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Study of Nitric Oxide and Some Hormonal Parameters in Hypertensive Men

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ABSTRACT

We examined the associate of some sex steroid and reproductive hormones including (testosterone(T) , estradiol (E2) and prolactin (PRL) with nitric oxide (NO) in hypertensive men.The sample of this study divided into three main groups (30/group) as following : 1st group (40-45) years , 2nd groups (50-55) years and 3rd group (60-65) years , each of these three main groups divided into two subgroups: 1st subgroup (15 normotensive men) , 2nd subgroup (15 hypertensive men) . Results revealed : testosterone levels reduced significantly ($p \leq 0.05$) for all samples in different subgroups and groups during hypertension . Both estradiol (E_2) and prolactin (PRL) increased significantly ($p \leq 0.05$) for all samples in different subgroups and groups . Nitric oxide reduced significantly ($p \leq 0.05$) for all samples in different subgroups and main groups (except the third subgroup and the third group in hypertensive) . These findings are inconsistent with the hypothesis that nitric oxide be associated with low testosterone highestradiol and prolactin levels and hypertension .

Key words : Testosterone , Estradiol , Prolactin , Nitric oxide.

INTRODUCTION

Age advancement has long been considered as an independent risk factor for a wide variety of disease states (sverdlov et al. , 2014) , progressive age is the most important risk factor for cancer overall , Alzheimer's disease, diabetes, Parkinson disease (Belikov, 2019) , a heart and arterial system , atherosclerosis, myocardial infarction, stroke and hypertension (Lakatta and Levy, 2003 ; North and Sinclair, 2012) . Furthermore , Lotti and Maggi (2018) , reported that the progressive age associated with impaired male sexual and reproductive health . That mean changes in the secretion of sex hormones (van den Beld et al., 2018) , especially T hormone in men , which their levels be 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 T 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 ; vodo et al., 2013 ; Hotta , 2019).

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Meanwhile, androgen levels in hypertensive men be decreased and a close relationship between androgen levels and hypertension have been hypothesized in older men , therefore , low T 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 coworkers (2020) mentioned that hypertension be regulated by the NO molecule , and impaired NO bioactivity is an important component of hypertension .

NO is a simple but pluripotent molecule , generated by Nitric Oxide Synthase (NOS) in nearly all types of mammalian cells , low-molecular-weight , highly lipophilic , free radical , it is extremely reactive , readily forming other nitrogen oxides , with very short half-life (5s) and travel only limited distances before being oxidized (Levine et al., 2012 ; wu, 2020) , predominantly synthesized in the vascular endothelium so it has long been known as endothelium-derived relaxing factor powerful antioxidant , anti-inflammatory molecule . It is a vasodilator , modulating vascular tone blood pressure and hemodynamics , a role exploited by nitrate donor therapy for angina , heart failure , pulmonary hypertension , erectile dysfunction (Levine et al., 2012) .

NO production and NOS be regulate by T levels and there are some reports showed that the low NO be associated with T deficiency production by altering the expression and activity of NOS (Hotta et al., 2019) .

In view of forgoing , we just to shed some light on the role of NO and its association with T , E2 and PRL hormones in hypertensive men(Tahmasebi et al.,2021) .

MATERIALS AND METHODS

The samples included the participants were recruited from the hypertension outpatient clinic at the Al-Sadr Teaching Hospital in Maysan province , Iraq . This sample followed the criteria of essential hypertension (systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg) , and excluded all participants which have cardiac vesicular disease and metabolic syndrome . The sample of this study divided into three main groups (30/group) as following : 1st group 40-45years . 2nd groups 50-55 years . 3rd group 60-65 years , and each of these three main groups divided also , into two subgroups as the following : 1st subgroup (15 normotensive men) . 2nd subgroup (15 hypertensive men) . The blood samples prepared by the usual procedure in order to measure testosterone , estradiol and prolactin via competitive test principle using monoclonal antibodies directed against the specific hormones (T , E2 , PRL) was used by using the hormones kits (Roch /Germany) . Nitric oxide was evaluated by using enzyme-linked immunosorbent assay (ELISA) system , with human NO kit (YH BOSEARCH SHANGHAI /CHINA) , according to the manufacturer's instructions . Ambulatory BP Measurement BP was obtained using Mercury pressure device (Alpk2 / Japan) . 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 a t-test for the subgroups and Duncan's test for the groups

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at (p-value ≤ 0.05) significant level . Tests were two tailed , a P-value less than 0.05 denoted the presence of a statistically significant difference .

RESULTS

Hormonal parameters within subgroups

Results revealed that the T levels in hypertensive men (3.213 ± 0.682 ng/ml) , (2.66 ± 0.682ng/mL) and (2.079 ± 0.52 ng/ml) decreased significantly ($p \leq 0.05$) in comparison with the normotensive men (4.56 ± 0.59 ng/ml) , (3.799 ± 0.659 ng/mL) and (2.639 ± 0.5474 ng/ml) for the three subgroups (Figure 1) .

The Levels of E_2 (36.29±0.775 pg/ml) (38.18±0.85 pg/mL) and(39.29±0.848 pg/ml) are increased significantly ($p \leq 0.05$) in hypertensive men in comparison with normotensive men (32.84±1.539 pg/ml), (35.26±1.426 pg/mL) and (36.44±1.446 pg/ml) for the three subgroups . **(Figure 2)**

The PRL levels (6.209±0.436 ng/ml) , (6.97±0.94 ng/mL) and(8.512±1.042 ng/ml) increased significantly ($p \leq 0.05$) in hypertensive men in comparison with the normotensive men (5.603±0.993 ng/ml), (6.226±0.792 ng/mL) and (6.861±0.508 ng/ml)for the three subgroups .**(Figure 3)**

Hormonal parameters for the main hypertensive groups :

Results revealed that the T levels in the third group (2.079333 ± 0.521118 ng/ml) decreased significantly ($p \leq 0.05$) in comparison with the second group (2.660667 ± 0.68228 ng/ml) and with the first group(3.213333 ± 0.682345ng/ml) , The T levels in the second group (3.799±0.66 ng/ml)decreased significantly ($p \leq 0.05$) in comparison with the first group (3.213333 ± 0.682345ng/ml).**(Figure 5)**

Results revealed that the E_2 and PRL levels in the third group (39.29±0.848 pg/ml) , (8.512±1.042ng/ml) increased significantly ($p \leq 0.05$) in comparison with the second group (38.18±0.85pg/ml) , (6.974±0.944 ng/ml) and with the first group (36.29±0.775pg/ml) (6.209±0.436ng/dl) respectively .The E_2 and PRL levels in the second group (38.18±0.85pg/ml) , (6.974±0.944ng/ml) increased significantly ($p \leq 0.05$) in comparison with the first group (36.29±0.775pg/ml) ,(6.209±0.436ng/dl) respectively. **(Figure 5)**

Nitric oxide within subgroups :

Levels of NO had decreased significantly ($p \leq 0.05$) (0.435±0.022µmol/L) (0.415±0.02µmol/L) (0.406±0.012µmol/L) in hypertensive men in comparison with normotensive men. (0.465±0.031µmol/L) , (0.443±0.011µmol/L) and (0.416±0.019 µmol/L) . **(Figure 4)**

Nitric oxide between hypertensive main groups :

Results revealed that the NO levels in the first group (0.435±0.022 µmol/L) increased significantly ($p \leq 0.05$) in comparison with the second group (0.415±0.02 µmol/L) and

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with the third group(0.406±0.012µmol/L) , The NO levels in the second group (0.415±0.02 µmol/L)had no significant difference ($p \leq 0.05$) in comparison with the third group (0.406±0.012µmol/L) . **(Figure 6)**

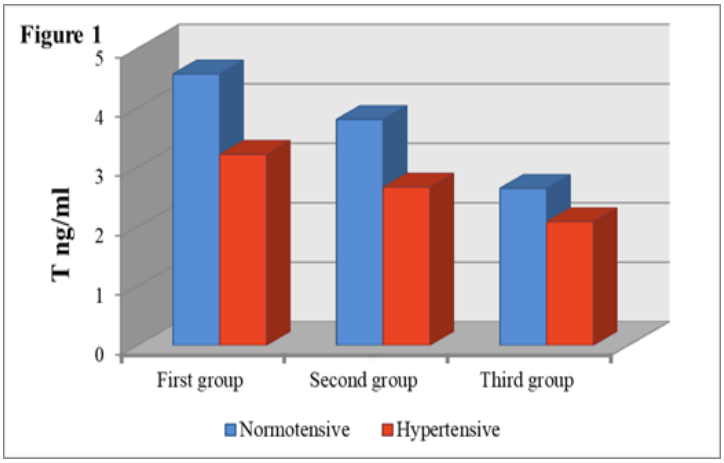

Figure (1): Testosterone levels within subgroups for different groups.
The values represent the Mean ± SD . Blue color represents the normotensive men . Red color represents the hypertensive men .

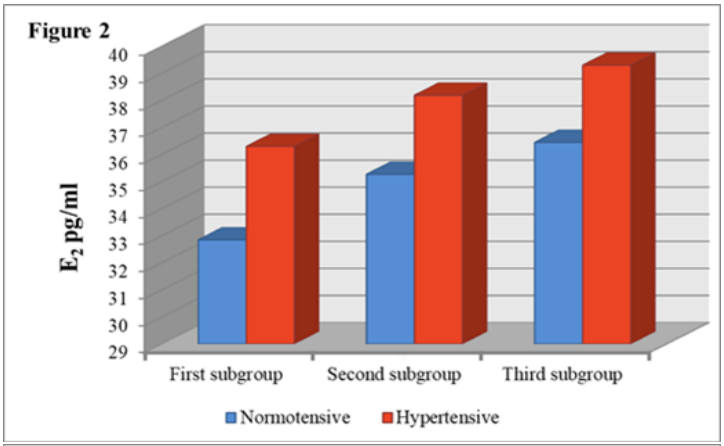

Figure (2) : Estradiol levels within subgroups for different groups .
The values represent the Mean ± SD . Blue color represent the normotensive men . Red color represent the hypertensive men .

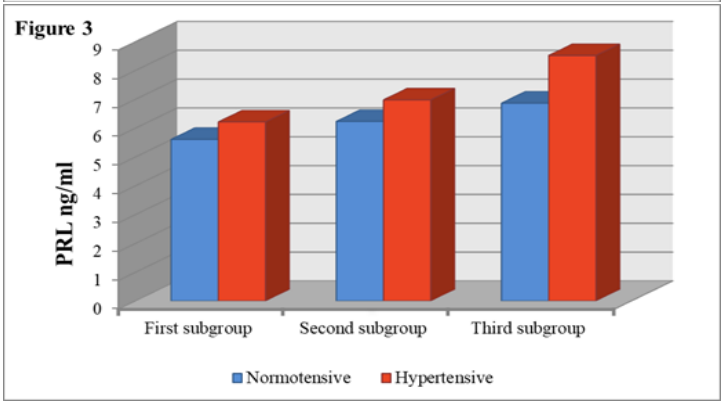

Figure (3) : Prolactin levels within subgroups for different groups .
The values represent the Mean ± SD . Blue color represent the normotensive men . Red color represent the hypertensive men

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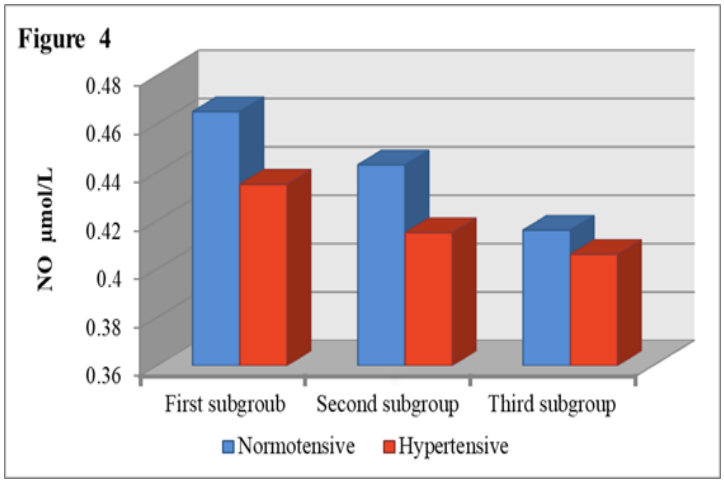

Figure (4) : Nitric oxide levels within subgroup for different groups .
The values represent the Mean ± SD . Blue represents normotensive . Red color represent the hypertensive

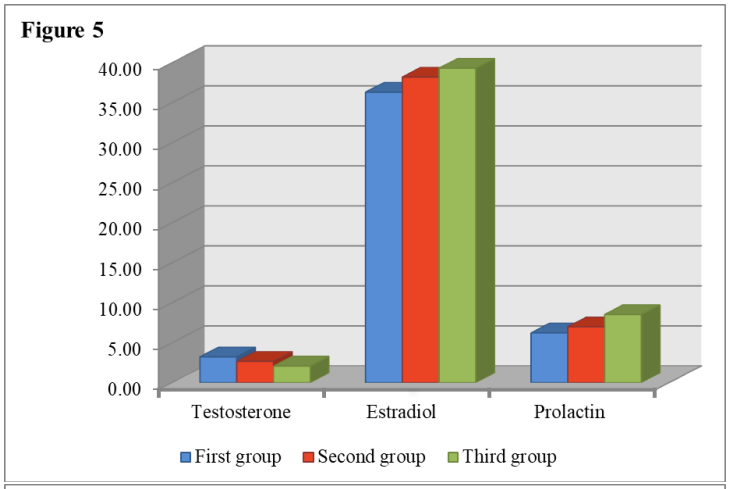

Figure (5) : The levels of hormonal parameters in the hypertensive men for different groups .
The values represent the Mean ± SD . Red color represents first group . Blue color represent the second group . Green color represents the third group

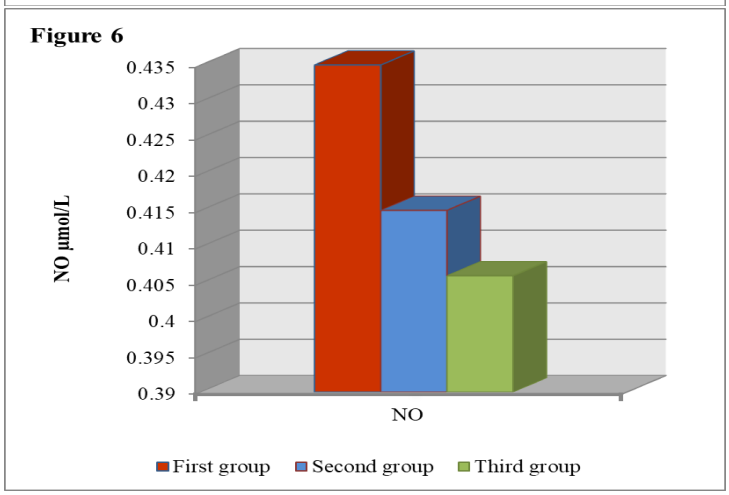

Figure (5) : The levels of NO in the hypertensive men for different groups .
The values represent the Mean ± SD . Red color represents first group . Blue color represent the second group . Green color represents the third group

DISCUSSION

The current results demonstrated that testosterone levels reduced significantly in hypertensive men in comparison with normotensive men for all subgroups in different main groups**(Figure 1)** .

The present finding related with reduction of T levels that associated with the hypertension might be attributed to dysfunction in the endothelial layer , and this dysfunction leading to many

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aspects problems including the low blood flow and reducing the stimulation of the testes resulting in the decline of T production .

Several studies have demonstrated that the vasodilation effects of T on vascular and non-vascular smooth muscle probably mediated via inhibition of L-type calcium channels (Scragg*et al*., 2004; Orshall and Khalil , 2004 ; Hall *et al*, 2006) , therefore , T 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 are a vasodilator response due to T treatment at physiological concentrations in radial artery patients undergoing bypass surgery (Seyrek*et al*., 2007; Kelly and Jones, 2013) , Furthmore , T may exert antihypertensive effects by inducing the relaxation of aorta in rat and rabbit animals (Perusquía*et al*., 2017) .

Endre and his colleagues (1996) agreed this reduction in T levels during hypertension due to testosterone's effect on blood pressure regulation, or the fact that high blood pressure adversely affects steroidogenesis or clearance, or the fact that there are genes involved in blood pressure regulation that also affect steroidogenesis , this later possibility is supported by data demonstrating that men with a family history of hypertension have been shown to have a lower levels of T than normal men .

In addition , Colli and his colleagues (2019) showed that in rat , that the hypertension is a main cause for the testicular damage associated to alterations in the local microcirculation , increased reactive oxygen species (ROS) activity , increased expression of testicular hypoxia-induced proteins , impaired mitochondrial .

Kapasi and his colleagues (1996) , showed that the isolated leydig cells in hypertensive rats secreted more T than the isolated leydig cells in normal rats due to more Atrial natriuretic peptides (ANP) stimulation in hypertensive rats . ANP stimulating the synthesis and release of leydig cells T and luteinizing hormone (Pandey, 2014) .

Nevertheless , many studies pointed that no changes occurred in the T 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 , 2016) .

The current results demonstrated that E_2 levels increased significantly in hypertensive men in comparison with normotensive men for all subgroups in different main groups**(Figure 2)** .

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 T (Cooke *et al*., 2017) . Most circulating 17β-estradiol in men is produced from aromatization of T , predominantly in adipose tissue (Finkelstein *et al*., 2013) .

On the other hand , the present study revealed that hypertension have a significant effect on the levels of E_2 hormone in different groups and subgroups , these findings are related with the increasing levels of E_2 and decreasing levels of T associated with the progressive age respectively , and might be caused due to the high activity of aromatization process companioned

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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) , conclude 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 , IL1 , IL6, and IL10 are all elevated in acute illness and have been demonstrated to increase aromatase activity in vitro (Simpson *et al*., 2002) .

The current results demonstrated that prolactin levels increased significantly in hypertensive men In comparison with normotensive men for all subgroups in different main groups**(Figure 3)** .

To discuss these significant high increase in prolactin levels it must be linked some studied parameters such as E_2 increment and T reduction during hypertension , that they reflect the picture of prolactin hormone during these studies circumstances .

High normal PRL 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) , PRL receptors were discovered in atherosclerotic lesions of the coronary arteries (Roselli , *et al*., 2008) , which further indicates the probable role of PRL in atherosclerosis . The association of daily fluctuations of circulating PRL with decreased endothelial function in men with arterial hypertension was also described (Stamatelopoulos *et al*., 2011) .

It is conceivable that long-term effects of estrogens regulate the prolactin cell mass, thus explaining hypothetically the remarkable decrease in serum PRL concentration after menopause and possibly also the moderate increase in elderly men by enhanced bioavailable E_2 (Roelfsema*et al*., 2012) . Estradiol may be one of the mediators that stimulated PRL secretion (Roelfsema*et al*., 2012; Messini*et al*., 2010) , and this mediation may be enhancing the effect of Thyrotropin-releasing hormone (TRH) and inhibiting that of dopamine (Levine and Muneyyirci-Delale , 2018) . Prolactin levels appeared to be significantly higher in hypertensive men with lower T , significant correlation of PRL level with parameters of arterial stiffness (Grabowska-Markowska*et al*. , 2019) . Hyper secretion of PRL in men has been associated with decreased sexual desire , infertility , reduction of T and erectile dysfunction (Maria , 2016) . New fathers had lower levels of T but higher levels of PRL than new paired males (Wang *et al*., 2018) .

Physiologic levels of PRL in males enhance luteinizing hormone-receptors in leydig cells , resulting in T secretion, which leads to spermatogenesis (Hair *et al*., 2002) .

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Hyperprolactinemia induces hypogonadism by interfering with the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus , and in turn decreases the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary the resulting decrease in serum T (Zeitlin and Rajfe , 2000) .

It is well known that estrogens induce the proliferation of lactotropic cells , as well as enhance the production and secretion of PRL (Cosma*et al*., 2008; Kalleinen*et al*., 2008) , and this may explain why hyperprolactinaemia is characterized by a female preponderance (Colao*et al*., 2003) . Because of a small number of studies providing contrasting results, the role of androgens in the regulation of PRL production and secretion is far from being understood (Krysiak*et al*., 2020). The numbers of lactotropes did not differ between castrate and testosterone-replaced castrate male rats (Ho *et al*., 1988) . The strength of lactotroph function was found to depend on susceptibility of androgen to aromatization (Haug*et al*., 1976) . Unlike non-aromatizable androgens , aromatizable androgens stimulated PRL release (Barrado*et al*., 2014).

The present general findings showed a significant (with some exception) decrease in nitric oxide levels related with hypertension in all different subgroups , and main groups (**Figure 4 , 6**) .

To shed some light about the nitric oxide result discussion it be remarkably to realize and discuss the major factor that influenced of these nitric oxide molecules such as hypertension , T levels and pro-inflammatory cytokines , these all factor are related defiantly with the fluctuation of NO levels .

Wu (2020) mentioned 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 most potent vasodilating substance (endothelium-derived relaxing factor) , which participates in modulation of vascular resistance and heart function , thus , regulation of blood pressure (Kunes et al. 2004) . More of NO molecules be inactivation associated with hypertension or its bioavailability be decreased as a consequence of impaired 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) report the improvements in endothelial function with T treatment were conducted in middle-aged and older men (Nori et al.,2021).

Elevation in TNFα via the reduction of testosterone levels in hypertensive men (Alnajdi and khalifa, 2021) .TNFα decreases the bioavailability of NO both by reducing its production , and by enhancing its removal , decreased NO generation results from TNFαmediated 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 whichperoxynitrite is generated (Urschel and Cicha , 2015; Qasim M T and Al-Mayali ,2019) .

The molecular mechanism underlying the effect of IL6 to decrease the nitric oxide bioavailability by increasing the half-life , therefore , the proteins levels of caveolin-1, the increased caveolin-1 proteins bind more eNOS and consequently decrease eNOS activation by reducing the 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 colleagues (2009) , showed that CRP inhibit endothelial nitric oxide synthase (eNOS) expression , indicating a role for CRP in the production of NO .In conclusion nitric oxide playing an important role during hypertension through the gradual deficiency in testosterone levels .

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