

# Testosterone Levels in Hypertensive Nigerian Men

[Hipertansif Nijeryali Erkeklerde Testosteron Düzeyleri]

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## ABSTRACT

The study aimed to determine the serum testosterone levels and the influence of obesity and cigarette smoking on testosterone levels in hypertensive men.

Serum testosterone levels and blood pressure were measured in 40 hypertensive males and 40 age-matched non hypertensive healthy males in Calabar, Southern Nigeria, using the enzyme labeled immunosorbent assay (ELISA) technique and sphygmomanometric methods. The body mass indices (BMI) and personal data were obtained.

The mean testosterone levels of hypertensives and non-hypertensives were found as  $2.50 \pm 1.60$ , and  $9.00 \pm 2.80$  ng/ml, respectively. The testosterone levels of hypertensives were significantly lower than those of the normotensives ( $p < 0.05$ ). There was also a significant reduction in the testosterone levels of smoking hypertensives than those in non-smoking hypertensives ( $p < 0.05$ ). A significant variation was observed between the testosterone levels and the BMI, along with the finding that the testosterone levels decreased by increasing BMI ( $P < 0.05$ ). No age related decrease was observed in the testosterone levels in all subjects of the study.

Hypertension, obesity and cigarette smoking are associated with lowered testosterone levels in men. The nature of the relationship between hypertension and testosterone levels needs further investigation.

**Key words:** Serum, testosterone, hypertensives, non-hypertensives.

## ÖZET

Bu çalışma hipertansif erkeklerde serum testosteron düzeyleri ve buna obezite ile sigara içmenin etkisinin belirlenmesi amaçlamıştır. Güney Nijerya'da Calabar'da ELISA tekniğiyle ve sfigmomanometrik yöntemlerle 40 hipertansif erkek ve aynı yaş grubundaki sağlıklı kişilerde serum testosteron düzeyleri ve kan basıncı ölçülmüştür. Vücut kitle indeksi (BMI) ve kişisel veriler elde edilmiştir. Hipertansif ve hipertansif olmayan kişilerde ortalama testosteron düzeyleri  $2.50 \pm 1.60$ ng/ml ve  $9.00 \pm 2.80$  ng/ml olarak ölçüldü. Hipertansiflerde testosteron düzeyinin normotansiflere göre önemli miktarda düşük olduğu saptandı ( $p < 0.05$ ). Ayrıca, sigara içen hipertansif kişilerin testosteron düzeyinin sigara içmeyenlerinkinden önemli miktarda düşük olduğu bulundu ( $p < 0.05$ ). Testosteron düzeyi ile BMI arasında önemli bir fark olduğu ve BMI arttıkça testosteron düzeyinin azaldığı belirlendi ( $p < 0.05$ ). Kişilerin tümünde yaş ile testosteron düzeyi arasında bir ilişki gözlenmedi. Erkeklerde, hipertansiyon, obezite ve sigara içme ile düşük testosteron düzeyi ilişkilidir. Hipertansiyon ile testosteron düzeyi arasındaki ilişkinin daha ileri düzeyde araştırılması gerekmektedir.

**Anahtar Kelimeler:** serum, testosteron, hipertansifler, normotansifler

## INTRODUCTION

Hypertension is a risk factor for the development of renal and peripheral vascular diseases, which, often culminates to death. The prevalence is higher among blacks than in Caucasians and blacks suffer a proportionately higher morbidity and mortality from hypertension (WHO-ISH, 1999). Predisposing factors ranges from obesity, stress and presence of other ailments as renal, and endocrine disorders. The incidence of hypertension has been reported to be higher in males than in females (Reckelhoff *et al*, 1999). Studies using the technique of ambulatory blood pressure monitoring have shown that blood pressure is higher in men than in women of similar ages (Wiinberg *et al*, 1995, Khoury *et al*, 1992). The mechanisms responsible for the increase in blood pressure in the males are unknown, but androgens have been shown to have a potential role in both humans and animals. Studies have also shown that after the onset of puberty, boys have higher blood pressure than those of the age-matched girls (Bachman *et al*, 1987, Harshfield *et al*, 1994). Men with low testosterone levels have been reported to have higher blood pressure (Dobryeki *et al*, 2003, Svartberg *et al*, 2004) and low testosterone levels correlated with the higher blood pressure (Fogari *et al*, 2002). Low testosterone levels have also been associated with increased risk of cardiovascular diseases and stroke (Robert and Griffth, 2003).

Smoking and alcoholism have been shown to lower testosterone levels, cause hypertension and increase the risk of vascular aneurysm in men (Gaspur, 2002). High testosterone levels may therefore be protective against arteriosclerosis, especially in men over 60 years of age (Swartz, 1988). The actual nature of the relationship between testosterone levels and blood pressure is being elucidated.

This work therefore was designed to evaluate the serum testosterone levels in hypertensive and non-hypertensive Nigerian men.

## SUBJECTS AND METHODS

### Study Design

The study subjects included the hypertensives attended the hypertension clinic of the University of Calabar Teaching Hospital (UCTH) and non-hypertensives selected from apparently healthy individuals attended the Staff Clinic of the hospital. Informed consent was obtained from the subjects before their recruitment into the study. The Ethics Committee of University of Calabar Teaching Hospital approved the study protocol. The inclusion criteria for the study were as follows; 30-75 years of age at the time of the study, known hypertension for the subjects of the hypertensive group for the past

five years diagnosed according to the 1999 World Health Organization-International Society for hypertension diagnostic criteria for hypertension (WHO-ISH, 1999). Exclusion criteria were habitual alcohol consumption, strenuous exercise and secondary hypertension.

### Selection of subjects

Subjects were randomly selected from the population specified above based on fulfillment of the inclusion criteria. A total of eighty subjects were recruited for the study. Forty known hypertensive patients comprising of 30 non-smokers and 10 moderate smokers, who smoke an average of 5 sticks of cigarette per day for the past five years were included in the study. The smokers were selected based on the information given in the questionnaire. Forty non-smoking, non-hypertensive apparently healthy volunteers were used as controls.

Body weight and height were measured and used to calculate the BMI, which was used as a measure of relative body weight.

Blood pressures of subjects were taken at three intervals one month prior to sample collection to rule out the undiagnosed hypertension in control subjects.

A structured questionnaire was used to obtain data on occupation, physical activity, lifestyle pattern as smoking and alcohol consumption, past and present illness and medication.

### Sample collection

Five milliliters of venous blood samples were taken from the subjects between 8am –10am on the day of the test. The blood was dispensed into plain bottles, allowed to clot and centrifuged at 3000 revolutions per minute for five minutes to extract the serum which was stored frozen and used for the determination of the total serum testosterone.

### Methods

Testosterone was determined with an enzyme immunoassay method using Microwell Testosterone EIA commercial test kit by Syntron Bioresearch, Inc. A microwell reader was used for the measurement of absorbance of the samples at 450nm. No significant variation in results was observed when samples were analyzed immediately and after storage with this method.

Expected normal values were 3.0-10.0ng/ml for adult males.

Systolic and diastolic blood pressures were measured using a sphygmomanometer and stethoscope.

## Statistical analysis

The significance of difference between the two groups was tested using the t-test analysis, while variation among groups was determined using analysis of variance.

**Table 1:** Mean Age, Body Mass Index (BMI), Blood pressure and Testosterone levels in hypertensives and non-hypertensive Men.

| Subjects                | Age (years)     | BMI* (Kg/m <sup>2</sup> ) | Systolic (mmHg)  | Diastolic (mmHg) | Testosterone (ng/ml) |
|-------------------------|-----------------|---------------------------|------------------|------------------|----------------------|
| Normotensives<br>n = 40 | 45.00<br>±14.00 | 25.00<br>±3.10            | 114.50<br>±7.00  | 71.88<br>±8.88   | 9.00<br>±2.80        |
| Hypertensives<br>n = 40 | 50.00<br>±17.00 | 25.78<br>±4.30            | 178.20<br>±26.00 | 104.00<br>±11.00 | 2.50<br>±1.60        |
| P value**               | p> 0.05         | p> 0.05                   | p<0.05           | p<0.05           | p<0.05               |

\*BMI: Body mass index

\*\* t-test analysis was used.

p<0.05 is significant

**Table 2:** Effect of Cigarette smoking on testosterone levels in hypertensive males.

| Subjects              | Testosterone (ng/ml) |
|-----------------------|----------------------|
| Smokers<br>n = 10     | 2.00 ± 4.20          |
| Non smokers<br>n = 30 | 2.74 ± 6.40          |
| P value*              | p<0.05               |

\* t-test analysis was used.

p<0.05 is significant

**Table 3:** Relationship between BMI and testosterone levels in hypertensive males.

| Subjects                            | BMI* (Kg/m <sup>2</sup> ) | Testosterone (ng/ml) |
|-------------------------------------|---------------------------|----------------------|
| Normal weight<br>BMI < 25<br>n = 22 | 21.15 ± 2.32              | 2.60 ± 1.40          |
| Preobese<br>BMI 25-29.90<br>n = 12  | 26.90 ± 1.44              | 2.16 ± 1.70          |
| Obese<br>BMI > 30<br>n = 6          | 30.97 ± 1.25              | 2.08 ± 1.40          |
| P value**                           | P<0.05                    | p<0.05               |

\*BMI: Body mass index

\*\* Analysis of variance (ANOVA) was used.

p<0.05 is significant

## RESULTS and DISCUSSION

The male sex hormone testosterone has been implicated in the aetogenesis of hypertension. This may be as a result of higher prevalence of hypertension in men than in women (Reckelhoff *et al*, 1999). The greater incidence of hypertension and coronary artery disease in men compared to women has been related in part to gender differences in vascular tone resulting from sex hormone induced stimulation of the endothelial dependent mechanisms of vascular relaxation and inhibition of mechanisms of vascular smooth muscle contraction (Orshal and Khalili, 2003).

In this study, the testosterone levels of normotensive men were significantly higher than those of their hypertensive counterparts (Table 1). Higher testosterone levels have been previously demonstrated in normotensive males than hypertensive males by Fogari *et al*, 2002 and Svartberg *et al*, 2004. Testosterone has been reported to play a regulatory role in counteracting visceral fat accumulation (Marin and Arver, 1998) thus the association of low testosterone level with high blood pressure is thought to be mediated by obesity (Svartberg *et al*, 2004). Testosterone is known as the androgen of greatest concentration in cardiac tissues which increases and strengthens the cardiac muscle mass and makes it more resistant to death during ischemia. It has been demonstrated that it leads to improved cardiac output and subsequently improved blood pressure (Robert and Berkow, 1987). Thus we may assume that the higher testosterone levels may account for the normal blood pressure seen in non-hypertensive males. The mechanism underlying this phenomenon is still uncertain. Testosterone receptors have been identified in the endothelial cells and vascular smooth muscles (Higashiura *et al*, 1997). Studies have shown that testosterone induces endothelial dependent vascular relaxation (Chou *et al*, 1996, Costeralla *et al*, 1996) by modifying the synthesis, release and bioactivity of one or more of the relaxing factors, such as nitric oxide and prostacyclin. Nitric oxide produced from the transformation of L-arginine to L-citrulline by the enzyme nitric oxide synthase (NOS), is a powerful vasodilator and relaxant of vascular smooth muscles (Nigel and John, 1991, Palmer *et al*, 1988), modifies vascular smooth muscle proliferation (Garg and Hashid, 1989) and prevents platelet activation (Radonski *et al*, 1987). Nitric oxide therefore plays important role in regulation of vascular tone (Orshal and Khalili, 2003). Testosterone also is shown to activate the rennin-angiotensin system to produce angiotensin II, a potent vasoconstrictor (Reckelhoff, 2001) that influences the vascular tone (Davis and Hall, 1999). Alterations in vascular tone play a major role in the control of blood pressure and the coronary circulation and thereby the incidence of hypertension and coronary artery disease,

suggesting that low testosterone levels may lead to impairment of endothelial function, reduced availability of nitric oxide, inability to maintain vascular tone and hence hypertension (Virdis *et al*, 2002).

Smoking habit seems to affect the testosterone levels of the hypertensive population of the study. Smoking hypertensive males were reported to have significantly lower testosterone levels than non-smoking hypertensives. Smoking is known as a risk factor for hypertension since it has some effects on red cell and plasma viscosity, packed cell volume and plasma protein concentrations (Dacie and Lewis, 2001, Vantiel *et al*, 2002, Khaw and Barrett-Connor, 1988). Development of hypertension as a result of smoking explains the lower testosterone levels seen in hypertensive smokers in the present study. Lower testosterone levels (Gaspur, 2002) and significantly higher free testosterone levels have been demonstrated in smoking healthy men when compared to their non-smoking counterparts (Vermeulen *et al*, 1996) suggesting that the effect of cigarette smoking on the serum testosterone levels is still unclear.

Our data showed that serum testosterone levels varied significantly with the body mass indices of the hypertensives in an inverse manner. The higher the BMI, the lower the testosterone level and vice versa. This result agrees with the findings of Giagulli *et al*, 1994, Vermeulen *et al*, 1996, Lima *et al*, 2000, Allen *et al*, 2002, who also reported that the total testosterone

and free testosterone levels are dependent on the degree of obesity. They concluded that massively obese men (BMI>35.1 kg/m<sup>2</sup>) are considered as candidates for consistently having low free testosterone and total testosterone levels. A functional decrease in luteinizing hormone pulse amplitude and serum luteinizing hormone levels in massively obese men were reported to be related to the decreased androgens levels possibly caused by a negative action of excess amounts of circulating leptin (Lima *et al*, 2000).

No age related differences was observed in the testosterone levels in all subjects studied. Significant inverse relationships between testosterone and age in normotensive men and a non-significant trend in hypertensive ones have been previously reported (Fogari *et al*, 2002). Similar findings were also demonstrated by Allen *et al*, 2002 and Vermeulen *et al*, 1996.

The findings of this work have shown that hypertensive men have lower testosterone levels than non-hypertensive men. Social habits such as cigarette smoking, and obesity are also associated with the low levels of testosterone, while age seems to have no significant effect on serum testosterone levels. The actual mechanism underlying the relationship between testosterone and hypertension has not been properly elucidated and hence needs further investigation at the molecular level. However, abstinence from cigarette smoking and weight reduction diet may improve the androgen levels in hypertensive men.

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