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# The effect of supraphysiologic doses of testosterone on fasting total homocysteine levels in normal men

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**Abstract**

Elevated total homocysteine (tHcy) levels are associated with increased risk for atherosclerotic cardiovascular disease. tHcy levels are higher in men than in women, and estrogen replacement therapy may reduce tHcy levels in postmenopausal women. The effect of androgenic hormones on tHcy levels in men has not been examined. The present study determined the effect of supraphysiologic doses of testosterone, with or without its aromatization to estradiol, on fasting tHcy levels in 14 normal male weightlifters aged 19–42 years. Subjects received testosterone enanthate (200 mg/week intramuscularly), the aromatase inhibitor, testolactone (1 g/day orally), or both drugs together in a crossover design. Each treatment lasted 3 weeks and each treatment was separated by a 4-week washout. Both testosterone regimens increased serum testosterone levels, whereas estradiol increased only during testosterone alone. Mean tHcy levels were not significantly altered when testosterone was given alone or together with testolactone. Testolactone did not significantly influence tHcy levels. We conclude that short-term, high-dose testosterone administration does not affect fasting tHcy levels in normal men. © 1997 Elsevier Science Ireland Ltd.

*Keywords:* Estrogen; Homocysteine; Men; Testosterone

## 1. Introduction

Homocysteine is a sulphur-containing amino-acid formed by the demethylation of dietary methionine. Homocysteine may promote atherosclerosis by injuring the vascular endothelium, [1] and elevated total homocysteine (tHcy) levels are associated with increased risk for atherosclerotic cardiovascular disease [2]. The observation that fasting tHcy concentrations are higher in men than in women has led to the suggestion that sex steroid hormones may influence tHcy levels [3]. Evidence to support this idea comes from studies showing that estrogen plus progestin replacement therapy [4] and the estrogen agonist tamoxifen [5] reduce tHcy concentrations in postmenopausal women. Others have documented an inverse correlation between serum estradiol concentrations and postmethionine tHcy levels in premenopausal women, [4] but to our knowledge the effect of androgenic hormones on tHcy concentrations in men has not been examined.

The androgenic hormone testosterone is normally aromatized to estradiol in liver, muscle, and adipose tissue, and peripheral aromatization of testosterone is the major source of circulating estrogen in men [6]. We have previously examined the effect of testosterone aromatization on serum lipids [7] in men by administering testosterone alone or in combination with the aromatase inhibitor testolactone. Because of the observations that tHcy concentrations are higher in men than in women [3] and that female sex hormones may affect tHcy levels [4,5], we used stored plasma samples from this prior study to determine the effects of testosterone and its aromatization to estradiol on fasting tHcy levels in men.

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## 2. Materials and methods

### 2.1. Study subjects

Fourteen healthy non-smoking men between 19 and 42 years of age (Mean ± S.D.; 27.1 ± 7.4 years) provided written informed consent and completed the study. Their mean body weight was 86.6 ± 20.6 kg before the study, and body fat estimated from the sum of three skinfold measurements [8] was 12.7 ± 6.9% (Table 1). All men had been weightlifting for approximately 6 years and exercised at least three times per week. None of the subjects had a history of renal, hepatic, or vascular disease, and no subject averaged more than one alcoholic beverage daily or took regular medications. All men denied current and prior androgen use. Baseline urinalysis confirmed that subjects had not recently used either anabolic–androgenic steroids or testosterone [7]. The subjects were instructed to maintain their habitual level of physical activity and to avoid altering their dietary habits and nutritional supplement use during the study. Subjects were reimbursed for participation as approved by The Miriam Hospital Clinical Research Review Board.

### 2.2. Study design

Subjects were randomly assigned to a counterbalanced cross-over design involving three treatments: testosterone enanthate (E.R. Squibb and Sons, Princeton, NJ), 200 mg/week intramuscularly (i.m.); oral testolactone (E.R. Squibb and Sons), 250 mg four times daily (QID); and both testosterone enanthate, 200 mg/week i.m. and testolactone, 250 mg QID. This testosterone dose has been recently studied as a male contraceptive [9]. Each treatment lasted 3 weeks, and treatments were separated by a 4-week washout period. Blood samples were obtained from an antecubital vein before and after each 3 week treatment, between 06:00 and 09:00, after a 12-h fast, and before testosterone injections. Plasma samples were collected in pre-chilled evacuated tubes that contained heparin as an anticoagulant and were immediately placed on ice. These samples were separated by centrifugation usually within 1 h (maximum 2 h), and frozen and stored at −70°C until analyzed.

Table 1
Baseline characteristics of study subjects

| | Mean | S.D. |
|---|---|---|
| Age (years) | 27.1 | 7.4 |
| Weight (kg) | 86.6 | 20.7 |
| Body fat (%) | 12.7 | 6.9 |
| Testosterone (nmol/l) | 19.0 | 5.9 |
| Estradiol (pmol/l) | 145.7 | 52.7 |
| Homocysteine (μmol/l) | 5.0 | 1.1 |

### 2.3. Biochemical assays

Fasting tHcy levels were measured in plasma with the fluorimetric method of Vester and Rasmussen [10], except that 20% methanol was used in buffer B in the high-performance liquid chromatography (HPLC) procedure. lnterassay variation for tHcy determinations was avoided by analyzing all samples for an individual subject in a single assay or autoanalyzer run. Intra-assay coefficient of variation for tHcy was less than 6.0%. Serum testosterone and estradiol were assayed in duplicate using radioimmunoassay kits (Diagnostic Products, Los Angeles, CA).

### 2.4. Statistical analysis

Data were analyzed with a treatment-by-time analysis of variance with repeated measures on both factors. When interaction effects were significant, the effect of time for each treatment and the differences between treatments at each measurement point were tested statistically. A modified Bonferroni procedure was employed to adjust for multiple comparisons [11]. Pearson simple and partial correlation coefficients were computed to examine the associations between baseline serum testosterone and estradiol levels and fasting tHcy concentrations with and without adjustment for baseline age and body weight.

## 3. Results

Pretreatment serum testosterone concentrations were in the normal physiologic range for young adult men. Serum testosterone levels increased by 38% when testosterone was given alone and by 102% when testosterone and testolactone were combined ($P < 0.01$ for both; Fig. 1). Testosterone also produced a 43% increase ($P < 0.01$) in serum estradiol levels, an effect that was not observed when testosterone and testolactone were administered together (Fig. 1). Testolactone alone did not significantly change either testosterone or estradiol levels. These results indicate that testolactone inhibited aromatase activity and blocked the conversion of exogenous testosterone to estradiol.

Mean pretreatment tHcy levels (5.0 ± 1.1 μmol/l: range, 3.3–7.0 μmol/l) were lower than previously reported values for men of a similar age [12]. We suspect that most of this difference is due to habitual use of B-vitamin-containing supplements, a common practice among athletes. Fasting tHcy concentrations were not significantly altered when testosterone was given alone or when testosterone and testolactone were combined (Table 2). Testolactone did not significantly affect plasma tHcy levels. Baseline serum testosterone ($r = -0.10$; $P = 0.74$) and estradiol ($r = -0.13$; $P = 0.65$) con-

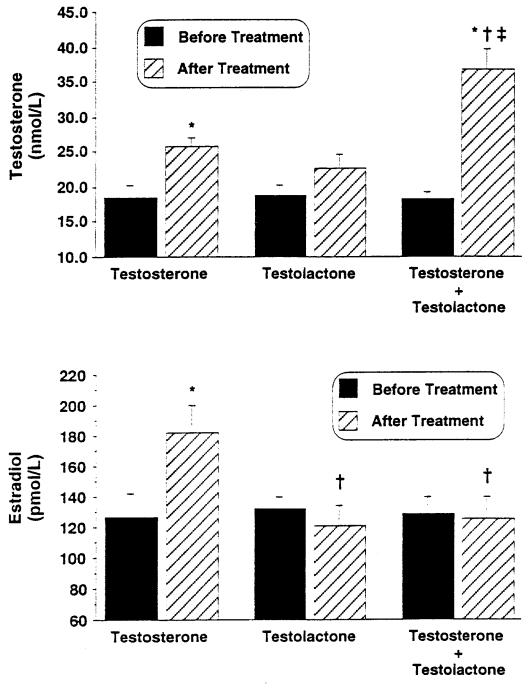


Fig. 1. Mean ( ± S.E.) serum testosterone (upper panel) and estradiol (lower panel) levels before and 3 weeks after treatment with testosterone, testolactone, and testosterone plus testolactone. * Significant difference ($P < 0.01$) from pretreatment levels. † Significant difference ($P < 0.01$) from testosterone treatment. ‡ Significant difference ($P < 0.01$) from testolactone treatment.

centrations were not strongly associated with tHcy levels. Similar correlation coefficients were observed after controlling for baseline age and body weight. These results indicate that testosterone, with or without its aromatization to estradiol, does not influence fasting tHcy levels in men.

## 4. Discussion

The original aim of this study was to examine the effects of testosterone, with or without aromatization to estradiol, on high-density lipoprotein cholesterol levels in men [7]. Recent reports suggesting that estrogens may decrease the atherogenic amino-acid tHcy in postmenopausal women [4,5] prompted us to examine the effects of testosterone, testolactone or the combination on fasting tHcy levels. Because supraphysiologic doses of testosterone increase estrogen levels in men, the use of testolactone provided an opportunity to examine the androgenic effects of testosterone with and without its aromatization to estradiol. Testosterone alone increased serum estradiol levels by 43%, whereas estradiol levels did not change significantly when testosterone and testolactone were combined. Fasting tHcy levels were not significantly altered during either testosterone condition, however, suggesting that testosterone and its aromatization to estradiol does not affect tHcy concentrations in eugonadal men.

The observation that fasting tHcy concentrations are higher in men than in women [3] has led to the suggestion that sex steroid hormones may explain the gender difference in tHcy levels [3]. Support for this idea comes from studies showing that estrogen plus progestin replacement therapy [4] and the estrogen agonist tamoxifen [5] reduce tHcy concentrations in postmenopausal women. Others have documented an inverse correlation between serum estradiol concentrations and post-methionine tHcy levels in premenopausal women [4]. The present study is the first, to our knowledge, to examine the effects of androgenic hormones on tHcy levels in men. Serum testosterone and estradiol levels were not strongly associated with pretreatment tHcy levels. Furthermore, testosterone administration, with or without its aromatization to estradiol, did not significantly alter tHcy levels. These results suggest that neither testosterone nor estradiol have an important influence on tHcy levels in normal young men, and raise the possibility that factors other than testosterone contribute to higher fasting tHcy levels in men than in women.

A more likely explanation for the gender difference in fasting tHcy levels may relate to the fact that creatine–creatinine production is directly coupled to *s*-adenosylhomocysteine generation from *s*-adenosylmethionine [13], and that lean body mass and creatine–creatinine production tend to be higher in men than in women. Indeed, plasma tHcy levels correlate directly with serum creatinine concentrations in men and women [14]. Moreover, the gender difference in tHcy levels disappeared in one recent study when men and women were matched for serum creatinine concentrations [14]. These results suggest that the higher mean fasting tHcy levels in men compared with women are most likely a result of the direct relationship between homocysteine production and creatine–creatinine synthesis [14].

The present study has several limitations. First, a longer treatment duration and larger sample size may be required to reveal a testosterone effect on mean fasting tHcy levels. Two previous reports demonstrating that female sex hormones decrease plasma tHcy levels in women included at least 27 study subjects [4,5], and a significant decline in tHcy in one study was not detectable until after 3–4 months of treatment [5]. Therefore, the present study may have underestimated the true magnitude of testosterone's effect on tHcy levels. Since fasting tHcy levels are only weakly related to the tHcy response to methionine loading [15], we also cannot exclude the possibility that testosterone administration influences post-methionine thcy levels. Finally, all of our study subjects had normal total testosterone levels, so it is possible that men with hypogonadism would have experienced a change in

Table 2
Plasma homocysteine concentrations before and after 3 weeks of testosterone, testolactone, and testosterone plus testolactone

| | Testosterone | Testolactone | Testosterone + Testolactone |
|---|---|---|---|
| Homocysteine ($\mu$mol/l) | | | |
| Before | $4.7 \pm 1.1$ | $4.6 \pm 0.8$ | $4.8 \pm 1.1$ |
| After | $4.6 \pm 0.9$ | $4.9 \pm 1.1$ | $4.8 \pm 0.9$ |

Values are mean $\pm$ S.D. There were no significant differences within or between treatments.

thcy with testosterone treatment. Additional studies are needed to examine these possibilities.

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