Testosterone alters iron metabolism and stimulates red blood cell production independently of dihydrotestosterone

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1Research Service, 2Geriatric Research, Education, and Clinical Center, and 3Research Pharmacy, Malcom Randall Veterans Affairs Medical Center, University of Florida, Gainesville, Florida; Departments of 4Applied Physiology and Kinesiology, 5Biostatistics, and 6Health Outcomes and Policy, University of Florida, Gainesville, Florida; and 7Department of Kinesiology, University of Rhode Island, Kingston, Rhode Island

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Beggs LA, Yarrow JF, Conover CF, Meuleman JR, Beck DT, Morrow M, Zou B, Shuster JJ, Borst SE. Testosterone alters iron metabolism and stimulates red blood cell production independently of dihydrotestosterone. Am J Physiol Endocrinol Metab 307: E456–E461, 2014. First published July 29, 2014; doi:10.1152/ajpendo.00184.2014.—Testosterone (T) stimulates erythropoiesis and regulates iron homeostasis. However, it remains unknown whether the type II 5α-reductase of T to dihydrotestosterone (DHT) mediates these androgenic effects, as it does in some other tissues. Our purpose was to determine whether inhibition of type II 5α-reductase (via finasteride) alters red blood cell (RBC) production and serum markers of iron homeostasis subsequent to testosterone- enantihate (TE) administration in older hypogonadal men. Sixty men aged ≥60 yr with serum T <300 ng/dl or bioavailable T <70 ng/dl received treatment with TE (125 mg/wk) vs. vehicle paired with finasteride (5 mg/day) vs. placebo using a 2 × 2 factorial design. Over the course of 12 mo, TE increased RBC count 9%, hematocrit 4%, and hemoglobin 8% while suppressing serum hepcidin 57% (P < 0.001 for all measurements). Most of the aforementioned changes occurred in the first 3 mo of treatment, and finasteride coadministration did not significantly alter any of these effects. TE also reduced serum ferritin 32% (P = 0.002) within 3 mo of treatment initiation without altering iron, transferrin, or transferrin saturation. We conclude that TE stimulates erythropoiesis and alters iron homeostasis independently of the type II 5α-reductase enzyme. These results demonstrate that elevated DHT is not required for androgen-mediated erythropoiesis or for alterations in iron homeostasis that would appear to support iron incorporation into RBCs.

iron; testosterone; finasteride; hypogonadal; hematocrit; hepcidin

Iron metabolism and erythropoiesis are intrinsically interrelated because incorporation of iron into the heme group of erythrocytes is necessary for oxygen transport (38). It is well established that testosterone (T) regulates erythropoietic activity in men (32, 33). Clinically, this remains an important concept given the five- to 13-fold higher prevalence of anemia in hypogonadal men compared with their eugonadal counterparts (14) and by the ability of T replacement therapy (TRT) to elevate hematocrit (HCT) and hemoglobin (HGB) in androgen-deficient men (4, 12, 25). In this regard, classical studies have established that androgen-stimulated erythropoiesis is mediated by erythropoietin (EPO) (32, 33), as evidenced by the complete inhibition of erythropoiesis in androgen-treated animals receiving anti-EPO antibody (15, 31). However, androgen may also indirectly support erythropoiesis by altering iron homeostasis (5) via the suppression of hepcidin (3, 4), a negative regulator of the iron transporter ferroportin (38). Hepcidin binds to and internalizes ferroportin within cells, limiting transport of intracellular iron into the circulation (28). Elevated hepcidin underlies anemia of chronic disease (38), and androgen-induced hepcidin suppression increases splenic ferroportin expression, which effectively increases iron absorption and iron incorporation into red blood cells (RBCs) in mice (17). Interestingly, androgen-induced hepcidin suppression occurs in a dose-dependent manner within 7 days of TRT initiation in men (4), preceding the time frame in which HGB is elevated subsequent to androgen administration (4). Additionally, the androgen-induced suppression of hepcidin appears independent of EPO, given that T-stimulated hepcidin suppression persists in mice administered anti-EPO antibody (17). However, the mechanisms underlying this effect require further elucidation.

In addition, many of the biological effects of T are mediated by the type II 5α-reductase enzyme (22) that converts T to dihydrotestosterone (DHT), a more potent and longer-acting androgen (37). This is an important clinical concept given the increasing prevalence of TRT in older hypogonadal men and our recent findings that pharmacological 5α-reductase inhibition prevents prostate enlargement (a primary clinical concern associated with TRT) without inhibiting the musculoskeletal or lipolytic benefits of this therapy (10). Interestingly, hepatocytes (the predominant source of circulating hepcidin) express type II 5α-reductase (27), and human liver extracts actively convert T to DHT (16), indicating that 5α-reductase influences hepatic androgen metabolism. Additionally, 5α-reductase is thought to be involved in erythropoiesis in mammals (23). However, few studies have examined the influence of type II 5α-reductase on iron homeostasis or androgen-stimulated erythropoiesis in humans. The primary purpose of this study was to determine whether type II 5α-reductase activity influences androgen-stimulated erythropoiesis in elderly hypogonadal men. A secondary purpose was to determine the role of type II 5α-reductase in regulating iron homeostasis subsequent to androgen administration.

METHODS

Study design. The 52-wk, double-blind, randomized controlled trial (RCT) involved men aged ≥60 yr with a serum T concentration of ≤300 ng/dl or bioavailable fractions of T (BioT) of ≤70 ng/dl. Participants were randomized to receive one of four treatments: 1) vehicle-placebo, 2) vehicle-finasteride, 3) T-enanthate (TE)-placebo,
or 4) TE-finasteride using a 2 × 2 factorial design. Treatment lasted for 12 mo and consisted of Proscar (5 mg/day po finasteride), placebo and Delatestryl (125 mg/wk im TE), or vehicle. Both drugs were administered in FDA-approved doses. Proscar and matching placebo were donated by Merck, Delatestryl was donated by Novartis, and matching vehicle was prepared by WestLab Pharmacy (Gainesville, FL). The study was approved by the Institutional Review Board at the University of Florida. All participants provided written, informed consent.

Individuals underwent screening to determine eligibility, including structured medical history, blood acquisition (performed twice between 0800 and 1000, separated by 30 min), and a physical exam, as reported previously by us (10). Two blood samples were obtained during screening as per the Endocrine Society’s recommendations (6).

To ensure participant safety, we excluded individuals who failed the Mini-Cog test indicating dementia, those with a history of prostrate or breast cancer, those with severe benign prostatic hyperplasia (BPH), and those with an American Urological Association/International Prostate Symptom Score (AUA/IPPSS) of ≥25, class 3 or 4 congestive heart failure, sleep apnea, HCT, hematocrit; HGB, hemoglobin. To convert values for testosterone to nmol/l, multiply by 0.0347. To convert values for DHT to nmol/l, multiply by 0.0344.

RESULTS

The primary outcomes from this RCT, including musculoskeletal and prostate findings, sex hormone concentrations, and clinical laboratory values, have been reported previously (10). Briefly, TE administration (i.e., the combined effects of groups 3 and 4) elevated nadir T and BioT 1.8- and 2.2-fold, respectively, over baseline. TE also elevated E2 and BioE2, representing 1.7- and 2.2-fold increases, respectively, over baseline. Finasteride coadministration did not significantly affect the aforementioned increases. TE also elevated serum DHT 2.4-fold, and finasteride administration (i.e., the combined effects of treatments 2 and 4) lowered DHT by 65%.

Baseline values for this study are reported in Table 1 and the 12-mo treatment effects in Tables 2 and 3. TE administration

Table 1. Baseline characteristics for participants receiving vehicle-placebo, vehicle-finasteride, TE-placebo, or TE-finasteride

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vehicle Placebo</th>
<th>Vehicle Finasteride</th>
<th>TE Placebo</th>
<th>TE Finasteride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>70.8 ± 9.7</td>
<td>69.5 ± 9.2</td>
<td>69.2 ± 8.0</td>
<td>64.2 ± 4.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30.5 ± 3.4</td>
<td>28.8 ± 3.9</td>
<td>29.9 ± 4.6</td>
<td>31.1 ± 2.5</td>
</tr>
<tr>
<td>Testosterone, ng/dl</td>
<td>264 ± 92</td>
<td>241 ± 112</td>
<td>246 ± 73</td>
<td>243 ± 147</td>
</tr>
<tr>
<td>BioT, ng/dl</td>
<td>25 ± 15</td>
<td>19 ± 13</td>
<td>21 ± 6</td>
<td>22 ± 23</td>
</tr>
<tr>
<td>RBC, cells/mcl</td>
<td>4.5 ± 0.5</td>
<td>4.7 ± 0.8</td>
<td>4.7 ± 0.8</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>HCT, %</td>
<td>40 ± 3.9</td>
<td>41 ± 3.9</td>
<td>43 ± 3.0</td>
<td>42 ± 2.8</td>
</tr>
<tr>
<td>HGB, g/dl</td>
<td>135 ± 1.3</td>
<td>138 ± 1.5</td>
<td>147 ± 1.4</td>
<td>146 ± 0.9</td>
</tr>
<tr>
<td>Hepcidin, ng/ml</td>
<td>48 ± 35</td>
<td>42 ± 22</td>
<td>53 ± 26</td>
<td>48 ± 35</td>
</tr>
<tr>
<td>Iron, µg/dl</td>
<td>88 ± 32</td>
<td>83 ± 38</td>
<td>82 ± 33</td>
<td>96 ± 35</td>
</tr>
<tr>
<td>Transferrin, µg/dl</td>
<td>247 ± 37</td>
<td>250 ± 25</td>
<td>249 ± 31</td>
<td>277 ± 36</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>36 ± 3.3</td>
<td>34 ± 4.8</td>
<td>34 ± 4.2</td>
<td>35 ± 2.8</td>
</tr>
<tr>
<td>Ferritin, ng/ml</td>
<td>142 ± 76</td>
<td>154 ± 69</td>
<td>186 ± 118</td>
<td>222 ± 271</td>
</tr>
</tbody>
</table>

Table 2. Change in RBC count, HCT, HGB, and hepcidin over 12 mo of treatment as a result of TE treatment [i.e., combined effects of treatments 1 and 2 (no TE) vs. 3 and 4 (with TE), respectively]

<table>
<thead>
<tr>
<th>Estimate</th>
<th>P Value</th>
<th>Interaction (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 mo AHEPCIDIN, ng/ml</td>
<td>-29 ± 8.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>12 mo AHECT, %</td>
<td>4.1 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>12 mo AHB, g/dl</td>
<td>1.1 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>12 mo AHB, cells/mcl</td>
<td>0.4 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values represent means ± SE. The interaction test was performed only when a significant effect was observed.
increased RBC count 9% \((P < 0.001)\), HCT 4% \((P < 0.001)\), and HGB 8% \((P < 0.001)\) and reduced serum hepcidin 57% \((P < 0.001)\) over 12 mo, with the vast majority of these changes occurring in the first 3 mo of treatment (Fig. 1, A–D). Finasteride coadministration did not significantly alter any of the aforementioned variables. A small increase in HGB that likely resulted from the increase present in the TE-finasteride group was also observed in the finasteride-treated groups \((P = 0.034)\), but no significant effect was seen in the placebo-finasteride group (interaction \(P = 0.065\), trend). Finasteride treatment did not significantly alter any other variables.

Given the large changes in RBCs and hepcidin occurring primarily within the first 3 mo of TE treatment, we limited subsequent analyses to this time frame. Within the first 3 mo of treatment, TE reduced serum ferritin 32% \((P = 0.002)\), and finasteride coadministration did not significantly alter this effect (Table 4). Neither TE nor finasteride significantly altered serum iron, transferrin, or transferrin saturation. No associations were present between baseline hepcidin and the magnitude of HCT/HGB change or between the 3-mo change in hepcidin and the magnitude of HCT/HGB change in groups receiving TE. The baseline to 3-mo change in E2 was significantly and positively correlated to the changes in RBC count and HGB (see Table 5). However, changes in T, BioT, DHT, and bioestradiol did not show similarly significant correlations.

**DISCUSSION**

T induces direct biological effects via interactions with androgen receptors (ARs) and/or indirect effects via AR or estrogen receptor (ER) activation following \(5\alpha\)-reduction to DHT or aromatization to E2. In this regard, T functions as a hormone and as a prohormone for more potent androgenic and estrogenic sex steroids. As such, determining the mechanism(s) through which administered T produces tissue- and/or cell-specific effects remains biologically significant and clinically important. One of the well-established functions of T is the regulation of erythropoiesis (32, 33). However, it remains unknown whether the \(5\alpha\)-reduction of T to DHT mediates the effects of androgens on RBC production and iron homeostasis. The primary findings of this study are that finasteride (a type II
Table 4. Change in serum ferritin, iron, and transferrin over the initial 3 mo of treatment as a result of TE treatment [i.e., combined effects of treatments 1 and 2 (no TE) vs. 3 and 4 (with TE), respectively]

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>P Value</th>
<th>Interaction (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Mo Δferritin, ng/ml</td>
<td>-132 ± 32</td>
<td>0.002</td>
<td>0.450</td>
</tr>
<tr>
<td>3-Mo Δiron, μg/dl</td>
<td>-3 ± 11</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>3-Mo Δtransferrin, mg/dl</td>
<td>11 ± 8</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>3-Mo Δtransferrin saturation, %</td>
<td>-1.0 ± 4.6</td>
<td>NS</td>
<td></td>
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</tbody>
</table>

Values represent means ± SE. The interaction test was performed only when a significant effect was observed.

5α-reductase inhibitor) does not significantly inhibit T-induced erythropoiesis or androgen-mediated alterations in iron homeostasis. Specifically, TE administration elevated RBC and HGB production independently of finasteride, and finasteride (alone) did not significantly reduce HCT despite a >65% reduction in circulating DHT. In addition, we provide the first evidence demonstrating that type II 5α-reductase activity is not required for T-induced hepcidin suppression in elderly hypogonadal men. We also observed that TE administration resulted in suppressed serum ferritin without alterations in serum iron or transferrin. Collectively, these findings suggest that T regulates erythropoiesis and alters iron homeostasis in a manner that may not require action of the type II 5α-reductase enzyme or elevated systemic DHT.

Recently, our laboratory (10) and others (2, 7, 29) have demonstrated that the musculoskeletal and lipolytic benefits of TRT do not require action of the type II 5α-reductase enzyme or systemically elevated DHT. In contrast, prostate enlargement (2, 8–10, 26, 36) and other putative side effects resulting from androgen administration (e.g., male-pattern baldness or acne) (18) are mediated primarily by type II 5α-reductase. However, the role of the type II 5α-reductase enzyme in mediating androgen-induced erythropoiesis has received little attention in the literature. Herein, we report that 125 mg/wk TE (a supraphysiological TRT dose that is within the FDA-approved range) increases RBC production in older hypogonadal men, which supports meta-analysis data indicating an average HCT increase of 3.2% in men receiving a range of doses and various forms of TRT (13). Additionally, our results extend the above-mentioned findings by demonstrating that finasteride coadministration does not significantly inhibit TE-induced erythropoiesis in older hypogonadal men, which increases the clinical relevance of this combination pharmacological therapy because hypogonadal men present with an increased prevalence of anemia compared with age-matched eugonadal men (14) and because older men exhibit greater HCT elevations in response to TRT than younger men (12). However, TRT also increases the risk for polycythemia (i.e., HCT >54%), which is the most common side effect associated with this therapy (11) and which potentially increases stroke risk (35). Importantly, the incidence of polycythemia remained very low within the cohort of men receiving TE in our study despite the fact that we administered TE in a dose that produces transient supraphysiological T concentrations, and in all cases HCT returned to baseline following discontinuation of TRT.

Red blood cell production also requires adequate iron availability. However, the ability of T to suppress hepcidin (a negative regulator of the iron transporter ferroportin) was only recently identified as a mechanism through which androgens increase iron absorption and iron incorporation into RBCs (17, 24). Herein, we observed that TE suppressed serum hepcidin in a magnitude that was similar to previous clinical trials (3, 4). Additionally, our results are the first to indicate that finasteride does not significantly interfere with this effect, demonstrating that type II 5α-reductase is not a mediator of T-stimulated hepcidin suppression. Interestingly, we also observed a large reduction in serum ferritin that occurred within 3 mo of PE administration and corroborates the findings of others (4), suggesting that T increases iron utilization, likely as a result of increased erythropoiesis. Regardless, serum iron, transferrin, and transferrin saturation, common clinical markers of iron availability, were not altered significantly within this time frame.

Several recent clinical trials have attempted to identify serum markers, which when measured at baseline will predict the magnitude of subsequent T-induced increases in HCT/HGB. Such a marker would be valuable given that polycythemia is the most common adverse event in men undergoing TRT and is a predisposing factor for cerebrovascular events. In this regard, Bachman et al. (3) reported that men with the highest quartile of hepcidin change subsequent to TRT experienced the greatest risk for erythrocytosis. In contrast to these findings, we found no associations between baseline hepcidin or the 3-mo change in hepcidin and the change in HCT/HGB in men receiving TE. As such, we are unaware of any marker that has consistently been shown to predict the magnitude of androgen-stimulated erythropoiesis prior to TRT initiation, which underlies the continued necessity to exclude men with elevated basal HCT (i.e., >50%) from TRT and the importance for regular HCT monitoring throughout the duration of TRT as recommended by the Endocrine Society Clinical Guidelines (6).

Interestingly, we also observed that TE administration elevated circulating E2 and BioE2 and that the magnitude of change in E2 was correlated to the increases in RBC count and...
HGB. These findings raise the possibility that estrogens may mediate several of the effects we observed. In this regard, estrogen has been shown to suppress hepcidin, and an estrogen response element is present in the promotor region of the hepcidin gene (20, 21). However, we find it highly unlikely that E2 mediated the erythropoietic effects of T given that T administration results in elevated RBC count and HGB in aromatase-deficient men (30) and that RBC count and HGB are elevated following T plus letrozole (a potent aromatase inhibitor) treatment in boys with constitutional delay of puberty (19). Similarly, preclinical evidence from our laboratory indicates that trenbolone (a nonaromatizable and non-5α-reducible T analog) and TE elevate HGB in an identical manner in orchietomized rats (26, 36). Together, these results demonstrate that aromatase activity is not necessary for androgen-stimulated erythropoiesis, although the possibility remains that the suppression of hepcidin was at least partially influenced by the elevated E2 following TE administration.

One limitation of our study is that we did not evaluate serum EPO. Several previous clinical trials have failed to detect alterations in serum EPO following TRT (25) even when T is administered well above the physiological range (12). However, others have reported that fluoxymesterone (an orally active synthetic androgen) produced a five- to 10-fold increase in urinary EPO within 4 days in both anemic and hypogonadal men (1), and a number of classical studies have demonstrated that anti-EPO antibody ablates androgen-stimulated erythropoiesis in animals (32, 33), which demonstrates that EPO is required for androgen-induced erythropoiesis. The inconsistencies in the aforementioned studies may be explained by the rather transient nature of androgen-stimulated EPO synthesis and by the high degree of variability in EPO between individuals. In this regard, Bachman et al. (4) recently observed elevated circulating EPO in older hypogonadal men 1 mo after TRT initiation, with values gradually declining toward baseline after several months. In contrast, we find it unlikely that androgen-stimulated EPO production mediates the hepcidin suppression resulting from TRT, because hepcidin is rapidly suppressed after T administration (3) and T-stimulated hepcidin suppression persists in mice administered anti-EPO antibody (17). Regardless, some controversy remains surrounding the mechanism(s) through which androgens initially stimulate erythropoiesis in animals (32, 33), which demonstrates that EPO is required for androgen-induced erythropoiesis.

In conclusion, we provide the first-ever evidence indicating that finasteride coadministration does not prevent T-stimulated erythropoiesis in older hypogonadal men and that elevated circulating DHT is not required for androgen-induced alterations in iron homeostasis. In this manner, our results provide further evidence supporting the viability of T plus finasteride coadministration as an alternative to traditional TRT, especially given the high prevalence of anemia in hypogonadal elderly men compared with their eugonadal counterparts. Future research focused on evaluating the mechanism(s) underlying androgen-stimulated erythropoiesis and androgen-induced alterations in iron homeostasis remains warranted and may provide insight into novel markers that predict the magnitude of androgen-stimulated erythropoiesis prior to the initiation of TRT.

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DISCLOSURES
The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS

REFERENCES
ANDROGEN-INDUCED ERYTHROPOIESIS IS INDEPENDENT OF DHT


