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ORIGINAL ARTICLE

# Association of stress, hostility and plasma testosterone levels

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**Abstract**

**OBJECTIVES:** Many studies assessing the role of sex hormones, like testosterone, on stress and hostility factors have been primarily conducted in selected atypical populations such as violent criminals as well as androgen users and abusers. Therefore, the main aim of the current study was to investigate the association between testosterone levels and two psychosocial variables: stress and hostility in a cohort of healthy individuals who were members of a health maintenance organization (HMO).

**METHODS:** At five quarterly visits, psychosocial scales and blood draws were collected. Psychological stress was measured by using several scales that assessed different types of stress, including daily hassles, major life events and perceived stress. Similarly, different aspects of hostility were measured, among them cynicism, hostile affect and aggressive responding. Plasma collected from each visit was used for testosterone level determinations.

**RESULTS:** Testosterone levels were significantly associated with stress in both males and females. However, whereas this association exhibited a "threshold effect" in males, it demonstrated a direct and continuous linear relationship between these variables in females. Hostility was not correlated with testosterone levels in neither males nor females.

**CONCLUSIONS:** These results suggest that testosterone levels in normal males and females may be more reflective of an intricate balance between physiological responding and emotional coping to stressors than the hostility profile of the individual.

## Introduction

The relationship between hormones and biobehavioral factors has long been of interest to researchers. The potential relevance of gonadal hormones in supporting expression of certain behaviors have been keenly considered in both animal and human studies [45,7,29,4,34]. Testosterone levels, in particular, have been linked to several psychosocial and behavioral variables, including hostile feelings, anger, impulsivity and aggression [29,11,22]. In animal studies, the data linking high levels of testosterone to increases in aggression has been consistently confirmed in a

variety of vertebrate species [3]. For example, in rhesus monkeys, males exhibited an annual cycle in their levels of aggression which corresponded with maximal levels of testosterone and the season when these behaviors naturally escalate in the wild [45]. These and many other observations have led researchers to examine levels of testosterone in humans most prone to exhibiting aggression. As such, studies on violent offenders [29], past inmates [9,4,25], alcoholics [41,42] and the mentally ill [4] are replete. In addition, as testosterone administration as treatment for hypogonadal men and the use and abuse of anabolic steroids have evolved, other atypical populations are being studied for accompanying behavioral changes [39,6,37]. Although these studies have provided valuable insights as to the neurobiology of these extreme populations, they provide little for the more typical male and female populations. Therefore, in the present study we attempted to address the association between testosterone, stress and hostility measures in normal men and women without manipulation of their intrinsic hormonal status.

## Method

***Subjects:*** This study is part of an ancillary study to the Seasonal Variation of Cholesterol Levels (SEASONS) study [5] an NHLBI-funded study designed to evaluate how blood cholesterol levels vary with the seasons. Subjects for the ancillary study were a subgroup of 178 SEASONS participants. Of these 178 participants, 132 had complete data for testosterone, stress and hostility measures.

SEASONS participants were recruited from the Fallon Healthcare System, an HMO serving Worcester, Massachusetts and surrounding communities. Additional individuals of Hispanic descent, the largest minority community in Worcester, MA, were recruited from outside the HMO population to increase the diversity of the SEASONS' study sample. Five of the 132 subjects analyzed were recruited outside of Fallon. Eligibility criteria excluded individuals taking cholesterol-lowering medications, individuals actively on lipid-lowering or weight-control diets, and individuals with possible causes of secondary hypercholesterolemia (e.g., hypothyroidism, pregnancy), and chronic life-threatening illness (e.g., cancer, or renal or heart failure). Eligible HMO members were called by a telephone recruiter and invited to take part in the study. During the telephone call, a brief overview of the study was presented and eligibility was determined. Participants agreed to complete quarterly assessments (once per season) over the course of one year. Assessments included physiological and laboratory measures and completion of psychosocial questionnaires (described below). All subject recruitment and data collection procedures were approved by the Institutional Review Boards (IRBs) of the Fallon Healthcare System and the University of Massachusetts Medical School. Each subject signed an approved informed consent prior to entering the study. Participants for this ancillary study were recruited consecutively between December 1995 and April 1997 prior to their second SEASONS study visit, and required to sign a separate consent form, which also was approved by the IRBs of the above-referenced institutions.

***Physiological and Laboratory Measures*:** Participants attended five quarterly clinic visits where psychosocial scales (previously mailed to the participants' homes) were collected by a research assistant. In addition, fasting blood samples were drawn by a phlebotomist (between 8–11 a.m) on the morning of each of their visits to the clinic. Blood was centrifuged at 5,000xg for 15 min and plasma was collected. The plasma samples were stored at −80º C until assays were performed. Plasma testosterone levels were determined with a RIA kit obtained from ICN Biomedicals Inc. catalog number 07-289102 (Costa Mesa, CA). The specificity of the testosterone antiserum was 100% for testosterone, approximately 7 % for 5 alpha-dihydrotestosterone and less than 0.10% for hydroxytestosterone, estrone, progesterone, and corticosterone.

***Psychosocial Measures*:** Assessed psychosocial constructs included hostility and stress. Hostility was assessed using a subset of items from the Cook-Medley Hostility scale [5]. This item subset assesses three components of the hostility construct: cynicism (hostile beliefs), hostile affect (emotional experiences) and aggressive responding (aggressive behaviors). The entire 50-item scale has shown high levels of consistency with Cronbach's alphas ranging from .80 to .82 for both men and women [36], high test-retest reliability (r = .84 over 4 years) [35], and good evidence of construct validity [30].

Overall psychological stress was measured by using several scales that assess different types of stress (daily hassles, major life events and perceived stress). These measures were later combined into a simple composite score as described below.

The Hassles Scale [24] was used as a measure of daily objective stressors in the areas of work, health, family, friends, environment, practical considerations, and chance occurrences. In this scale, subjects are asked to indicate whether they experienced any of 117 hassles during the previous month, and how stressful these hassles were for them. The cumulative severity score used in this analysis consists of the sum of all hassles endorsed by the participants as being "extremely" stressful. This scale has demonstrated good psychometric properties including good internal consistency, test-retest reliability and concurrent validity [24,44].

A Life Events Checklist [12] also was used. This instrument contains a list of major stressful life events (41 items) that might have occurred in the life of a subject over the last 12 months. This scale was constructed by selecting events that had a relatively high frequency of occurrence in the population and those that had potentially negative impact from a more exhaustive List of Recent Experiences [23]. Given that the study assessments were conducted quarterly, the time-period assessed was modified to "the past three months."

The Perceived Stress Questionnaire (PSQ) [26] is a 30-item instrument used to assess physical, emotional and cognitive perceptions associated with the experi-

ence of stress during the previous month. The PSQ has favorable psychometric characteristics including high internal consistency, test-retest reliability and predictive and constant validity [26].

In an effort to obtain a single overall measure of psychological stress, a single composite score was constructed to represent the level of stress of each participant during each study quarter. Quartiles for each scale were calculated and an index was assigned based on quartiles of each scale: 1 was assigned if a subject was in the first quartile, 2 if a subject was in the second quartile, 3 if a subject was in the third quartile and 4 if a subject was in the forth quartile. The sum of indexes from each scale was termed the stress index score. This score ranged from 3 to 12. A principal component analysis showed that all three stress scales formed a component with loading of 0.93, 0.71, and 0.88, and the component explained 71% of variance, suggesting that the scales measured a common underlying concept of overall psychological stress. Cronbach's alpha coefficient was 0.77.

***Analysis*:** The distributions of patient characteristics were summarized using percentages and mean ± standard deviation. As testosterone levels were varied by gender, all analyses were stratified by gender. Comparisons of differences between males and females were conducted using Chi-square tests for categorical variables and two sample t-tests for continuous variables.

Testosterone data were averaged within subject for the cross-sectional analysis. The distributions of testos-

**Table 1:** Sample Characteristics, The Seasons Stress Study, Worcester, Massachusetts USA.

| | **Total (n=132)** | **Males (n=72)** | **Females (n=60)** | **p-value*** |
|---|---|---|---|---|
| *Categorical* | n (%) | n (%) | n (%) | |
| **MARITAL STATUS** | | | | 0.003 |
| Married/Living w/Part. | 106 (80.92) | 63 (91.30) | 43 (71.67) | |
| Other | 23 (17.83) | 6 (8.70) | 17 (28.33) | |
| **ETHNICITY** | | | | 0.34 |
| White | 116 (91.34) | 64 (94.12) | 52 (88.14) | |
| Other | 11 (8.66) | 4 (5.88) | 7 (11.86) | |
| **EDUCATIONAL LEVEL** | | | | 0.54 |
| High School or less | 23 (17.69) | 10 (14.29) | 13 (21.67) | |
| Some College | 47 (36.15) | 26 (37.14) | 21 (35.00) | |
| >=Bachelor degree | 60 (46.15) | 34 (48.57) | 26 (43.33) | |
| **EMPLOYMENT STATUS** | | | | 0.27 |
| Full-time/Part-time | 109 (83.83) | 61 (87.14) | 48 (80.00) | |
| Unemployed | 21(16.15) | 9 (12.86) | 12 (20.00) | |
| **SMOKING STATUS (CURRENT)** | | | | 0.26 |
| Yes | 20(16.00) | 13 (19.40) | 7 (12.07) | |
| No | 105 (84.00) | 54 (80.60) | 51 (87.93) | |
| *Continuous* | **Mean (SD), Median** | **Mean (SD), Median** | **Mean (SD), Median** | |
| **AGE (YEARS)** | 48.41 (10.83), 48.00 | 48.49 (11.03), 49.00 | 48.32 (10.67), 48 | 0.93 |
| **TESTOSTERONE (NG/ML)** | 3.30 (2.74), 3.47 | 5.45 (1.87), 5.03 | 0.72 (0.37), 0.65 | <0.001 |
| **HOSTILITY MEASURES** | | | | |
| Cynicism | 3.89 (3.92), 3.44 | 4.40 (0.36), 6.75 | 3.26 (0.34), 2.67 | 0.03 |
| **STRESS MEASURES** | | | | |
| Hassle cumulative severity | 34.87 (32.39), 25 | 28.40 (3.05), 16.25 | 42.54 (4.86), 31.50 | 0.01 |
| Life events | 1.84 (1.46), 1.50 | 1.66 (0.14), 1.5 | 2.06 (0.23), 1.67 | 0.12 |
| Perceived stress | 0.34 (0.18), 3.11 | 0.30 (0.02), 0.28 | 0.38 (0.02), 0.36 | 0.009 |
| Stress index | 7.46 (2.56), 7.25 | 6.96 (0.28), 6.29 | 8.05 (0.34), 8.00 | 0.01 |

NOTE: Numbers in categories do not always add to 132 due to missing values for some demographic data.
*P-value for test of differences between males and females based on a chi-square test for categorical variables (except ethnicity where a Fisher's exact test was used) and two sample t-tests for continuous variables.

terone and stress measures were examined for normality. Smoothing curves [10] were used to determine the appropriate bivariate relationship between stress measures and testosterone levels.

In some cases there was an indication of a nonlinear association between testosterone and some predictors – an indication of a possible "threshold" effect. We used several models to fit the data and these models resulted in consistent results which increased our confidence in the nonlinear effect. We present the linear analysis and quadratic analysis ($y = \alpha + \beta_1 x + \beta_2 x^2$) in tables. Fractional polynomial fits – which allows for a more complex functional form on the right hand side of the equation – resulted in almost identical curves to the simpler quadratic fit. Nested models were tested using likelihood ratio tests. A generalized estimating equation model was fit to the longitudinal data but provided no additional information, replicating the associations estimated in the average models.

All analyses were carried out in SAS (SAS version 8.0, SAS institute inc.) and Stata (version 8.0, Stata Corporation, Inc., College Station, Texas).

## Results

Seventy-two men and 60 women with testosterone measurements and stress and hostility measurements for at least one quarter were used in this analysis. A comparison between the included (n=132) and excluded (n=46) subjects revealed no significant differences between the two groups in terms of gender, age, marital status, employment status, and smoking status. However, an unadjusted statistically significant difference in educational levels, and ethnicity was found between these two groups, with more educated white subjects in our study group compared to those excluded from the analysis.

Descriptive categorical characteristics and continuous characteristics of the study participants were examined (*Table 1*). The average age was 48 years (SD=10.83) and participants were predominately white, married, educated, and employed in white collar occupations (e.g., managerial, scientific, or office work).

Average testosterone level for men was 5.45 ng/ml (SD=1.87) and for women 0.72 ng/ml (SD=0.37). Hostility scores were more pronounced in males than in females, with males reporting higher hostility levels ($p < 0.03$). Females experienced more "extremely" stressful hassles and had greater perceived stress ($p<0.01$ and $p < 0.009$ respectively) than males. These differences contributed to a higher overall stress score – in females compared to males ($p<0.01$).

*Table 2* presents the results from cross-sectional regression model analysis that examine the associations between testosterone, hostility and stress measures for male and female participants. Hostility measures were not associated with testosterone levels for either gender. A similar lack of association between testosterone levels and stress measures, including hassle, cumulative severity, and life events, were also observed in males. However, the severity of cumulative hassles was significantly correlated with testosterone levels in females ($p < 0.03$).

Furthermore, additional stress measures including perceived stress ($p<0.004$) and the overall stress index ($p < 0.02$) were associated with testosterone levels in females. Graphical exploration of the association of testosterone and the stress index indicated a non-linear

**Table 2:** Cross-sectional Regression Model Predicting Testosterone Using Psychosocial Measures by Gender, SEASONS Stress Study, Worcester, MA 1994-1999

| | Males (n=72)48 | | Females (n=60) | |
|---|---|---|---|---|
| | **Coefficient (SE)** | **P-value*** | **Coefficient (SE)** | **P-value*** |
| **HOSTILITY MEASURES** | | | | |
| Cynicism | 0.06 (0.07) | 0.38 | 0.02 (0.02) | 0.27 |
| **STRESS MEASURES** | | | | |
| *Linear Models* | | | | |
| Hassle cumulative severity | 0.006 (0.009) | 0.51 | 0.003 (0.001) | 0.03 |
| Life events | 0.11 (0.19) | 0.57 | 0.03 (0.028) | 0.28 |
| Perceived stress score | 1.67 (1.3) | 0.20 | 0.80 (0.26) | 0.004 |
| Stress Index Score | 0.13 (0.04) | 0.15 | 0.04 (0.02) | 0.02 |
| *Quadratic Models* | | | | |
| Hassle cum. Severity | 0.04 | P=0.24+ | | |
| Hassle cum. Severity$^2$ | –0.001 | | | |
| Life events | 0.96 | P=0.18+ | | |
| Life events$^2$ | –0.20 | | | |
| Perceived stress | 8.75 (5.01) | P=0.15+ | | |
| Perceived stress$^2$ | –9.91 (6.78) | | | |
| Stress index | 1.33 (0.60) | P=0.05+ | | |
| Stress index$^2$ | –0.08 (0.04) | | | |

*P-value was from test of $H_0$:ß=0.
+ P-value for test vs null model

association in males. A quadratic model was fit (and was consistent with other more complex models) and indicated an association in males with a "threshold effect" ($p \leq 0.05$), a positive association with a plateau (Figure 1). The figure depicts a direct association between the stress index and testosterone at lower range and this pattern plateaus as the stress index increases.

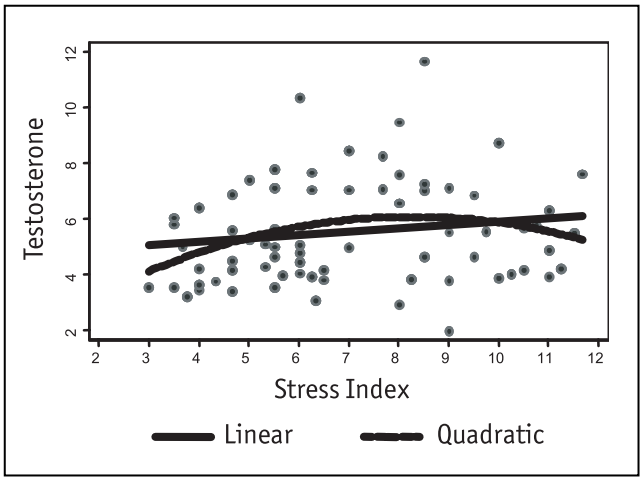


**Figure 1:** Scatter plot of Testosterone values vs Stress Index for Males. Solid line is the linear regression line fit. Dotted line is the quadratic regression line fit.

## Discussion

The present study assessed the correlation between testosterone, stress and hostility in a group of normal men and women. The lack of known testosterone therapy, androgen abuse and criminal behavior in this population may have facilitated the more subtle associations (or lack of association) between normal testosterone levels and the psychosocial factors studied over an extended period in both men and women.

Although males exhibited more hostility than females, testosterone levels were not linked to hostility measures in either males or females. This is in opposition to earlier reports showing a high degree of association between increased testosterone levels, hostility and aggression in males [29,41,14,9]. However, it should be highlighted that, in general, the above reports involved comparisons between violent and control populations [25,41,14] or men administered varying levels of testosterone in different chemical forms [31,38,32]. The current results are more in concert with those of a recent publication of other researchers [29,1] showing that testosterone may have no significant effect on mood in eugonadal men or when hormone levels are within normal range. Their analysis held true for measures including hostile cognitions, affect and behavior [29]. Therefore, the current working premise articulated by several researchers is that the adverse psychological effects or negative mood states may be more directly associated with levels of testosterone [2] that is outside the normal physiological range seen in normal young men. Another contributing factor may be the utilization of externally infused coping styles by some males. For example, music, parental care and the involvement in stable romantic relationships correlated with decreases in testosterone levels in males [20,18,8], suggesting that beyond a certain level of stress "normal" males (without externally manipulated testosterone levels) with the opportunity to do so may switch to more adaptive coping styles and modulate their levels of testosterone.

Although fewer studies have evaluated the effects of testosterone on aggressive behavior in females [42,27,40,17,15], the results are similarly inconsistent with the current report and may be similarly reflective of the different atypical populations involved [40,13,16]. In these studies, the populations included women who were compared to transsexual males [13], those exhibiting criminal behavior [16], and others with body image challenges [13]. In a more recent study of aggression and testosterone in a typical female population, researchers found testosterone may be related to regulation of aggression proneness in women in mid-cycle [17].

The measure of various types of stress and the use of a "stress index" or composite score allowed us to examine the association between testosterone and overall stress. Testosterone and stress were positively and linearly associated among females. In the case of males, the association between testosterone and stress was apparently non-linear, characterized by a positive association at lower levels of stress index with a subsequent leveling off at higher values. This complex interaction between the stress index and testosterone in males is supported by reports indicating that levels of testosterone are suppressed or enhanced by psychological and physical stressors [19,33]. This is interesting since human males engaged in a competitive sport may experience accompanying changes in testosterone levels after winning or having home field advantage [28,21]. Similarly, animal studies have shown that winning or losing a fight can increase or decrease testosterone levels, respectively [34,43].

The two main strengths of the present study are the multiple time points for data collection and the utilization of the composite stress index measures. For example, one of the most profound discoveries of the current study supports the hypothesis that testosterone levels (over an extended period of time) may be associated with stress factors, although the nature of this association may be different for males than females. One possible limitation of this study is the absence of data examining the role of the hormonal cycle status on the testosterone-stress association in females. However, taken together, the present study demonstrates that acute levels of the sex steroids, testosterone, may be reflective of overall stressors encountered and that individual emotional responses to or coping with such stressors.

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