelial dysfunction and atherosclerosis through ROS , a downstream product of cellular and soluble immune factors , consequently , ROS stimulates pro-inflammatory cytokine secretion , increasing IL 6 expression and decreasing NO availability, these cytokines (IL-

6) trigger hypertensive responses to angiotensin II infusion even in normotensive individuals (Tanase *et al.,* 2019).

In men , the role of androgen as an anti-inflammatory hormone is further demonstrated by testosterone supplementation trials , which show that proinflammatory markers are suppressed in both young and old hypogonadal men (Maggio *et al.,* 2006 ; Vodo *et al.,* 2013).

There is benefit of testosterone as anti- inflammation in hypertensive and aging men as indicated by the study of Kalinchenko and his colleagues ( 2010 ) , were is testosterone therapy reduced significantly ( except IL 6) the levels of TNF α and CRP.

Corcoran and his colleagues (2010) , found both physiological and supraphysiological concentrations of testosterone reduced the expression and secretion of TNF α and reduced the expression of IL-1β, but did not affect IL 6 or CRP expression .

Cytokines like IL-6 and TNF, on the other hand, have been proven in numerous inflammatory experimental trials to inhibit testosterone release via altering the hypothalamic-pituitary-gonadal axis action (Malkin *et al.*, 2004 ; Norata *et al.,* 2006).

There is evidence supporting the immunosuppressive effect of androgens , the incidence of autoimmune diseases is higher in androgen-deficient men (Tengstrand *et al.,* 2002).

It has been proposed that androgens' immunosuppressive activity is due to either a direct effect on the expression of inflammatory genes (Asirvatham *et al.,* 2006) , or indirect effect through inhibition of nuclear factor-kB activation (Vignozzi *et al.,* 2012) , in addition , Bianchi ( 2019) mentioned that

testosterone administration showed a reduction in many inflammatory cytokines by its action as a primary anti-inflammatory effects .

## 5.3 : Nitric oxide

The present general findings showed a significant (with some exception ) decrease in nitric oxide levels related with aging and hypertension in all different subgroups and groups .(Table 4.2 , Table 4.6, Table 4.10) .

To shed some light on the nitric oxide in result discussion , it is remarkably to realize and discuss the major factor that influenced these nitric oxide molecules such aging hypertension , testosterone levels and pro inflammatory cytokines ,these all factor are related defiantly with the fluctuation of nitric oxide levels and in the same time , nitric oxide is a continual in some of each of these factors .

Studies in the human being and experimental models revealed that constitutive production of nitric oxide (NO) is reduced with aging) to an extent which is commensurate with adverse impact on cardiac vascular outcomes (Torregrossa *et al.,* 2011; Sverdlov *et al.,* 2014).

Furthermore , Wu and his colleagues ( 2021) concluded that nitric oxide plays an important role in keeping up the vascular health as well as for the controlled blood pressure . NO is well-known as the most potent vasodilator (endothelium-derived relaxing factor) , which aids in the control of blood pressure by modulating vascular resistance and heart function ( Kunes *et al.,* 2004) . More of NO molecules are inactive associated with hypertension or its bioavailability and decreased as a result of NO poor synthesis by eNOS (Pinheiro *et al.,* 2017)

Testosterone deficiency may contribute to NO deficiency through its direct effect on the expression of NOS , or its effect on Sphingosine-1-

phosphate (S1P) , or through its relationship with the Endothelial progenitor cells (EPCs) (Hotta *et al.,* 2019) . Moreau and his colleagues (2020) mentioned that the improvements in endothelial role with testosterone therapy was observed on men in their forties and fifties. TNFα decreases the bioavailability of NO both by reducing its production , and by enhancing its removal , decreased NO generation results from TNFα mediated inhibition of endothelial NO synthase (eNOS) expression and activity , TNFα signaling suppresses gene promoter activity and destabilizes eNOS mRNA , thus reducing eNOS protein expression mediated by TNFR1 (Yoshizumi *et al.,* 2003 ; Neumann *et al.,*2004). Moreover, TNFα decreases NO bioavailability by accumulation of the endogenous eNOS inhibitor ADMA (asymmetric dimethylarginine) , and by enhanced removal of NO, for example via its reaction with superoxide , in which peroxynitrite is generated ( Urschel and Cicha , 2015).

The molecular mechanism underlying the effect of IL 6 to decrease nitric oxide bioavailability by raising the half-life , thereby increasing caveolin-1 protein levels , which bind more eNOS and, as a result, decrease eNOS activation by decreasing Ser1177 phosphorylation (Hung *et al.,* 2010).

CRP has the ability to attenuate NO production with a marked reduction in vitro angiogenesis , cell migration , and capillary-like tube formation by CRP at concentrations known to cause cardiovascular risk (Sproston and Ashworth , 2018).

Eisenhardt and his coworkers ( 2009) reported that the Adhesion molecules increase with CRP concentration, while nitric oxide molecules decrease .

## 5.4 : Lipid profile

The present findings concerning lipid profile revealed that the parameters TC, TG , LDL and VLDL increased significantly and HDL reduced significantly during age and hypertension when they be compared between subgroups (Table 4.3) . A similar significant increased is showen in these parameters (TC, TG, LDL and VLDL) in hypertensive and normotensive samples during the progressive age (between groups) (Table 4.7 ), and the same findings concerning the significant ( except the hypertensive ) reduction in HDL in normotensive and hypertensive sample during the progressive age (between groups ) (Table 4.3 ; Table 4.7; Table 4.11) .

These finding include TC , TG , LDL , HDL and the reduction in testosterone hormone levels during both hypertension and progressive age are in consistence with the facts referred with all the bad parameters (TC, TG, LDL , VLDL ) and the good parameter (HDL) increased and deceased respectively during hypertension and progressive age .

Epidemiological studies found that the plasma levels of HDL cholesterol decrease with increasing age , but in elderly they are unchanged or increased slightly (Walter , 2009). The decreased levels of HDL cholesterol can be secondary to inflammatory, hormonal, and metabolic changes (obesity, hypertension , diabetes mellitus) , which are more prevalent with aging ( Kolovou *et al.,* 2011) . A low HDL cholesterol levels is one of the strongest predictors of premature coronary heart disease and stroke , in contrast to high LDL cholesterol , low HDL cholesterol remains a powerful risk predictor into old age (Walter , 2009).

In the Rancho Bernardo's Study in 1997 using prospective data from 50- to 93-year-old probands , it was estimated that HDL cholesterol declines by approximately 1% per year (Walter , 2009).

Impaired lipolysis are more frequent at advanced age leading to many probable dysfunction for example , impair Reverse Cholesterol Transport (RCT) by various mechanisms , some change in food consumption and in activity , and inflammatory processes in aged people may cause many changes in HDL cholesterol (Walter, 2009) , beside , the testosterone reduction itself has an impairment for the lipoprotein lipase (LPL) activity and RCT (Wu and Eckardstein , 2003).

In the other hand , in older men hepatic synthesis of VLDL and their conversion to LDL particles increase , and in the same time the catabolism of VLDL and LDL particles decrease due to reduced expression of LDL receptors and activity of lipoprotein lipase (Li *et al.,* 2008) .The hepatic lipase levels increase after the exogenous administration of testosterone (Langer *et al.,* 2002).

Gyllenborg and his colleagues (2001) , after evaluation of 508 healthy men aged 41 to 72 years , they concluded that testosterone declined slightly in these ages and had an adverse correlation with the arterial blood pressure , VLDL and TG .

The low levels of testosterone and high levels of free androgens index are associated with the atherogenic lipid profile in men (Zmuda *et al.,* 1997; Andersen *et al.,* 2000).

Furthermore , testosterone stimulated lipoprotein lipolysis lipase (which are responsible of triglyceride catabolism and storage ) via the β-adrenergic receptors leading to decrees in fat storage , however , in aging men this action is diminished due to insufficient levels of testosterone and increased androgen index ( Kolovou *et al.,* 2011).

Mancuso and Bouchard (2019) mentioned that testosterone improve lipolysis and suppress lipid accumulation .

Increased lipolysis of Triglyceride-rich lipoproteins in proximity to the endothelium increases endothelial layer permeability , and causes a pro-inflammatory state in endothelial cells , manifested by increased TNFa secretion, adhesion molecule expression, and oxidative stress induction (Wang et al., 2009) , Testosterone stimulates β-adrenergic receptors with consequent augmentation of lipolysis and suppression of lipoprotein lipase ( Kolovou *et al.,* 2011) .

Present finding concerning lipid profile revealed that testosterone concentration correlated positively with HDL (Agledahl *et al.,* 2008 ; Mäkinen *et al.,* 2008) , negatively with triglycerides , and total cholesterol ( Finkle *et al.,* 2014 ; Roth *et al.,* 2014) and VLDL (Vaidya *et al.,* 2008; Snyder *et al.,* 2014).

Furthermore , Isidori and his colleagues (2005) found that androgen therapy in the ageing men stimulated a reduction in total body fat mass , an increased in fat free mass , a small decreased in total cholesterol.

Hassan ( 2010 ) showed that in castrated rats a significant increase in the levels of total cholesterol , triglycerides , low density lipoprotein , and when testosterone administration , it caused a significant decrease in the levels of total cholesterol , triglycerides , and low density lipoproteins .

In the other hand , a mong coronary artery disease (CAD) patients , hypertention and atherosclerosis , a significant positive association was found between testosterone and HDL , whereas a negative association was found with LDL (Wickramatilake *et al.,* 2013 ; Attyha *et al.,* 2013 ; Choudhury *et al.,* 2014 ) .

The body's ability to deal properly with fat decreases, which contributes to the dysfunction represented in its distribution sites as a result of age, where it accumulates in the muscles and liver, which leads to low-grade inflammation, insulin resistance and metabolic syndrome as a result of the defect in the construction of adipose tissue derivatives, including adipokines which regulate the inflammation (Mancuso and Bouchard , 2019).

Additionally, prolactin hormone levels in our current study revealed a significant increase both in hypertension and progressive age between sub group and groups , and this high increment in prolactin levels which is an additive agent beside all above factors and agents that interact with lipolysis and adipogensis leading to this picture of lipid profile . Ruiz-Herrera and his colleagues ( 2017) , reported that prolactin is produced by adipose tissue and stimulates adipogenesis and inhibits lipolysis . Treatment to hyperprolactinaemia was accompanied by a decrease in TC and LDL (Auriemma *et al.*, 2013 ; Schwetz *et al.*, 2017) , and a decrease in the rest of the criteria , triglycerides , high-density lipoprotein (Auriemma *et al.*, 2013).

## 5.5: Total protein and its fractions

The present findings concerning total protein ( TP ) and its fractions revealed that TP and albumin decreased significantly , in the same manner , globulins had a general tendency to be increased during progressive age and hypertension between subgroups and groups (Table 4.4, Table 4.8, Table 4.12).

Progressive age and hypertension beyond all the changes leading to organs dysfunction particularly decreased protein intake , malabsorption , gastrointestinal disorders , liver dysfunction resulting in significant reduction in total protein and albumin in current findings , therefore the less efficient in the role of some vital organs . In other hand , the slight increase tendency in

studied globulin and the gamma globulin significant increase indicated an inflammatory state due to hypertension and progressive age , in consistence with the high levels of CRP the findings of the present study associated with hypertension and progressive age , besides the main role of some hormones ( testosterone , estradiol ) in these immunoglublulines parameters .

Tian and his colleagues ( 2014) showed in the elderly, serum total protein levels possibly decrease gradually with age . The effect of ageing on the serum levels of albumin , globulin and total protein was found to be negative in both sexes in all the progressive decades of life and it was not significant (Devi and Kumar , 2012). Ihara and his coworkers (2001) , found in Japanese men from age 30-69 , albumin reduced and alpha and a globulins increased in terms of percent composition . The results of albumin agree with Weaving and his colleagues (2016) found that the albumin values started to decrease from the age group of 41–45 years (42 g/L) .Similar findings have been reported about the albumin concentration be increased from childhood to the third decade from which it slowly decreased during life , and the same results for elderly (Gomi *et al.,* 2007; Miyake *et al.,* 2011) .

Age-related dysfunction , which may cause a special disorder in the digestive system and in the liver , leading to a decrease in the amount of total protein produced (Tian *et al.,* 2014) . Beside that elderly individuals become weaker and are often susceptible to infections , fever , diarrhea , fractures , and other ailments , which induce the consumption of large amounts of serum protein as age increases (Wengreen *et al.,* 2004) .

In hypertensive men , serum total proteins significantly is reduced (Memon *et al.,* 2017) . In the other hand , Wahed and his colleagues ( 2019) mentiend that the albumin band may be decreased with increased gamma zone in chronic inflammation . Kanda and his coworkers (1996) found the inhibitory

effect of testosterone in vitro on spontaneous immunoglobulin IgG , IgM production by human peripheral blood mononuclear cells (PBMC). In humans , androgen treatment inhibits B cell hyperactivity and immunoglobulin production by mononucleated cells (Kanda *et al.*, 1997) . Estrogen enhances antibody production by B cells (Medina et al. 1993 ;Verthelyi and Ahmed , 1994) . Androgen deficiency appears to enhance B cell responses , leading to an increased production of immunoglobulins possibly by oestrogenic influences , and ART appears to inhibit immunoglobulin synthesis in Klinefelter's syndrome (KS) (Kocar *et al.*, 2000 ).

On the other hand, the correlation between testosterone and the rest of the parameters of this study (table 13) indicated the close relationship for the influence of this hormone on these parameters during hypertension and progressive age, representing that this hormone playing a regulation role and has many impacts on these parameters positively or negatively leading to reduced or regressed the functions of the cells and tissues and promotes many disorders here or there in the body during these two bad conditions hypertension and progressive age .

It has been hypothesized that when individuals are chronically confronted with stressful conditions in daily life (e.g., poverty, crime, poor housing), they will engage in unhealthy behaviors (e.g., smoking, alcohol use and abuse, drug use, and overeating, especially of comfort foods) that help to alleviate the resulting symptoms of stress , However, these same behaviors silently contribute to physical health morbidities and early mortality. Thus, the engaging in unhealthy behaviors alleviates the symptoms of stress and the possible biological cascade to mental disorders while simultaneously combining with the effects of poor living conditions to contribute to the

development of physical health ailments and chronic physical health disorders later in life (Jackson *et al.,* 2010).

Levels of stress increased with increasing age and health-related stress is highly prevalent in older men(Osmanovic-Thunström *et al.,* 2015).

Depending on the local regulatory environment at the time of stress, prolactin level can either increase or decrease, vasopressin and peptide histidine isoleucine may be involved in the secretion of prolactin during stress, still , the teleological significance of change in the prolactin level is uncertain,it may affect the immune system or some aspect of homeostasis (Ranabir and Reetu, 2011)

Besid that , In males, with stress there can be decreased sperm count, motility and altered morphology, ejaculatory disorders, impotence and oligospermia may be associated with psychological factors in male infertility (Ranabir and Reetu, 2011).

In the past 20 years, war and human rights violations have led to high rates of exposure to traumatic events among the Iraqi population. Due to the ongoing violence, many physicians and mental health professionals have left Iraq in recent years (Wagner *et al.,* 2012 ).

Stress in Iraq is increasing causing more health problems , and one of them is hypertension .

# *Conclusions and Recommedations*

## Conclusions :

1- Testosterone hormone playing a regulation role and has many impacts on the study parameters positively or negatively leading to reduced or regressed the functions of the cells and tissues and promotes many disorders here or there in the body during these two bad conditions hypertension and progressive age .

2- Progressive age and hypertension considered as independent factor that influenced all the hormonal and biochemical parameter as the following :

a- A significant decrease in testosterone levels .

b- A significant increase in estradiol and prolactin levels .

c- A significant increase in TNFα , IL6 , CRP concentrations .

d- A significant decrease in nitric oxide .

e- A significant increase in lipid profile parameters (except HDL ) levels .

f- A significant decrease in total protein and albumin with slight increase in globulins .

3- The reduction in present testosterone levels associated with the increase estradiol , prolactin , pro-inflammatory markers , and the nitric oxide reduction during progressive age and hypertension .

2- These finding indicated a decline in some of health progression .

## Recommendations

The results of the present study included the following recommendations :

1- Additional studies to investigate :

A- More samples with deferent ages , in different experimental design with many slices of patients .

B- Other precise parameters related with the molecular genetic of hormonal receptor , and more processes involvement in the actions of these hormones .

2. Studying additional physiological and pathological states (e.i. stress , obesity , diabetes) in elderly people .

3. Studying the fertility and infertility both for male and female related with progressive age .

# *REFRENCES*

**Aflyatumova, G. N., Nigmatullina, R., Sadykova, D. I., Chibireva, M. D., Fugetto, F., & Serra, R. (2018).** Endothelin-1, nitric oxide, serotonin and high blood pressure in male adolescents. *Vascular Health and Risk Management*, 14: 213–223.

**Agita, A., & Alsagaff, M. T. (2017).** Inflammation, immunity, and hypertension. *Acta Med Indones*, 49(2) :158-165.

**Agledahl, I., Skjærpe, P. A., Hansen, J. B., & Svartberg, J. (2008).** Low serum testosterone in men is inversely associated with non-fasting serum triglycerides: the Tromsø study. *Nutrition, Metabolism and Cardiovascular Diseases* ,18(4) : 256-262.

**Ahmad, A., Dempsey, S. K., Daneva, Z., Azam, M., Li, N., Li, P. L., & Ritter, J. K. (2018).** Role of nitric oxide in the cardiovascular and renal systems. *International Journal of Molecular Sciences*, 19(9):2605.

**Akdoğan, M., Tamer, M. N., Cüre, E., Cüre, M. C., Köroğlu, B. K., & Delibaş, N. (2007).** Effect of spearmint (Mentha spicata Labiatae) teas on androgen levels in women with hirsutism. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 21(5): 444-447.

**Akishita, M., Fukai, S., Hashimoto, M., Kameyama, Y., Nomura, K., Nakamura, T., Ogawa, S., Lijima,K., Eto, M., & Ouchi, Y. (2010).** Association of low testosterone with metabolic syndrome and its components in middle-aged Japanese men. *Hypertension Research*, 33(6): 587-591.

**Andersen, A. G., Jensen, T. K., Carlsen, E., Jørgensen, N., Andersson, A. M., Krarup, T., Keiding, N., & Skakkebaek, N. E. (2000).** High frequency of sub-optimal semen quality in an unselected population of young men. *Human Reproduction*, 15(2) : 366-372.

**Andersen, M. L., & Tufik, S. (2008).** The effects of testosterone on sleep and sleep-disordered breathing in men: its bidirectional interaction with erectile function. *Sleep medicine reviews*, 12(5):365-379.

**Andersson, K. E. (2018).** PDE5 inhibitors–pharmacology and clinical applications 20 years after sildenafil discovery. *British Journal of Pharmacology*, 175(13): 2554-2565.

**Antonelli, M., & Kushner, I. (2017).** It's time to redefine inflammation. *The Federation of American Societies For Experimental Biology Journal*, *31*(5): 1787-1791.

**Araujo, A. B., Dixon, J. M., Suarez, E. A., Murad, M. H., Guey, L. T., & Wittert, G. A. (2011).** Endogenous testosterone and mortality in men: a systematic review and meta-analysis. *The Journal of Clinical Endocrinology & Metabolism*, *96*(10), 3007-3019.

**Ashton, M. C. (2018).** Individual differences and personality. 3rded, *Academic Press*.p, 607

**Asirvatham, A. J., Schmidt, M., Gao, B., & Chaudhary, J. (2006).** Androgens regulate the immune/inflammatory response and cell survival pathways in rat ventral prostate epithelial cells. *Endocrinology*, 147(1) : 257-271.

**Atlantis, E., Martin, S. A., Haren, M. T., O'Loughlin, P. D., Taylor, A. W., Anand-Ivell, R., Ivell, R., Wittert,G.A., & Florey Adelaide Male Ageing Study. (2009).** Demographic, physical and lifestyle factors associated with androgen status: the Florey Adelaide Male Ageing Study (FAMAS). *Clinical Endocrinology*, *71*(2) : 261-272.

**Attyha, I. N., Turki, E. M., & Husan, J. M. (2013).** Effect of hyperlipidemia in the atherosclerotic patients on sex hormones. *Al-Kufa University Journal for Biology*, 5(2)： 198-202.

**Aukrust, P., Liabakk, N. B., Lien, E., Espevik, T., and Frøland, S. S. (1994).** Serum levels of tumor necrosis factor-α (TNFα) and soluble TNF receptors in human immunodeficiency virus type 1 infection-correlations to clinical, immunologic, and virologic parameters. *Journal of Infectious Diseases*, 169(2): 420-424.

**Auriemma, R. S., Granieri, L., Galdiero, M., Simeoli, C., Perone, Y., Vitale, P., Pivonello,C., Negri,M., Mannarino,T., Giordano, C., Gasperi, M., Colao, A., & Pivonello, R. (2013).** Effect of cabergoline on metabolism in prolactinomas. *Neuroendocrinology*, 98(4) : 299-310.

**Aversa, A., Isidori, A. M., De Martino, M. U., Caprio, M., Fabbrini, E., Rocchietti-March, M., Frajeset , G., & Fabbri, A. (2000).** Androgens and penile erection: evidence for a direct relationship between free

testosterone and cavernous vasodilation in men with erectile dysfunction. *Clinical Endocrinology*, 53(4): 517-522.

**Bachorik, P. S. (2000).** 12. Measurement of low-density-lipoprotein cholesterol. *Handbook of lipoprotein testing*, 245-263.

**Barrado, M. J. G., Blanco, E. J., Hernández, M. C., Osma, M. C. I., Carretero, M., Herrero, J. J., Burks, D.J., & Carretero, J. (2014).** Local transformations of androgens into estradiol by aromatase P450 is involved in the regulation of prolactin and the proliferation of pituitary prolactin-positive cells. *PloS one*, 9(6) : 1-9

**Barrett, K. E., Boitano, S., Barman, S. M., Brooks, H. L. & Yuan, J. (2016).** Ganong's review of medical physiology. 26ed, *McGraw-Hill Education*.pp,913-975.

**Bayer, S. R., Alper, M. M., and Penzias, A. S. (2011).** The Boston In Vitro Fertilization Handbook of Infertility: A Practical Guide for Practitioners Who Care for Infertile Couples. 3rded, *Academic Press*.p, 218.

**Baylis, D., Bartlett, D. B., Patel, H. P., & Roberts, H. C. (2013)**. Understanding how we age: insights into inflammaging. *Longevity & Healthspan*, 2(1) : 1-8.

**Bektas, A., Schurman, S. H., Sen, R., & Ferrucci, L. (2018).** Aging, inflammation and the environment. *Experimental Gerontology*, 105:10-18.

**Belchetz, P. E., Barth, J. H., & Kaufman, J. M. (2010).** Biochemical endocrinology of the hypogonadal male. *Annals of Clinical Biochemistry*, 47(6):503-515.

**Belikov, A. V. (2019).** Age-related diseases as vicious cycles. *Ageing Research Reviews*, 49: 11-26.

**Bennett, N. C., Gardiner, R. A., Hooper, J. D., Johnson, D. W., & Gobe, G. C. (2010).** Molecular cell biology of androgen receptor signalling. *The international journal of Biochemistry & Cell Biology*, 42(6):813-827.

**Bernichtein, S., Touraine, P., & Goffin, V. (2010).** New concepts in prolactin biology. *The Journal of Endocrinology*, *206*(1), 1-11.

**Bernini, G., Versari, D., Moretti, A., Virdis, A., Ghiadoni, L., Bardini, M., Taurino, C., Domenico, C., Taddei, S., & Salvetti, A. (2006).** Vascular

reactivity in congenital hypogonadal men before and after testosterone replacement therapy. *The Journal of Clinical Endocrinology & Metabolism*, 91(5):1691-1697.

**Bianchi, V. E. (2019).** The anti-inflammatory effects of testosterone. *Journal of the Endocrine Society*, 3(1): 91-107.

**Bird, B., & Zilioli, S. (2017).** Testosterone. In V. A. Weekes-Shackelford and T. K. Shackelford (Eds.), Encyclopedia of evolutionary psychological science (pp. 1-3). Cham, Switzerland: *Springer International Publishing AG*.

**Black, S., Kushner, I., & Samols, D. (2004).** C-reactive protein. *Journal of Biological Chemistry*, 279(47) : 48487-48490.

**Blaya, R., Thomaz, L. D. G. R., Guilhermano, F., Paludo, A. D. O., Rhoden, L., Halmenschlager, G., & Rhoden, E. L. (2016).** Total testosterone levels are correlated to metabolic syndrome components. *The Aging Male*, 19(2): 85-89.

**Bobjer, J., Katrinaki, M., Tsatsanis, C., Giwercman, Y. L., & Giwercman, A. (2013).** Negative association between testosterone concentration and inflammatory markers in young men: a nested cross-sectional study. *PLoS One*, 8(4) : 1-8 .

**Bowcock, A. M., Kidd, J. R., Lathrop, G. M., Daneshvar, L., May, L. T., Ray, A., Sehgal, P.B., Kidd, K.K., & Cavalli-Sforza, L. L. (1988).** The human "interferon-β2/hepatocyte stimulating factor/interleukin-6" gene: DNA polymorphism studies and localization to chromosome 7p21. *Genomics*, *3*(1): 8-16.

**Bozzola, M., Bozzola, E., Montalbano, C., Stamati, F. A., Ferrara, P., & Villani, A. (2018).** Delayed puberty versus hypogonadism: a challenge for the pediatrician. *Annals of Pediatric Endocrinology & Metabolism*, 23(2): 57.

**Braga-Basaria, M., Dobs, A. S., Muller, D. C., Carducci, M. A., John, M., Egan, J., & Basaria, S. (2006).** Metabolic syndrome in men with prostate cancer undergoing long-term androgen-deprivation therapy. *Journal of Clinical Oncology*, 24(24) : 3979-3983.

**Brocker, C., Thompson, D., Matsumoto, A., Nebert, D. W., & Vasiliou, V. (2010).** Evolutionary divergence and functions of the human interleukin (IL) gene family. *Human Genomics*, 5(1):1-26.

**Brüünsgaard, H., & Pedersen, B. K. (2003).** Age-related inflammatory cytokines and disease. *Immunology and Allergy Clinics*, 23(1):15-39.

**Buford, T. W. (2016).** Hypertension and aging. *Ageing Research Reviews*, *26* : 96-111.

**Carré, J. M., & Putnam, S. K. (2010).** Watching a previous victory produces an increase in testosterone among elite hockey players. *Psychoneuroendocrinology*, 35(3) : 475-479.

**Casey, A., Benson, H., & O'Neill, B. E. (2006).** The Harvard medical school guide to lowering your blood pressure. 1st ed , *McGraw-Hill*.p,3

**Cau, S. B. D. A., Carneiro, F. S., & Tostes, R. (2012).** Differential modulation of nitric oxide synthases in aging: therapeutic opportunities. *Frontiers in Physiology*, 3(218):1-11

**Chistiakov, D. A., Myasoedova, V. A., Melnichenko, A. A., Grechko, A. V., & Orekhov, A. N. (2018).** Role of androgens in cardiovascular pathology. *Vascular Health and Risk Management*, 14: 283–290

**Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo Jr, J. L., Jones ,D.W., Materson, B.J., Operil,S., Wrightjr, J.T., Roccella, E.J., & National High Blood Pressure Education Program Coordinating Committee. (2003).** The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. *Hypertension*, 42(6), 1206-1252.

**Chou, T. C., Yen, M. H., Li, C. Y., & Ding, Y. A. (1998).** Alterations of nitric oxide synthase expression with aging and hypertension in rats. *Hypertension*, 31(2):643-648.

**Choudhury, K. N., Mainuddin, A. K. M., Wahiduzzaman, M., & Islam, S. M. S. (2014).** Serum lipid profile and its association with hypertension in Bangladesh. *Vascular Health and Risk Management*, 10: 327–332.

**Chrissobolis, S., Miller, A. A., Drummond, G. R., Kemp-Harper, B. K., & Sobey, C. G. (2011).** Oxidative stress and endothelial dysfunction in cerebrovascular disease. Frontiers in bioscience (Landmark edition), 16:1733-1745.

**Chuaiphichai, S., Crabtree, M. J., Mcneill, E., Hale, A. B., Trelfa, L., Channon, K. M., & Douglas, G. (2017).** A key role for tetrahydrobiopterin-dependent endothelial NOS regulation in resistance arteries: studies in endothelial cell tetrahydrobiopterin-deficient mice. *British Journal of Pharmacology*, 174(8): 657-671.

**Chuang, S. Y., Hsu, P. F., Chang, H. Y., Bai, C. H., Yeh, W. T., & Pan, H. W. (2013).** C-reactive protein predicts systolic blood pressure and pulse pressure but not diastolic blood pressure: the Cardiovascular Disease Risk Factors Two-Township Study. *American Journal of Hypertension*, 26(5): 657-664.

**Chuffa ,L. G. A., Fioruci-Fontanelli B. A., Porto-Amorim E.M., ℘ Amorim , J. P. d. A. (2013 ).** Effects of Estradiol on Male and Female Reproductive Tissues and the Influence of Endocrine Disruptors. R. Palmeri ℘ S. Grimauo (Eds). Estradiol synthesis , health effects and drug interactions (pp.69-98). New York : *Nova Science Publishers*.

**Chung, H. Y., Kim, D. H., Lee, E. K., Chung, K. W., Chung, S., Lee, B., ... & Yu, B. P. (2019).** Redefining chronic inflammation in aging and age-related diseases: proposal of the senoinflammation concept. *Aging and Disease*, 10(2) : 367–382.

**Chung, H. Y., Sung, B., Jung, K. J., Zou, Y., & Yu, B. P. (2006).** The molecular inflammatory process in aging. *Antioxidants & Redox Signaling*, 8(3-4) : 572-581.

**Clapp, C., Torner, L., Gutierrez-Ospina, G., Alcantara, E. V. A., Lopez-Gomez, F. J., Nagano, M., Kelly,P.A., Mejia,S., Morales, M.A., & de la Escalera, G. M. (1994).** The prolactin gene is expressed in the hypothalamic-neurohypophyseal system and the protein is processed into a 14-kDa fragment with activity like 16-kDa prolactin. *Proceedings of the National Academy of Sciences*, 91(22) : 10384-10388.

**Cohen, P. G. (2001).** Aromatase, adiposity, aging and disease. The hypogonadal-metabolic-atherogenic-disease and aging connection. *Medical Hypotheses*, *56*(6), 702-708.

**Colao, A., Di Sarno, A., Cappabianca, P., Briganti, F., Pivonello, R., Di Somma, C., Faggiano, A., Biondi, B., & Lombardi, G. (2003).** Gender differences in the prevalence, clinical features and response to

cabergoline in hyperprolactinemia. *European Journal of Endocrinology*, *148*(3), 325-331.

**Colli, L. G., Belardin, L. B., Echem, C., Akamine, E. H., Antoniassi, M. P., Andretta, R. R., Mathias, L.S., Rodrigues, S.F.D., Bertolla, R.P., & De Carvalho, M. H. C. (2019).** Systemic arterial hypertension leads to decreased semen quality and alterations in the testicular microcirculation in rats. *Scientific Reports*, 9(1) :1-12.

**Cooke, P. S., Nanjappa, M. K., Ko, C., Prins, G. S., & Hess, R. A. (2017).** Estrogens in male physiology. *Physiological Reviews*, 97(3) : 995-1043.

**Corcoran, M. P., Meydani, M., Lichtenstein, A. H., Schaefer, E. J., Dillard, A., & Lamon-Fava, S. (2010).** Sex hormone modulation of proinflammatory cytokine and CRP expression in macrophages from older men and postmenopausal women. *The Journal of Endocrinology*, 206(2): 217.

**Corona, G., & Maggi, M. (2010).** The role of testosterone in erectile dysfunction. *Nature Reviews Urology*, *7*(1): 46-56.

**Corona, G., Wu, F. C., Rastrelli, G., Lee, D. M., Forti, G., O'Connor, D. B., O'Neill, T.W., Pendleton, N., Bartfai,G., Boonen,S., Casanueva, F.F., Finn,J.D., Huhtaniemi , I .T., Kula , K., Punab , M., Vanderschueren , D., Rutter, M.K., Maggi, M., & EMAS Study Group. (2014).** Low prolactin is associated with sexual dysfunction and psychological or metabolic disturbances in middle-aged and elderly men: the European Male Aging Study (EMAS). *The Journal of Sexual Medicine*, 11(1): 240-253.

**Dabbous, Z., & Atkin, S. L. (2018).** Hyperprolactinaemia in male infertility: Clinical case scenarios. *Arab journal of urology*, *16*(1), 44-52.

**Danylovych, G. V., Bohach, T. V., & Danylovych, Y. V. (2018).** The biosynthesis of nitric oxide from L-arginine. Nitric oxide formation features and its functional role in mitochondria. *The Ukrainian Biochemical Journal*, 90(1) : 3-24.

**Davies, K. P. (2015).** Development and therapeutic applications of nitric oxide releasing materials to treat erectile dysfunction. *Future science OA*, 1(1).

**Devi, R., & Kumar, M. P. (2012).** Effect of ageing and sex on the ceruloplasmin (Cp) and the plasma protein levels. *Journal of Clinical and Diagnostic Research*, *6*(4), 577-580.

**Dinh, Q. N., Drummond, G. R., Sobey, C. G., & Chrissobolis, S. (2014).** Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. *BioMed Research International*, 2014(1):1-11.

**Donato, A. J., Gano, L. B., Eskurza, I., Silver, A. E., Gates, P. E., Jablonski, K., & Seals, D. R. (2009).** Vascular endothelial dysfunction with aging: endothelin-1 and endothelial nitric oxide synthase. *American Journal of Physiology-Heart and Circulatory Physiology*, 297(1) : H425-H432.

**Doumas, M., Boutari, C., & Viigimaa, M. (2016).** Arterial hypertension and erectile dysfunction: an under-recognized duo. *The Journal of Clinical Hypertension*, 14(4) : 1-7.

**Duan, T., Fan, K., Chen, S., Yao, Q., Zeng, R., Hong, Z., Peng,L., Shao,Y., & Yao, B. (2016).** Role of peroxiredoxin 2 in H2O2-induced oxidative stress of primary Leydig cells. *Molecular Medicine Reports*, *13*(6): 4807-4813.

**Eisenhardt, S. U., Thiele, J. R., Bannasch, H., Stark, G. B., & Peter, K. (2009).** C-reactive protein: how conformational changes influence inflammatory properties. *Cell Cycle*, 8(23): 3885-3892.

**Erenpreiss, J., Fodina, V., Pozarska, R., Zubkova, K., Dudorova, A., & Pozarskis, A. (2019).** Prevalence of testosterone deficiency among aging men with and without morbidities. *The Aging Male*. 23(5): 901-905.

**Fahie-Wilson, M., and Smith, T. P. (2013).** Determination of prolactin: the macroprolactin problem. *Best Practice & Research Clinical Endocrinology & Metabolism*, *27*(5):725-742.

**Ferreira, V. L., Borba, H. H., Bonetti, A. D. F., Leonart, L., & Pontarolo, R. (2018).** Cytokines and interferons: types and functions. *Autoantibodies and Cytokines*, *13*: 65–97.

**Ferrucci, L., Corsi, A., Lauretani, F., Bandinelli, S., Bartali, B., Taub, D. D., Guralnik, J.M., & Longo, D. L. (2005).** The origins of age-related proinflammatory state. *Blood*, 105(6): 2294-2299.

**Finkelstein, J. S., Lee, H., Burnett-Bowie, S. A. M., Pallais, J. C., Yu, E. W., Borges, L. F., Brent, F., Jones, M.D., Christopher, V., Barry, M.P.H., Kendra, E., Wulczyn, B.A., Bijoy, J., Thomas, M.D., & Leder, B. Z. (2013).** Gonadal steroids and body composition, strength, and sexual function in men. *New England Journal of Medicine*, *369*(11): 1011-1022.

**Finkle, W. D., Greenland, S., Ridgeway, G. K., Adams, J. L., Frasco, M. A., Cook, M. B., Fraumeni , J. F., & Hoover, R. N. (2014).** Increased risk of non-fatal myocardial infarction following testosterone therapy prescription in men. *PloS one*, 9(1) : 1-7

**Firtser, S., Juonala, M., Magnussen, C. G., Jula, A., Loo, B. M., Marniemi, J., Viikari, J. S.A.,Toppari, J., Perheentupa, A., Hutri-Kähönen, N.,& Raitakari, O. T. (2012).** Relation of total and free testosterone and sex hormone-binding globulin with cardiovascular risk factors in men aged 24–45 years. The Cardiovascular Risk in Young Finns Study. *Atherosclerosis*, 222(1) : 257-262.

**Fitzgerald, P., & Dinan, T. G. (2008).** Prolactin and dopamine: what is the connection? A review article. *Journal of Psychopharmacology*, 22(2) : 12-19.

**Florentino, I. F., Silva, D. P., Silva, D. M., Cardoso, C. S., Moreira, A. L., Borges, C. L., Soares, C. M. , Galdino, P. M.,Lião, L. M., Ghedini, P. C., Menegattie, R., & Costa, E. A. (2017).** Potential anti-inflammatory effect of LQFM-021 in carrageenan-induced inflammation: the role of nitric oxide. *Nitric Oxide*, 69 : 35-44.

**Fogari, R., Preti, P., Zoppi, A., Fogari, E., Rinaldi, A., Corradi, L., & Mugellini, A. (2005).** Serum testosterone levels and arterial blood pressure in the elderly. *Hypertension Research*, *28*(8), 625-630.

**Franceschi, C. (2007).** Inflammaging as a major characteristic of old people: can it be prevented or cured?. *Nutrition Reviews*, *65*(S3), S173-S176.

**Franceschi, C., & Campisi, J. (2014).** Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, 69(S1):S4–S9.

**Friedewald , W.T., Levy, R.I. and Fredrickson , S.D.,(1972).** Estemation of the concentration of low –density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge . *Clinical Chemistry* , 18 (6):499-502.

**Fukai, S., Akishita, M., Miyao, M., Ishida, K., Toba, K., & Ouchi, Y. (2010).** Age-related changes in plasma androgen levels and their association with cardiovascular risk factors in male Japanese office workers. *Geriatrics & Gerontology International,* 10(1) : 32-39.

**Giovannini, S., Onder, G., Liperoti, R., Russo, A., Carter, C., Capoluongo, E., Pahor, M., Bernabei, R., & Landi, F. (2011).** Interleukin-6, C-reactive protein, and tumor necrosis factor-alpha as predictors of mortality in frail, community-living elderly individuals. *Journal of the American Geriatrics Society,* 59(9) : 1679-1685.

**Glasser, S. P., Basile, J. N., & Lackland, D. T. (2009).** Does Prehypertension Represent an Increased Risk for Incident Hypertension and Adverse Cardiovascular Outcome?. *Hypertension,* 54(5) : 954-955.

**Goettsch, W., Lattmann, T., Amann, K., Szibor, M., Morawietz, H., Münter, K., Müller, S. P., Shaw,S., & Barton, M. (2001).** Increased expression of endothelin-1 and inducible nitric oxide synthase isoform II in aging arteries in vivo: implications for atherosclerosis. *Biochemical and Biophysical Research Communications*, 280(3) : 908-913.

**Gomi, I., Fukushima, H., Shiraki, M., Miwa, Y., Ando, T., Takai, K., & Moriwaki, H. (2007).** Relationship between serum albumin level and aging in community-dwelling self-supported elderly population. *Journal of Nutritional Science and Vitaminology*, 53(1) : 37-42.

**Gonzalez, C., Corbacho, A. M., Eiserich, J. P., Garcia, C., Lopez-Barrera, F., Morales-Tlalpan, V., Barajas-Espinosa, A. , Diaz-Muñoz, M., Rubio, R., Lin, S., Escalera, G. M.,& Clapp, C. (2004).** 16K-prolactin inhibits activation of endothelial nitric oxide synthase, intracellular calcium mobilization , and endothelium-dependent vasorelaxation. *Endocrinology*, *145*(12), 5714-5722.

**Grabowska-Markowska , J., Pawłowska, I., Ziółkowski, G., & Wójkowska-Mach, J. (2019).** Bacteraemia caused by ochrobactrum anthropi-unusual behavior. Wiadomosci Lekarskie (Warsaw, Poland: 1960), 72(3): 489-492.

**Guarner, V., & Rubio-Ruiz, M. E. (2015).** Low-grade systemic inflammation connects aging, metabolic syndrome and cardiovascular disease. *Aging and Health-A systems Biology Perspective*, 40: 99-106.

**Gyllenborg, J., Rasmussen, S. L., Borch-Johnsen, K., Heitmann, B. L., Skakkeb, N. E., & Juul, A. (2001).** Cardiovascular risk factors in men: the role of gonadal steroids and sex hormone-binding globulin. *Metabolism-Clinical and Experimental*, 50(8) : 882-888.

**Hair, W. M., Gubbay, O., Jabbour, H. N., & Lincoln, G. A. (2002).** Prolactin receptor expression in human testis and accessory tissues: localization and function. *Molecular Human Reproduction*, 8(7) : 606-611.

**Håkonsen, L. B., Thulstrup, A. M., Aggerholm, A. S., Olsen, J., Bonde, J. P., Andersen, C. Y., Bungum, M., Ernst, E. H., Hansen, M. L., Ernst, E. H.,& Ramlau-Hansen, C. H. (2011).** Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. *Reproductive Health*, 8(1) : 1-8.

**Hall, J., Jones, R. D., Jones, T. H., Channer, K. S., & Peers, C. (2006).** Selective inhibition of L-type Ca2+ channels in A7r5 cells by physiological levels of testosterone. *Endocrinology*, 147(6) : 2675-2680.

**Halmenschlager, G., Rhoden, E. L., & Riedner, C. E. (2011).** The influence of age on bioavailable and free testosterone is independent of body mass index and glucose levels. *World Journal of Urology*, *29*(4), 541-546.

**Handelsman, D.J.(2020)** Androgen Physiology, Pharmacology, Use and Misuse. https://www.ncbi.nlm.nih.gov/books/NBK279000 .

**Haring, R., Baumeister, S. E., Völzke, H., Dörr, M., Kocher, T., Nauck, M., & Wallaschofski, H. (2012).** Prospective inverse associations of sex hormone concentrations in men with biomarkers of inflammation and oxidative stress. *Journal of Andrology*, 33(5): 944-950.

**Härkönen, P. L., & Mäkelä, S. I. (2004).** Role of estrogens in development of prostate cancer. *The Journal of Steroid Biochemistry and Molecular Biology*, 92(4): 297-305.

**Harman, S. M., Metter, E. J., Tobin, J. D., Pearson, J., & Blackman, M. R. (2001).** Longitudinal effects of aging on serum total and free testosterone

levels in healthy men. *The Journal of Clinical Endocrinology & Metabolism*, *86*(2) :724-731.

**Hassan, A. (2010).** Effect of castration on some physiological aspect in rats: Effect of testosterone hormone. *Journal of Education and Science*, 23(3) : 28-39.

**Hauger-Klevene, J. H., Pinkas, M. B., & Gerber, S. U. S. A. N. A. (1981).** Blood pressure and prolactin: effects of guanfacine. Three-year follow-up study. *Hypertension*, *3*(6_pt_2) : II-222.

**Hauser, L. J., Jensen, E. L., Mirsky, D. M., & Chan, K. H. (2018).** Pediatric anosmia: A case series. *International Journal of Pediatric Otorhinolaryngology*, 110 : 135-139.

**Hermann, M., Flammer, A., & Lüscher, T. F. (2006).** Nitric oxide in hypertension. *The Journal of Clinical Hypertension*, 8 : 17-29.

**Herrera, M. D., Mingorance, C., Rodríguez-Rodríguez, R., & de Sotomayor, M. A. (2010).** Endothelial dysfunction and aging: an update. *Ageing Research Reviews*, 9(2) : 142-152.

**Hess, R. A. (2000).** Oestrogen in fluid transport in efferent ducts of the male reproductive tract. *Reviews of Reproduction*, 5(2), 84-92.

**Hotta, Y., Kataoka, T., & Kimura, K. (2019).** Testosterone deficiency and endothelial dysfunction: nitric oxide, asymmetric dimethylarginine, and endothelial progenitor cells. *Sexual Medicine Reviews*, 7(4) : 661-668.

**Hou, Y. C., Janczuk, A., and Wang, P. G. (1999).** Current trends in the development of nitric oxide donors. *Current Pharmaceutical Design*, *5*(6) : 417-442.

**Houston, M., & Hays, L. (2014).** Acute effects of an oral nitric oxide supplement on blood pressure, endothelial function, and vascular compliance in hypertensive patients. *The Journal of Clinical Hypertension*, 16(7) : 524-529.

**Howcroft, T. K., Campisi, J., Louis, G. B., Smith, M. T., Wise, B., Wyss-Coray, T., Augustine, A. D., McElhaney, J. E., R., Kohanski, & Sierra, F. (2013).** The role of inflammation in age-related disease. *Aging* (*Albany NY*), 5(1) : 84.

**Huhtaniemi, I. (2014).** Late-onset hypogonadism: current concepts and controversies of pathogenesis, diagnosis and treatment. *Asian Journal of Andrology*, *16*(2) : 192–202.

**Hung, M. J., Cherng, W. J., Hung, M. Y., Wu, H. T., & Pang, J. H. S. (2010).** Interleukin-6 inhibits endothelial nitric oxide synthase activation and increases endothelial nitric oxide synthase binding to stabilized caveolin-1 in human vascular endothelial cells. *Journal of Hypertension*, 28(5) : 940-951.

**Ignacak, A., Kasztelnik, M., Sliwa, T., Korbut, R. A., Rajda, K., & Guzik, T. J. (2012).** Prolactin–not only lactotrophin. A "new" view of the "old" hormone. *Jornal of Physiology and Pharmacology*, 63(5) : 435-43.

**Ihara, H., Toya, N., Kakinoki, T., Tani, A., Aoki, Y., Hashizume, N., Inada, Y., Namba, S., Urayama, I., & Yoshida, M. (2001).** Reference ranges for serum protein fractions as determined by capillary zone electrophoresis. *Seibutsu Butsuri Kagagu*, 45(1) : 69-74.

**Isidori, A. M., Giannetta, E., Greco, E. A., Gianfrilli, D., Bonifacio, V., Isidori, A., Lenzi,A., & Fabbri, A. (2005).** Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. *Clinical Endocrinology*, 63(3) : 280-293.

**Jackson, J. S., Knight, K. M., & Rafferty, J. A. (2010).** Race and unhealthy behaviors: chronic stress, the HPA axis, and physical and mental health disparities over the life course. *American Journal of Public Health*, *100*(5): 933-939.

**Jasuja, G. K., Travison, T. G., Davda, M., Murabito, J. M., Basaria, S., Zhang, A., Kushnir,M.M., Rockwood, A. L., Meikle, W., Pencina, M. J., Coviello, A., Rose, A. J., Agostino, R., Vasan, R. S., & Bhasin, S. (2013).** Age trends in estradiol and estrone levels measured using liquid chromatography tandem mass spectrometry in community-dwelling men of the Framingham Heart Study. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, 68(6) : 733-740.

**Javaroni, V., & Neves, M. F. (2012).** Erectile dysfunction and hypertension: impact on cardiovascular risk and treatment. *International Journal of Hypertension*, 2012:1-11.

**Jellum, E., Dollekamp, H., Brunsvig, A., & Gislefoss, R. (1997).** Diagnostic applications of chromatography and capillary electrophoresis. *Journal of Chromatography B: Biomedical Sciences and Applications*, *689*(1), 155-164.

**Jones, T. H. (2010).** Testosterone deficiency: a risk factor for cardiovascular disease?. *Trends in Endocrinology & Metabolism*, 21(8): 496-503.

**Kalfa, N., Gaspari, L., Ollivier, M., Philibert, P., Bergougnoux, A., Paris, F., & Sultan, C. (2019).** Molecular genetics of hypospadias and cryptorchidism recent developments. *Clinical Genetics*, 95(1) : 122-131.

**Kalinchenko, S. Y., Tishova, Y. A., Mskhalaya, G. J., Gooren, L. J., Giltay, E. J., & Saad, F. (2010).** Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: the double-blinded placebo-controlled Moscow study. *Clinical Endocrinology*, 73(5) : 602-612.

**Kanda, N., Tsuchida, T., & Tamaki, K. (1996).** Testosterone inhibits immunoglobulin production by human peripheral blood mononuclear cells. *Clinical & Experimental Immunology*, 106(2) : 410-415.

**Kanda, N., Tsuchida, T., & Tamaki, K. (1997).** Testosterone suppresses anti-DNA antibody production in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*, 40(9) : 1703-1711.

**Kapasi, A. A., Kumar, R., Pauly, J. R., & Pandey, K. N. (1996).** Differential expression and autoradiographic localization of atrial natriuretic peptide receptor in spontaneously hypertensive and normotensive rat testes: diminution of testosterone in hypertension. *Hypertension*, 28(5) : 847-853.

**Kataoka, T., & Kimura, K. (2019).** Estrogen for Male Function: Effect of Changes in the Sex Hormone Milieu on Erectile Function. A.K.Wahid(EDs). Estrogen.https://www.intechopen.com/books/6891 .

**Katayama, Y., Soya, H., Fujinaka, M., Mori, H., Tomita, A., and Kayahara, N. (2009).** Evaluation of New Homogeneous Assay Kit to Determine HDL-C with a High Reactivity with Cholesterol in Various Types of HDL. *Clinical Chemistry*, 55( 6): A83.

**Kaufman, J. M., & Vermeulen, A. (2005).** The decline of androgen levels in elderly men and its clinical and therapeutic implications. *Endocrine Reviews*, 26(6) : 833-876.

**Kelly, D. M., & Jones, T. H. (2013).** Testosterone: a vascular hormone in health and disease. *Jornal of Endocrinol*, 217(3) : R47-R71.

**Kelsey, T. W., Li, L. Q., Mitchell, R. T., Whelan, A., Anderson, R. A., & Wallace, W. H. B. (2014).** A validated age-related normative model for male total testosterone shows increasing variance but no decline after age 40 years. *PloS one*, 9(10) : 1-11.

**Keshet, R., & Erez, A. (2018).** Arginine and the metabolic regulation of nitric oxide synthesis in cancer. *Disease Models & Mechanisms*, 11(8):1-11

**Khaw, K. T., Dowsett, M., Folkerd, E., Bingham, S., Wareham, N., Luben, R., Welch, A., & Day, N. (2007).** *Clinical Perspective. Circulation*, 116(23): 2694-2701.

**Kocar, I. H., Yesilova, Z., Özata, M., Turan, M., Sengül, A., & Özdemir, I. Ç. (2000).** The effect of testosterone replacement treatment on immunological features of patients with Klinefelter's syndrome. *Clinical & Experimental Immunology*, 121(3), 448-452.

**Kolovou, G., Bilianou, H., Marvaki, A., & Mikhailidis, D. P. (2011).** Aging men and lipids. *American Journal of Men's Health*, 5(2) : 152-165.

**Krysiak, R., Szkróbka, W., & Okopień, B. (2020).** Endogenous testosterone determines metformin action on prolactin levels in hyperprolactinaemic men: A pilot study. *Basic & Clinical Pharmacology & Toxicology*, 126(2): 110-115.

**Kunes, J., Hojna, S., Kadlecova, M., Dobesova, Z., Rauchova, H., Vokurkova, M., Loukotova , J., Pechanova, O., & Zicha, J. (2004).** Altered balance of vasoactive systems in experimental hypertension: the role of relative NO deficiency. *Physiological Research*, 53(S1): S23-S34.

**Kupelian, V., Chiu, G. R., Araujo, A. B., Williams, R. E., Clark, R. V., & McKinlay, J. B. (2010).** Association of sex hormones and C-reactive protein levels in men. *Clinical Endocrinology*, 72(4) : 527-533.

**Laaksonen, D. E., Niskanen, L., Punnonen, K., Nyyssonen, K., Tuomainen, T. P., Salonen, R., Rauramaa, R., & Salonen, J. T. (2003).** Sex

hormones, inflammation and the metabolic syndrome: a population-based study. *European Journal of Endocrinology*, 149(6): 601-608.

**Lakatta, E. G., & Levy, D. (2003).** Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. *Circulation*, *107*(1) : 139-146.

**Lakoski, S. G., Cushman, M., Siscovick, D. S., Blumenthal, R. S., Palmas, W., Burke, G., & Herrington, D. M. (2011).** The relationship between inflammation, obesity and risk for hypertension in the Multi-Ethnic Study of Atherosclerosis (MESA). *Journal of Human Hypertension*, 25(2), 73-79.

**Langer, C., Gansz, B., Goepfert, C., Engel, T., Uehara, Y., von Dehn, G., ... & von Eckardstein, A. (2002).** Testosterone up-regulates scavenger receptor BI and stimulates cholesterol efflux from macrophages. *Biochemical and Biophysical Research Communication*s, 296(5) : 1051-1057.

**Lau, D. H., Nattel, S., Kalman, J. M., & Sanders, P. (2017).** Modifiable risk factors and atrial fibrillation. *Circulation*, 136(6) : 583-596.

**Laughlin, G. A., Barrett-Connor, E., & Bergstrom, J. (2008).** Low serum testosterone and mortality in older men. *The Journal of Clinical Endocrinology & Metabolism*, 93(1) : 68-75.

**Lee, D. L., Sturgis, L. C., Labazi, H., Osborne Jr, J. B., Fleming, C., Pollock, J. S., Manhiani, M., Imig, J. D., & Brands, M. W. (2006).** Angiotensin II hypertension is attenuated in interleukin-6 knockout mice. *American Journal of Physiology-Heart and Circulatory Physiology*, 290(3) : H935-H940.

**Lee, H. R., Kim, T. H., & Choi, K. C. (2012).** Functions and physiological roles of two types of estrogen receptors, ERα and ERβ, identified by estrogen receptor knockout mouse. *Laboratory Animal Research*, 28(2) : 71-76.

**Levine, A. B., Punihaole, D., & Levine, T. B. (2012).** Characterization of the role of nitric oxide and its clinical applications. *Cardiology*, 122(1): 55-68.

**Levine, S., & Muneyyirci-Delale, O. (2018).** Stress-induced hyperprolactinemia: pathophysiology and clinical approach. *Obstetrics and Gynecology International*, *2018*:1-6.

**Lewerin, C., Nilsson-Ehle, H., Jacobsson, S., Johansson, H., Sundh, V., Karlsson, M. K., Lorentzon, M., Barrett-Connor, E., Vandenput, L., Ohlsson, C.,& Mellström, D. (2014).** Serum estradiol associates with blood hemoglobin in elderly men: the MrOS Sweden study. *The Journal of Clinical Endocrinology & Metabolism*, 99(7) : 2549-2556.

**Lewington, S. (2002).** Prospective studies collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*, 360 : 1903-1913.

**Li, N., Ma, R., Wang, S., Zhao, Y., Wang, P., Yang, Z., Jin, L., Zhang, P., Ding, H., Bai, F., & Yu, J. (2019).** The potential role of testosterone in hypertension and target organ damage in hypertensive postmenopausal women. *Clinical Interventions in Aging*, 14 : 743–752.

**Li, Y., Sugiyama, E., Yokoyama, S., Jiang, L., Tanaka, N., & Aoyama, T. (2008).** Molecular Mechanism of Age-Specific Hepatic Lipid Accumulation in PPARα (+/−): LDLR (+/−) Mice, an Obese Mouse Model. *Lipids*, 43(4) : 301-312.

**Liu, Y. Z., Wang, Y. X., & Jiang, C. L. (2017).** Inflammation: the common pathway of stress-related diseases. *Frontiers in Human Neuroscience*, 11( 316):1-11.

**Lotti, F., & Maggi, M. (2018).** Sexual dysfunction and male infertility. *Nature Reviews Urology*, *15*(5) : 287-307.

**Maggio, M., Guralnik, J. M., Longo, D. L., & Ferrucci, L. (2006).** Interleukin-6 in aging and chronic disease: a magnificent pathway. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *61*(6) : 575-584.

**Mäkinen, J. I., Perheentupa, A., Irjala, K., Pöllänen, P., Mäkinen, J., Huhtaniemi, I., & Raitakari, O. T. (2008).** Endogenous testosterone and serum lipids in middle-aged men. *Atherosclerosis*, 197(2) : 688-693.

**Malkin, C. J., Pugh, P. J., Jones, R. D., Kapoor, D., Channer, K. S., & Jones, T. H. (2004).** The effect of testosterone replacement on

endogenous inflammatory cytokines and lipid profiles in hypogonadal men. *The Journal of Clinical Endocrinology & Metabolism*, 89(7) : 3313-3318.

**Mancuso, P., & Bouchard, B. (2019).** The impact of aging on adipose function and adipokine synthesis. *Frontiers in Endocrinology*, 10 : 137.

**Maranon, R., & Reckelhoff, J. F. (2013).** Sex and gender differences in control of blood pressure. *Clinical Science*, *125*(7), 311-318.

**Maria, M. (2016).** The Role of Prolactin in Men. *Endocrinology & Metabolic Syndrome*, 05(01):1-6.

**Matsumoto, A. M. (2002).** Andropause: clinical implications of the decline in serum testosterone levels with aging in men. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 57(2): M76-M99.

**Mattace-Raso, F. U., Verwoert, G. C., Hofman, A., & Witteman, J. C. (2010).** Inflammation and incident-isolated systolic hypertension in older adults: the Rotterdam study. *Journal of Hypertension*, 28(5): 892-895.

**Matz, R. L., De Sotomayor, M. A., Schott, C., Stoclet, J. C., & Andriantsitohaina, R. (2000).** Vascular bed heterogeneity in age-related endothelial dysfunction with respect to NO and eicosanoids. *British Journal of Pharmacology*, 131(2): 303-311.

**MB, D. J. H. (2000).** Androgen Physiology, Pharmacology and Abuse. *Endotext* . https://www.ncbi.nlm.nih.gov/books/NBK279000.

**Medina, K. L., Smithson, G., & Kincade, P. W. (1993).** Suppression of B lymphopoiesis during normal pregnancy. *The Journal of Experimental Medicine*, 178(5): 1507-1515.

**Memon, A. R., Shaikh, F., Memon, Z. A., & Majeed, A. B. A. (2017).** Assessment of Lipid Profile & Serum Total Protein in Patients with Hypertension. *Annals of PIMS*, 13(2):246-248.

**Michels, G., & Hoppe, U. C. (2008).** Rapid actions of androgens. *Frontiers in Neuroendocrinology*, 29(2): 182-198.

**Miller, A. L. (2006).** The Effects of a Sustained-Release L-Arginine Formulation on Blood Pressure and Vascular Compliance in 29 Healthy Individuals. *Alternative Medicine Review*, 11(1): 23–29.

**Minciullo, P. L., Catalano, A., Mandraffino, G., Casciaro, M., Crucitti, A., Maltese, G., Morabito, N., Lasco, A., Gangemi , S., & Basile, G. (2016).** Inflammaging and anti-inflammaging: the role of cytokines in extreme longevity. *Archivum Immunologiae Et Therapiae Experimentalis*, 64(2): 111-126.

**Miyake, M., Ogawa, Y., Yoshida, Y., & Imaki, M. (2011).** Seven-year large cohort study for the association of serum albumin level and aging among community dwelling elderly. *Journal of Analytical Bio-Science* , 34(4): 281- 286.

**Mohamad, N. V., Wong, S. K., Hasan, W. N. W., Jolly, J. J., Nur-Farhana, M. F., Ima-Nirwana, S., & Chin, K. Y. (2018).** The relationship between circulating testosterone and inflammatory cytokines in men. *The Aging Male*. 22(2):129–140

**Molinari, C., Grossini, E., Mary, D. A., Uberti, F., Ghigo, E., Ribichini, F., ... & Vacca, G. (2007).** Prolactin induces regional vasoconstriction through the β2-adrenergic and nitric oxide mechanisms. *Endocrinology*, 148(8), 4080-4090.

**Moon, H., Choi, I., Kim, S., Ko, H., Shin, J., Lee, K., , Sung , J., & Song, Y. M. (2017).** Cross-sectional association between testosterone, sex hormone-binding globulin and metabolic syndrome: The Healthy Twin Study. *Clinical Endocrinology*, 87(5): 523-531.

**Moreau, K. L., Babcock, M. C., & Hildreth, K. L. (2020).** Sex differences in vascular aging in response to testosterone. *Biology of sex Differences*, 11:1-14.

**Morelli, A., Filippi, S., Mancina, R., Luconi, M., Vignozzi, L., Marini, M., ... & Maggi, M. (2004).** Androgens regulate phosphodiesterase type 5 expression and functional activity in corpora cavernosa. *Endocrinology*, 145(5) : 2253-2263.

**Moretti, C., Lanzolla, G., Moretti, M., Gnessi, L., & Carmina, E. (2017).** Androgens and hypertension in men and women: a unifying view. *Current Hypertension Reports*, 19(5): 1-8.

**Morrisette-Thomas, V., Cohen, A. A., Fülöp, T., Riesco, É., Legault, V., Li, Q., Milot, E., Dusseault-Bélanger, F., & Ferrucci, L. (2014).**

Inflamm-aging does not simply reflect increases in pro-inflammatory markers. *Mechanisms of Ageing and Development*, 139: 49-57.

**Muller, M., den Tonkelaar, I., Thijssen, J. H., Grobbee, D. E., & van der Schouw, Y. T. (2003).** Endogenous sex hormones in men aged 40-80 years. *European Journal of Endocrinology*, 149(6): 583-590.

**Mulligan, T., Frick, M. F., Zuraw, Q. C., Stemhagen, A., & McWhirter, C. (2006).** Prevalence of hypogonadism in males aged at least 45 years: the HIM study. *International Journal of Clinical Practice*, 60(7): 762-769.

**Nakamura, T., Kawachi, K., Saito, Y., Saito, T., Morishita, K., Hoshino, J., Hosoi, T., Iwasaki, T., Ohyama, Y., & Kurabayashi, M. (2009).** Effects of ARB or ACE-inhibitor administration on plasma levels of aldosterone and adiponectin in hypertension. *International Heart Journal*, 50(4):501-512.

**Nakhai Pour, H. R., Grobbee, D. E., Bots, M. L., Verhaar, H. J., Muller, M., & Van Der Schouw, Y. T. (2007).** Circulating sex hormone levels and aortic stiffness in men . *Journal of the American Geriatrics Society*, 55(4):621-622.

**Nassar, G. N., & Leslie, S. W. (2018).** Physiology, testosterone . *Treasure Island, FL: StatPearls*. https://europepmc.org/article/NBK/nbk526128

**Naya, M., Tsukamoto, T., Morita, K., Katoh, C., Furumoto, T., Fujii, S., ... & Tsutsui, H. (2007).** Plasma interleukin-6 and tumor necrosis factor-α can predict coronary endothelial dysfunction in hypertensive patients. *Hypertension Research*, 30(6): 541-548.

**Nehring, S. M., Goyal, A., Bansal, P., & Patel, B. C. (2020).** C reactive protein (CRP). *Treasure Island, FL: StatPearls*.https://europepmc.org/books/n/statpearls/article18744/?extid=31747193&src=med

**Neumann, P., Gertzberg, N., & Johnson, A. (2004).** TNF-α induces a decrease in eNOS promoter activity. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 286(2): L452-L459.

**Nieschlag, E., & Nieschlag, S. (2014).** Testosterone deficiency: a historical perspective. *Asian journal of andrology*, 16(2): 161–168.

**Nieschlag, E., Behre, H. M., and Nieschlag, S. (2012).** Testosterone: action, deficiency, substitution. *Cambridge University Press*.4thed .pp 512.

**Norata, G. D., Tibolla, G., Seccomandi, P. M., Poletti, A., & Catapano, A. L. (2006).** Dihydrotestosterone decreases tumor necrosis factor-α and lipopolysaccharide-induced inflammatory response in human endothelial cells. *The Journal of Clinical Endocrinology & Metabolism*, 91(2): 546-554.

**North, B. J., & Sinclair, D. A. (2012).** The intersection between aging and cardiovascular disease. *Circulation Research*, *110*(8): 1097-1108.

**Nunes, K. P., Labazi, H., & Webb, R. C. (2012).** New insights into hypertension-associated erectile dysfunction. *Current Opinion in Nephrology and Hypertension*, 21(2): 163–170.

**O'Connell, T., Horita, T. J., & Kasravi, B. (2005).** Understanding and interpreting the serum protein electrophoresis. *American Family Physician*, *71*(1): 105-112.

**Oparil, S., Zaman, M. A., & Calhoun, D. A. (2003).** Pathogenesis of hypertension. *Annals of Internal Medicine*, 139(9):761-776.

**Orshal, J. M., & Khalil, R. A. (2004).** Gender, sex hormones, and vascular tone. American Journal of Physiology-Regulatory, *Integrative and Comparative Physiology*, 286(2): R233-R249.

**Osmanovic-Thunström, A., Mossello, E., Åkerstedt, T., Fratiglioni, L., & Wang, H. X. (2015).** Do levels of perceived stress increase with increasing age after age 65? A population-based study. *Age and Ageing*, *44*(5): 828-834.

**Pala, N. A., Laway, B. A., Misgar, R. A., & Dar, R. A. (2015).** Metabolic abnormalities in patients with prolactinoma: response to treatment with cabergoline. *Diabetology & Metabolic Syndrome*, 7(1): 1-6.

**Palmeri ,R. & Grimaudo , S.(2013).** Estradiol: Synthesis, Health Effects and Drug Interactions. *Nova Science Pub Inc*. 1st ed .pp 304.

**Palmeri, M., Misiano, G., Malaguarnera, M., Forte, G. I., Vaccarino, L., Milano, S., Scola, L., Caruso, C.,Motta, M.,Maugeri, D., & Lio, D. (2012).** Cytokine serum profile in a group of Sicilian nonagenarians. *Journal of Immunoassay and Immunochemistry*, 33(1): 82-90.

**Pandey, K. N. (2014).** Guanylyl cyclase/natriuretic peptide receptor-A signaling antagonizes phosphoinositide hydrolysis, Ca2+ release, and

activation of protein kinase C. *Frontiers in Molecular Neuroscience*, 7( 75):1-14

**Parameswaran, N., & Patial, S. (2010).** Tumor necrosis factor-α signaling in macrophages. *Critical Reviews™ in Eukaryotic Gene Expression*, 20(2): 87–103.

**Park, K. H., Kim, S. W., Kim, K. D., & Paick, J. S. (1999).** Effects of androgens on the expression of nitric oxide synthase mRNAs in rat corpus cavernosum. *British Journal of Urology International*, 83(3):327-333.

**Pechanova, O., Varga, Z. V., Cebova, M., Giricz, Z., Pacher, P., & Ferdinandy, P. (2015).** Cardiac NO signalling in the metabolic syndrome. *British Journal of Pharmacology*, 172(6):1415-1433.

**Peeters, A. C. T. M., Netea, M. G., Janssen, M. C. H., Kullberg, B. J., Van der Meer, J. W. M., & Thien, T. (2001).** Pro-inflammatory cytokines in patients with essential hypertension. *European Journal of Clinical Investigation*, 31(1): 31-36.

**Perusquía, M., Herrera, N., Ferrer, M., & Stallone, J. N. (2017).** Antihypertensive effects of androgens in conscious, spontaneously hypertensive rats. *The Journal of Steroid Biochemistry and Molecular Biology*, 167:106-114.

**Pinheiro, L. C., Tanus-Santos, J. E., & Castro, M. M. (2017).** The potential of stimulating nitric oxide formation in the treatment of hypertension. *Expert Opinion on Therapeutic Targets*, 21(5):543-556.

**Price, C. P., Trull, A. K., Berry, D., and Gorman, E. G. (1987)**. Development and validation of a particle-enhanced turbidimetric immunoassay for C-reactive protein. *Journal of Immunological Methods*, *99* (2) : 205-211.

**Puzianowska-Kuźnicka, M., Owczarz, M., Wieczorowska-Tobis, K., Nadrowski, P., Chudek, J., Slusarczyk, P.,Skalska,A., Jonas, M., Franek , E.,& Mossakowska, M. (2016).** Interleukin-6 and C-reactive protein, successful aging, and mortality: the PolSenior study. *Immunity & Ageing*, 13(1) : 1-12.

**Quiroz, Y., Johnson, R. J., & Rodríguez-Iturbe, B. (2012).** The role of T cells in the pathogenesis of primary hypertension. *Nephrology Dialysis Transplantation*, 27(S4): iv2-iv5.

**Ranabir, S., & Reetu, K. (2011).** Stress and hormones. *Indian Journal of Endocrinology and Metabolism*, *15*(1): 18.

**Raut, S., Deshpande, S., & Balasinor, N. H. (2019).** Unveiling the role of prolactin and its receptor in male reproduction. *Hormone and Metabolic Research*, 51(04): 215-219.

**Reckelhoff, J. F. (2005).** Sex steroids, cardiovascular disease, and hypertension: unanswered questions and some speculations. *Hypertension*, 45(2): 170-174.

**Rodríguez-Mañas, L., El-Assar, M., Vallejo, S., López-Dóriga, P., Solís, J., Petidier, R., Montes, M.,Nevado, J., Castro, M., Gómez-Guerrero, Peiró, C., & Sánchez-Ferrer, C. F. (2009).** Endothelial dysfunction in aged humans is related with oxidative stress and vascular inflammation. *Aging cell*, 8(3): 226-238.

**Roelfsema, F., Pijl, H., Keenan, D. M., & Veldhuis, J. D. (2012).** Prolactin secretion in healthy adults is determined by gender, age and body mass index. *PloS one*, 7(2): 1-10.

**Roselli, C. E., Bocklandt, S., Stadelman, H. L., Wadsworth, T., Vilain, E., & Stormshak, F. (2008).** Prolactin expression in the sheep brain. *Neuroendocrinology*, 87(4): 206-215.

**Roth, J. A., Etzioni, R., Waters, T. M., Pettinger, M., Rossouw, J. E., Anderson, G. L., Chlebowski, R. T., Manson, J. E., Hlatky, M., Johnson, K. C., & Ramsey, S. D. (2014).** Economic return from the Women's Health Initiative estrogen plus progestin clinical trial: a modeling study. *Annals of internal medicine*, 160(9): 594-602.

**Rubio-Ruiz, M. E., Perez-Torres, I., Soto, M. E., Pastelin, G., & Guarner-Lans, V. (2014).** Aging in blood vessels. Medicinal agents FOR systemic arterial hypertension in the elderly. *Ageing research reviews*, 18: 132-147.

**Ruiz-Herrera, X., de Los Ríos, E. A., Díaz, J. M., Lerma-Alvarado, R. M., Martínez de la Escalera, L., López-Barrera,F., Lemini, M., E., Arnold, Escalera, G. M., Clapp, C., & Macotela, Y. (2017).**

Prolactin promotes adipose tissue fitness and insulin sensitivity in obese males. *Endocrinology*, 158(1): 56-68.

**Sader, M. A., Griffiths, K. A., Skilton, M. R., Wishart, S. M., Handelsman, D. J., & Celermajer, D. S. (2003).** Physiological testosterone replacement and arterial endothelial function in men. *Clinical Endocrinology*, 59(1): 62-67.

**Sahakyan, K., Klein, B. E., Myers, C. E., Tsai, M. Y., & Klein, R. (2010).** Novel risk factors in long-term hypertension incidence in type 1 diabetes mellitus. *American Heart Journal*, 159(6): 1074-1080.

**Sanada, F., Taniyama, Y., Muratsu, J., Otsu, R., Shimizu, H., Rakugi, H., & Morishita, R. (2018).** Source of chronic inflammation in aging. *Frontiers in Cardiovascular Medicine*, 5: 1-5.

**Sartorius, G., Spasevska, S., Idan, A., Turner, L., Forbes, E., Zamojska, A., Allan, A. A., Ly, L. P., Conway, A. J.,McLachlan, R. I., & Handelsman, D. J. (2012).** Serum testosterone, dihydrotestosterone and estradiol concentrations in older men self-reporting very good health: The healthy man study. *Clinical Endocrinology*, 77(5): 755-763.

**Sawin, C. T., Carlson, H. E., Geller, A., Castelli, W. P., & Bacharach, P. (1989).** Serum prolactin and aging: basal values and changes with estrogen use and hypothyroidism. *Journal of Gerontology*, 44(4): M131-M135.

**Scheller, J., Chalaris, A., Schmidt-Arras, D., & Rose-John, S. (2011).** The pro-and anti-inflammatory properties of the cytokine interleukin-6. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1813(5): 878-888.

**Schiffrin, E. L. (2013).** The immune system: role in hypertension. *Canadian Journal of Cardiology*, 29(5) : 543-548.

**Schulster, M., Bernie, A. M., & Ramasamy, R. (2016).** The role of estradiol in male reproductive function. *Asian Journal of Andrology*, 18(3):435.

**Schultz,c . (2018).** Tumor Necrosis Factor. *General Internal Medicine and Clinical Innovations* , 3(3): 1-2 .

**Schwetz, V., Librizzi, R., Trummer, C., Theiler, G., Stiegler, C., Pieber, T. R., Obermayer-Pietsch,B.,&Pilz,S.(2017).** Treatment of

hyperprolactinaemia reduces total cholesterol and LDL in patients with prolactinomas. *Metabolic Brain Disease*, 32(1):155-161.

**Seals, D. R., Jablonski, K. L., & Donato, A. J. (2011).** Aging and vascular endothelial function in humans. *Clinical Science*, 120(9): 357-375.

**Sedger, L. M., & McDermott, M. F. (2014).** TNF and TNF-receptors: From mediators of cell death and inflammation to therapeutic giants–past, present and future. *Cytokine & Growth Factor Reviews*, 25(4): 453-472.

**Senchenkova, E. Y., Komoto, S., Russell, J., Almeida-Paula, L. D., Yan, L. S., Zhang, S., & Granger, D. N. (2013).** Interleukin-6 mediates the platelet abnormalities and thrombogenesis associated with experimental colitis. *The American Journal of Pathology*, 183(1): 173-181.

**Shen, J. K., Dong, A., Hackett, S. F., Bell, W. R., Green, W. R., & Campochiaro, P. A. (2007).** Oxidative damage in age-related macular degeneration. *Histology and Histopathology*, 22 :1301-1308.

**Shimodaira, M., Nakayama, T., Sato, N., Saito, K., Morita, A., Sato, I., ... & Izumi, Y. (2008).** Association study of aromatase gene (CYP19A1) in essential hypertension. *International Journal of Medical Sciences*, 5(1): 29-35.

**Siedel, J., Schmuck, R., Staepels, J., and Town, M. H. (1993).** Long term stable, liquid ready-to-use monoreagent for the enzymatic assay of serum or plasma triglycerides (GPO-PAP method). AACC meeting abstract 34. *Clinical Chemistry*, *39*: 1127.

**Sierra, C. (2020).** Hypertension and the risk of dementia. *Frontiers in Cardiovascular Medicine*, *7*:1-7.

**Simpson, E. R., Clyne, C., Rubin, G., Boon, W. C., Robertson, K., Britt, K., Speed, C., & Jones, M. (2002).** Aromatase—a brief overview. *Annual Review of Physiology*, 64(1): 93-127.

**Singh, T., & Newman, A. B. (2011).** Inflammatory markers in population studies of aging. *Ageing Research Reviews*, 10(3): 319-329.

**Skogastierna, C., Hotzen, M., Rane, A., & Ekström, L. (2014).** A supraphysiological dose of testosterone induces nitric oxide production and oxidative stress. *European Journal of Preventive Cardiology*, 21(8): 1049-1054.

**Snyder, P. J., Bhasin, S., Cunningham, G. R., Matsumoto, A. M., Stephens-Shields, A. J., Cauley, J. A., Gill, T. M., Barrett-Connor, E., Swerdloff, R. S., Wang, C., Ensrud, K. E., Lewis, C. E., Farrar, J. T., Cella, D., Rosen, R. C., Pahor, M., Crandall, J. P., Molitch, M. E., Cifelli, D., Dougar, D.,Fluharty, L., Resnick, S. M., Storer, T. W., Anton, S., Basaria, S., Diem, S. J., Hou, X., Mohler, E. R., Parsons, J. K.,Wenger, N. K., Zeldow, B., Landis, J. R., & Ellenberg, S. S. (2016).** Effects of testosterone treatment in older men. *New England Journal of Medicine*, 374(7): 611-624.

**Snyder, P. J., Ellenberg, S. S., Cunningham, G. R., Matsumoto, A. M., Bhasin, S., Barrett-Connor, E.,Gill,T.M., Farrar, J. T., Cella, D., Rosen, R. C., Resnick, S. M., Swerdloff, R. S., Cauley, J. A., Cifelli, D., Fluharty, L., Pahor, M., Ensrud, K. E., Lewis, C. E., Molitch, M. E., Crandall, J. P., Wang, C., Budoff, M. J., Wenger, N. K., Mohler, E. R., Bild, D.E., Cook, N. L., Keaveny, T. M., Kopperdahl, D. L., Lee, D., Schwartz, A. V., Storer, T.W., Ershler, W. B., Roy,C.N., Raffel, L. J., Romashkan, S., & Hadley,E. (2014).** The Testosterone Trials: seven coordinated trials of testosterone treatment in elderly men. *Clinical Trials*, 11(3): 362-375.

**Snyder, P.J.( 2019) .** Patient education:High Prolacin levels and Prolacinomas (Beyond the Basic) . https://www.uptodate.com/contents/high-prolactin-levels-and prolactinomas-beyond-the-basics .

**Spaziani, M., Mileno, B., Rossi, F., Granato, S., Tahani, N., Anzuini, A., Lenzi, A., & Radicioni, A. F. (2018).** Endocrine and metabolic evaluation of classic Klinefelter syndrome and high-grade aneuploidies of sexual chromosomes with male phenotype: are they different clinical conditions?. *European Journal of Endocrinology*, 178(4): 343-352.

**Spratt, D. I., Morton, J. R., Kramer, R. S., Mayo, S. W., Longcope, C., & Vary, C. P. (2006).** Increases in serum estrogen levels during major illness are caused by increased peripheral aromatization. *American Journal of Physiology-Endocrinology and Metabolism*, 291(3): E631-E638.

**Sproston, N. R., & Ashworth, J. J. (2018)**. Role of C-reactive protein at sites of inflammation and infection. *Frontiers in Immunology*, 9 (754):1-11.

**Stamatelopoulos, K. S., Georgiopoulos, G. A., Sfikakis, P. P., Kollias, G., Manios, E., Mantzou, E., Kyrkou, K., Zakopoulos, N., Papamichael, C. M.,& Alevizaki, M. (2011).** Pilot study of circulating prolactin levels and endothelial function in men with hypertension. *American Journal of Hypertension*, 24(5): 569-573.

**Stanhewicz, A. E., Wenner, M. M., & Stachenfeld, N. S. (2018).** Sex differences in endothelial function important to vascular health and overall cardiovascular disease risk across the lifespan. *American Journal of Physiology-Heart and Circulatory Physiology*, 315(6): H1569-H1588.

**Stuveling, E. M., Bakker, S. J., Hillege, H. L., Burgerhof, J. G., de Jong, P. E., Gans, R. O., & de Zeeuw, D. (2004).** C-reactive protein modifies the relationship between blood pressure and microalbuminuria. *Hypertension*, 43(4): 791-796.

**Sun, D., Huang, A., Yan, E. H., Wu, Z., Yan, C., Kaminski, P. M., Oury, T. D., Wolin, M.S., & Kaley, G. (2004).** Reduced release of nitric oxide to shear stress in mesenteric arteries of aged rats. *American Journal of Physiology-Heart and Circulatory Physiology*, 286(6): 2249-2256.

**Svechnikov, K., Izzo, G., Landreh, L., Weisser, J., & Söder, O. (2010).** Endocrine disruptors and Leydig cell function. *Journal of Biomedicine and Biotechnology*,2010:1-10.

**Sverdlov, A. L., Ngo, D. T., Chan, W. P., Chirkov, Y. Y., & Horowitz, J. D. (2014).** Aging of the nitric oxide system: are we as old as our NO?. *Journal of the American Heart Association*, 3(4): 1-13.

**Syntin, P., Chen, H., Zirkin, B. R., & Robaire, B. (2001).** Gene expression in Brown Norway rat Leydig cells: effects of age and of age-related germ cell loss. *Endocrinology*, 142(12):5277-5285.

**Tanaka, T., Narazaki, M., & Kishimoto, T. (2014).** IL-6 in inflammation, immunity, and disease. *Cold Spring Harbor perspectives in biology*, 6(10): 1-16.

**Tanase, D. M., Gosav, E. M., Radu, S., Ouatu, A., Rezus, C., Ciocoiu, M., … Floria, M. (2019).** Arterial Hypertension and Interleukins: Potential Therapeutic Target or Future Diagnostic Marker*? International Journal of Hypertension* , 2019:1–17.

**Tarbutton, P. N., & Gunter, C. R. (1974).** Enzymatic Determination of Total Cholesterol in Serum. *Clinical Chemistry, 20(6), 724–725.*

**Tengstrand, B., Carlström, K., & Hafström, I. (2002).** Bioavailable testosterone in men with rheumatoid arthritis—high frequency of hypogonadism. *Rheumatology,* 41(3): 285-289.

**Thienpont, L. M., Verhaeghe, P. G., Van Brussel, K. A., and De Leenheer, A. P. (1988).** Estradiol-17 beta quantified in serum by isotope dilution-gas chromatography-mass spectrometry:reversed-phase C18 high-performance liquid chromatography compared with immuno-affinity chromatography for sample pretreatment . *Clinical Chemistry*, 34(10):2066-2069.

**Thomas, M. P., & Potter, B. V. (2013).** The structural biology of oestrogen metabolism. *The Journal of Steroid Biochemistry and Molecular Biology*, *137*: 27-49.

**Tian, C. R., Qian, L., Shen, X. Z., Li, J. J., & Wen, J. T. (2014).** Distribution of serum total protein in elderly Chinese. *PloS one*, 9(6): 1-5

**Toma, A., Makonnen, E., & Yimer, G. (2014).** Nitric Oxide as a Target for Hypertension Management: a Review. *Journal of Pharmaceutical Research*, 13(2) : 27-33.

**Torkler, S., Wallaschofski, H., Baumeister, S. E., Völzke, H., Dörr, M., Felix, S., Rettig, R., Nauck, M.,& Haring, R. (2011).** Inverse association between total testosterone concentrations, incident hypertension and blood pressure. *The Aging Male*, 14(3) : 176-182.

**Torregrossa, A. C., Aranke, M., & Bryan, N. S. (2011).** Nitric oxide and geriatrics: Implications in diagnostics and treatment of the elderly. *Journal of Geriatric Cardiology:* JGC, 8(4) : 230−242.

**Travison, T. G., Araujo, A. B., Kupelian, V., O'Donnell, A. B., & McKinlay, J. B. (2007).** The relative contributions of aging, health, and lifestyle factors to serum testosterone decline in men. *The Journal of Clinical Endocrinology & Metabolism*, *92*(2) : 549-555.

**Tremellen, K., McPhee, N., & Pearce, K. (2017).** Metabolic endotoxaemia related inflammation is associated with hypogonadism in overweight men. *Basic and Clinical Andrology*, 27(1) : 1-9.

**Tsilidis, K. K., Rohrmann, S., McGlynn, K. A., Nyante, S. J., Lopez, D. S., Bradwin, G., Feinleib, M., Joshu, C. E., Kanarek, N., Nelson, W. G., Selvin , E., & Platz, E. A. (2013).** Association between endogenous sex steroid hormones and inflammatory biomarkers in US men. *Andrology*, 1(6): 919-928.

**Tsounis, D., Bouras, G., Giannopoulos, G., Papadimitriou, C., Alexopoulos, D., & Deftereos, S. (2014).** Inflammation markers in essential hypertension. *Medicinal Chemistry*, 10(7): 672-681.

**Urschel, K., & Cicha, I. (2015).** TNF-α in the cardiovascular system: from physiology to therapy. *International Journal of Interferon, Cytokine and Mediator Research*, 7: 9-25.

**Vaidya, D., Dobs, A., Gapstur, S. M., Golden, S. H., Hankinson, A., Liu, K., & Ouyang, P. (2008).** The association of endogenous sex hormones with lipoprotein subfraction profile in the Multi-Ethnic Study of Atherosclerosis. *Metabolism*, 57(6): 782-790.

**van den Beld, A. W., Kaufman, J. M., Zillikens, M. C., Lamberts, S. W., Egan, J. M., & van der Lely, A. J. (2018).** The physiology of endocrine systems with ageing. *The Lancet Diabetes & Endocrinology*, *6*(8): 647-658.

**Vanderschueren, S., Deeren, D., Knockaert, D. C., Bobbaers, H., Bossuyt, X., & Peetermans, W. (2006).** Extremely elevated C-reactive protein. *European Journal of Internal Medicine*, 17(6) : 430-433.

**Varadhan, R., Yao, W., Matteini, A., Beamer, B. A., Xue, Q. L., Yang, H., Manwani, B., Reiner, A., Jenny, N., Parekh, N., Fallin, M. D., Newman, A., Bandeen-Roche, K., Tracy, R., Ferrucci, L., & Walston, J. (2014).** Simple biologically informed inflammatory index of two serum cytokines predicts 10 year all-cause mortality in older adults. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, 69(2):165-173.

**Vermeulen, A., Kaufman, J. M., Goemaere, S., & Van Pottelberg, I. (2002).** Estradiol in elderly men. *The Aging Male*, 5(2): 98–102.

**Verthelyi, D., & Ahmed, S. A. (1994).** 17 beta-estradiol, but not 5 alpha-dihydrotestosterone, augments antibodies to double-stranded

deoxyribonucleic acid in nonautoimmune C57BL/6J mice. *Endocrinology*, 135(6): 2615-2622.

**Vignozzi, L., Cellai, I., Santi, R., Lombardelli, L., Morelli, A., Comeglio, P., Filippi, S., Logiodice , F., Carini, M., Nesi, G., Gacci, M., Piccinni, M., Adorini, L., & Maggi, M. (2012).** Antiinflammatory effect of androgen receptor activation in human benign prostatic hyperplasia cells. *Journal of Endocrinology*, 214(1) : 31- 43.

**Vingren, J. L., Kraemer, W. J., Ratamess, N. A., Anderson, J. M., Volek, J. S., & Maresh, C. M. (2010).** Testosterone physiology in resistance exercise and training. *Sports Medicine*,40(12) : 1037-1053.

**Vodo, S., Bechi, N., Petroni, A., Muscoli, C., & Aloisi, A. M. (2013).** Testosterone-Induced Effects on Lipids and Inflammation. *Mediators of Inflammation*, 2013: 1–8.

**Wadley, A. J., van Zanten, J. J. V., & Aldred, S. (2013).** The interactions of oxidative stress and inflammation with vascular dysfunction in ageing: the vascular health triad. *Age*, 35(3): 705-718.

**Wagner, B., Schulz, W., & Knaevelsrud, C. (2012).** Efficacy of an Internet-based intervention for posttraumatic stress disorder in Iraq: a pilot study. *Psychiatry Research*, *195*(1-2): 85-88

**Wahed, A., Quesada, A., & Dasgupta, A. (2019).** Hematology and coagulation: a comprehensive review for board preparation, certification and clinical practice.1st ed.*Acadmic pr*.p,117

**Walter, M. (2009).** Interrelationships among HDL metabolism, aging, and atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology*, 29(9):1244-1250.

**Wang, B., Li, L., He, Z., Wang, L., Zhang, S., Qiao, H., JIA, R., & Tai, F. (2018).** Effects of reproductive experience on paternal behavior, levels of testosterone, prolactin in serum and dendritic spines in medial prefrontal cortex of mandarin voles. *Integrative Zoology*, 13(6):711-722.

**Wang, L., Gill, R., Pedersen, T. L., Higgins, L. J., Newman, J. W., & Rutledge, J. C. (2009).** Triglyceride-rich lipoprotein lipolysis releases neutral and oxidized FFAs that induce endothelial cell inflammation. *Journal of Lipid Research*, *50*(2): 204-213.

**Wang, L., Manson, J. E., Gaziano, J. M., Liu, S., Cochrane, B., Cook, N. R., Ridker,P.M., Rifai, N., & Sesso, H. D. (2011).** Circulating inflammatory and endothelial markers and risk of hypertension in white and black postmenopausal women. *Clinical Chemistry*, 57(5): 729-736.

**Wang, X., & Stocco, D. M. (2005).** The decline in testosterone biosynthesis during male aging: a consequence of multiple alterations. *Molecular and Cellular Endocrinology*, 238(1-2): 1-7.

**Weaving, G., Batstone, G. F., & Jones, R. G. (2016).** Age and sex variation in serum albumin concentration: an observational study. *Annals of Clinical Biochemistry*, 53(1):106-111.

**Weichselbaum, C. T. (1946).** An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *American Journal of Clinical Pathology*, 16(3): 40-49.

**Wengreen, H. J., Munger, R. G., West, N. A., Cutler, D. R., Corcoran, C. D., Zhang, J., & Sassano, N. E. (2004).** Dietary protein intake and risk of osteoporotic hip fracture in elderly residents of Utah. *Journal of Bone and Mineral Research*, 19(4): 537-545.

**Wheeler, M. J. (1995).** The determination of bio-available testosterone. *Annals of Clinical Biochemistry*, *32*(4), 345-357.

**Wickramatilake, C. M., Mohideen, M. R., & Pathirana, C. (2013).** Association of serum testosterone with lipid abnormalities in patients with angiographically proven coronary artery disease. *Indian Journal of Endocrinology and Metabolism*, 17(6):1061-1065.

**Woods, J. A., Wilund, K. R., Martin, S. A., & Kistler, B. M. (2012).** Exercise, inflammation and aging. *Aging and Disease*, 3(1):130-140.

**World Health Organization. (2009).** Global health risks: mortality and burden of disease attributable to selected major risks. *World Health Organization*.https://apps.who.int/iris/bitstream/handle/10665/44203/9789241563871_eng.pdf .

**World Health Organization. (2013).** A global brief on hypertension: silent killer, global public health crisis: World Health Day 2013 (No. WHO/DCO/WHD/2013.2). *World Health Organization*. https://apps.who.int/iris/bitstream/handle/10665/79059/W?sequence=1

**Wu, F. C., & von Eckardstein, A. (2003).** Androgens and coronary artery disease. *Endocrine Reviews*, 24(2) : 183-217.

**Wu, F. C., Tajar, A., Beynon, J. M., Pye, S. R., Silman, A. J., Finn, J. D., Neill, T.O., Bartfai, G., Casanueva, F. F., Giwercman,G.A.,Han, T.S., Kula, K., lean, M.J., Pendleton, N., Punab,M., Boonen, S., Vanderschueren, D., Labrie, F., & Huhtaniemi, I. T. (2010).** Identification of late-onset hypogonadism in middle-aged and elderly men. *New England Journal of Medicine*, 363(2): 123-135.

**Wu, Y., Ding, Y., Ramprasath, T., & Zou, M. H. (2021).** Oxidative stress, GTPCH1, and endothelial nitric oxide synthase uncoupling in hypertension. *Antioxidants & Redox Signaling*, 34(9) :750-764.

**Wyczalkowska-Tomasik, A., Czarkowska-Paczek, B., Zielenkiewicz, M., & Paczek, L. (2016).** Inflammatory markers change with age, but do not fall beyond reported normal ranges. *Archivum Immunologiae et Therapiae Experimentalis*, 64(3) : 249-254.

**Xia, F., Wang, N., Han, B., Li, Q., Chen, Y., Zhu, C., Chen, Y., Pu, X., Cang, Z., Zhu, C. ,Lu, M., Meng,Y., Guo, H., Chen, C., Lin, D., Zheng, J., Kuang, L., Tu, W., Li, B., Hu, L., Shen, Z., & Lu, Y. (2017).** Hypothalamic-pituitary-gonadal axis in aging men and women: increasing total testosterone in aging men. *Neuroendocrinology*, 104(3) : 291-301.

**Yang, Y. M., Huang, A., Kaley, G., & Sun, D. (2009).** eNOS uncoupling and endothelial dysfunction in aged vessels. *American Journal of Physiology-Heart and Circulatory Physiology*, 297(5):1829-1836.

**Yeap, B. B., Knuiman, M. W., Divitini, M. L., Handelsman, D. J., Beilby, J. P., Beilin, J., McQuillan,B & Hung, J. (2014)**. Differential associations of testosterone, dihydrotestosterone and oestradiol with physical, metabolic and health-related factors in community-dwelling men aged 17–97 years from the Busselton Health Survey. *Clinical Endocrinology*, 81(1) : 100-108.

**Yin, J., Guo, Y. M., Chen, P., Xiao, H., Wang, X. H., DiSanto, M. E., & Zhang, X. H. (2018)**. Testosterone regulates the expression and functional activity of sphingosine-1-phosphate receptors in the rat corpus cavernosum. *Journal of Cellular and Molecular Medicine*, 22(3) : 1507-1516.

**Yoshizumi, M., Abe, J. I., Tsuchiya, K., Berk, B. C., & Tamaki, T. (2003).** Stress and vascular responses: atheroprotective effect of laminar fluid shear stress in endothelial cells: possible role of mitogen-activated protein kinases. *Journal of Pharmacological Sciences*, 91(3) : 172-176.

**Yu, X., Yang, Z., & Yu, M. (2010)**. Correlation of tumor necrosis factor alpha and interleukin 6 with hypertensive renal damage. *Renal Failure*, 32(4) : 475-479.

**Zeitlin, S. I., & Rajfer, J. (2000).** Hyperprolactinemia and erectile dysfunction. *Reviews in Urology*, 2(1) : 39 –42.

**Zhang, J. M., & An, J. (2007).** Cytokines, inflammation and pain. *International Anesthesiology Clinics*, 45(2) : 27-37

**Zhang, L., Curhan, G. C., & Forman, J. P. (2010).** Plasma prolactin level and risk of incident hypertension in postmenopausal women. *Journal of Hypertension*, 28(7) : 1400–1405.

**Zitzmann, M., & Nieschlag, E. (2007).** Androgen receptor gene CAG repeat length and body mass index modulate the safety of long-term intramuscular testosterone undecanoate therapy in hypogonadal men. *The Journal of Clinical Endocrinology & Metabolism* , 92(10) : 3844-3853.

**Zitzmann, M., Brune, M., & Nieschlag, E. (2002).** Vascular reactivity in hypogonadal men is reduced by androgen substitution. *The Journal of Clinical Endocrinology & Metabolism*, 87(11) : 5030-5037.

**Zmuda, J. M., Cauley, J. A., Kriska, A., Glynn, N. W., Gutai, J. P., & Kuller, L. H. (1997)**. Longitudinal relation between endogenous testosterone and cardiovascular disease risk factors in middle-aged men: a 13-year follow-up of former multiple risk factor intervention trial participants. *American Journal of Epidemiology*, 146(8) : 609-617.

# *APPENDICES*

**Questionnaire**

| Name of patient | Age | Addrss | Marrid | | Smoking | | Fasting | | Family history of hypertension | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Yes | No | Yes | No | Yes | No | Yes | No |
| | | | | | | | | | | |

**Clinical assessment**

| Clinical specimen | Blood | | | | |
|---|---|---|---|---|---|
| | | | | | |
| Clinical features | The Weight | The height | Medical therapy | The period of taking the treatment | The amount of pressure |
| | | | | | |

**ELISA kit**

The contents of ELISA kits are shown in the following :

**Table (3.3) :** TNF-alpha human ELISA kit components .

| NO | ITEM | Specifications |
|---|---|---|
| 1 | 1-5 callaborator :benzamidina and thymol | 5 lyophi |
| 2 | 96 coated well with monoclonal antibodies | 96 wells |
| 3 | Buffer for enzyme conjugate of tris maleate and serum of bovines albumin , EDTA plus thymol . | 1 *6 ml |
| 4 | Buffer for incubation : TRIS-Maleate plus serum bovines albumin, EDTA plus thymol | 1 *l 6 m |
| 5 | Chromogen TMB | 1 * 12 ml |
| 6 | Controls one and two plus thymol | 2 *lyophil |
| 7 | Stop solution: HCl | 1 * 12 m |
| 8 | TRIS-Maleate buffer with a serum albumin of bovine with thymol plus labled monoclonal antibodies with HRP | 1 * 0.75 ml |
| 9 | Wash Solution 200x (Tris-HCl) | 1 *10 ml |
| 10 | Zero collaborator : benzamidine and thymol | 2 lyophil. |

**Interleukin 6 ELISA kit components .**

| NO | Item | Specifications |
|---|---|---|
| 1 | Adhesive Strip | 4 |
| 2 | Biotin-antibody Diluent | 1 vial 15 ml |
| 3 | Biotin-antibody(100x) | 1 vial 120 µl |
| 4 | HRP-avidin (100 x) | 1 vial 120 µl |
| 5 | HRP-avidin Diluent | 1 vial 15 ml |
| 6 | plate | 96 well |
| 7 | Reagents | quantity |
| 8 | Sample buffer Diluent | 1 vial 50 ml |
| 9 | Standard (Freeze dried) | 2 |
| 10 | Stop Solution | 1 vial 10 ml |
| 11 | TMB Substrate | 1 vial 10 ml |
| 12 | Wash Buffer (25x) | 1 vial 20 ml |

Nitric oxide ELISA kit components .

| NO | **Item** | **Specifications** |
|---|---|---|
| 1 | Anti NO antibodies labeled with biotin | 1ml× one vial |
| 2 | Hermetic bag | 1 |
| 3 | plate | 96 well |
| 4 | reagent A | 6ml× one vial |
| 5 | reagent B | 6ml× one vial |
| 6 | Seal plate membrane | 2 |
| 7 | Standard dilution | 3ml× one vial |
| 8 | Standard solution(640µmol/L) | 0.5ml×one vial |
| 9 | Stop Solution | 6ml× one vial |
| 10 | Streptavidin-HRP | 6ml× one vial |
| 11 | Washing concentrate | (20ml×30)×one |

**Electrophoresis protein (6) kit components :**

| No | Item | Specifications |
|---|---|---|
| 1 | Buffer (ready to use) | 2 vials, 250 mL each |
| 2 | Container lids | 4 lids |
| 3 | Containers for used | 4 containers |
| 4 | Filters | 3 filters |
| 5 | Reagent containers | 1 pack of 125 pieces |
| 6 | Wash solution (stock solution) | 1 vial, 25 Ml |

# الخلاصة

**هدفت الدراسة الحالية إلى التعرف على بعض المتغيرات الهرمونية , الالتهابية والبايوكيمائية الكيميائية المرتبطة مع تقدم العمر وارتفاع ضغط الدم للرجال في محافظة ميسان. تضمنت عينة الدراسة 90 رجلاً تتراوح أعمارهم بين (40-65) سنة زاروا مستشفى الصدر التعليمي ومركز القلب للفترة من أكتوبر 2019 حتى فبراير 2020. تقسم العينة إلى ثلاث مجموعات رئيسية (30 / مجموعة) على النحو التالي :المجموعة الاولى 40-45 سنة ,المجموعة الثانية 50-55 سنة,**

**المجموعة الثالثة 60 - 65 سنة. وتنقسم كل مجموعة من هذه المجموعات الرئيسية الثلاث أيضًا إلى مجموعتين فرعيتين على النحو التالي: المجموعة الفرعية الأولى (15 رجلاً لا يعانون من ارتفاع ضغط الدم), المجموعة الفرعية الثانية (15 رجلاً يعانون من ارتفاع ضغط الدم).**

**اظهرت النتائج : انخفاض في مستويات هرمون التستوستيرون معنويا لجميع العينات في مختلف المجموعات الفرعية والمجموعات الرئيسية خلال كل من التقدم بالعمر وضغط الدم المرتفع . كما زاد كل من الاستراديول والبرولاكتين بشكل معنوي لجميع العينات في المجموعات الفرعية والمجموعات الرئيسية المختلفة خلال كل من التقدم بالعمر وضغط الدم المرتفع.**

**زادت مستويات عامل نخر الورم (TNF-α) والإنترلوكين 6 (IL6) والبروتين التفاعلي (CRP) بشكل معنوي لجميع العينات في المجموعات الفرعية والمجموعات الرئيسية المختلفة خلال كل من التقدم بالعمر وضغط الدم المرتفع (باستثناء مستويات CRP بين المجموعات السوية والتي لا تعاني من ارتفاع ضغط الدم).**

**انخفض أكسيد النيتريك (NO) معنويا (مع استثناء المجموعة الفرعية الثالثة و المجموعة الخاصة بأصحاب الضغط المرتفع الثالثة مقارنة بالمجموعة الثانية ) لجميع العينات في المجموعات الفرعية والمجموعات المختلفة خلال كل من التقدم بالعمر وضغط الدم المرتفع.**

**الكوليسترول الكلي (TC) ، الدهون الثلاثية (TG) ، الكوليسترول منخفض الكثافة (LDL) ، الكوليسترول منخفض الكثافة جداً (VLDL) زادت بشكل معنوي بينما انخفض الكوليسترول عالي الكثافة (HDL) بشكل معنوي لجميع العينات في المجموعات الفرعية المختلفة خلال كل من التقدم بالعمر وضغط الدم المرتفع . زاد TG بشكل معنوي لجميع العينات في المجموعات المختلفة خلال كل من التقدم بالعمر وضغط الدم المرتفع ، وزاد TC بشكل معنوي لجميع العينات في مجموعات الضغط الطبيعي ، وانخفض HDL بشكل معنوي لجميع العينات في مجموعات الضغط الطبيعي خلال كل من التقدم بالعمر وضغط الدم المرتفع .كما ارتفع VLDL معنوياً لجميع العينات في المجموعات الفرعية والمجموعات الرئيسية .**

**انخفض البروتين الكلي (TP) ومستويات الألبومين بشكل معنوي لجميع العينات في المجموعات الفرعية خلال كل من التقدم بالعمر وضغط الدم المرتفع . كما انخفض الالبومين معنوياً ايضا في مجموعات الضغط الطبيعي .**

**كما اظهر التستوستيرون ارتباطاً معنوياً تحت مستوى معنوية سالباً او موجباً مع اغلب معايير الدراسة لذوي الضغط الطبيعي والمرتفع .**

**الابعاد الفسيولوجية لنتائج الدراسة الحالية قد نوقشت استنادا الى عاملي ارتفاع ضغط الدم والتقدم بالعمر حيث ان العاملين الاخيرين لهما علاقة بنتائج الدراسة الحالية الهرمونية , المؤشرات الالتهابية و البيوكيماوية.**

وزارة التعليم العالي والبحث العلمي
جامعة ميسان
كلية العلوم
قسم علوم الحياة

# العلاقة بين التستوستيرون وهرمونات التكاثر الأخرى ، المعايير الالتهابية والكيموحيوية في الرجال كبار السن الأصحاء و المصابين بارتفاع ضغط الدم في محافظة ميسان

رسالة مقدمة

الى مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة ماجستير علوم في علوم الحياة

**من قبل**

**عذراء صبيح عبد النجدي**

بكالوريوس علوم حياة / جامعة ميسان (2003)

**بأشراف**

**الاستاذ الدكتور احمد عبود خليفة**

اذار 2021م

شعبان 1442هـ