

## Mechanism of Action of Androgens on Erythropoiesis – A Review

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### ABSTRACT

The effects of androgens on hematopoiesis are well documented. These drugs have been major pharmacological agents for stimulation of erythropoiesis before the recombinant hematopoietic growth factors were available. Their effects on red blood cells are best studied. They stimulate erythropoiesis and increase levels of red blood cells, hemoglobin and hematocrit. There are conflicting data on the effects of androgens on leucopoiesis and platelets. The stimulatory effect of androgens on erythropoiesis is a problem in patients with hypogonadism undergoing testosterone replacement. Increased blood viscosity in these patients is associated with increased risk of cardiovascular thrombotic events. Three major mechanisms for the effects of testosterone on erythropoiesis are proposed: increased erythropoietin set point, increased iron utilization and conversion of androgens in estrogens. Testosterone-induced increase in hematocrit and hemoglobin is probably associated with elevated erythropoietin levels, but the available data on the relationship between testosterone and erythropoietin are too contradictory. The effects of testosterone on erythropoiesis are accompanied by other mechanisms, such as reduced hepcidin probably by direct inhibition of its transcription. The direct erythropoietic effect of testosterone is likely mediated by inducing the synthesis of IGF-1 through a receptor-mediated mechanism although this mechanism can not completely explain the effects of testosterone on erythropoiesis. Interaction of androgens with some cytokines and growth factors may also influence hemopoiesis. It is contradictory if the effect of testosterone on erythropoiesis requires its conversion into estrogens or not. More experimental and clinical trials are needed to investigate the exact mechanism of stimulatory effect of androgens on erythropoiesis.

**Keywords:** testosterone, erythropoiesis, erythropoietin, hemoglobin.

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### INTRODUCTION

#### *Effect of androgens on erythropoietin*

The role of erythropoietin in testosterone-mediated stimulation of erythropoiesis is not entirely clear. The available data on the relationship between testosterone and the key regulator of erythropoiesis – erythropoietin are too contradictory. Initial suppositions are that administration of testosterone in people and experimental animals leads to increased plasma concentration of erythropoietin. Experimental data from the 1970s show that testosterone increases serum hemoglobin levels in laboratory animals<sup>1</sup>. It is assumed that this effect is mediated by stimulation of erythropoietin production in the kidneys<sup>2</sup>. Newer experimental data show that this mechanism is unlikely and androgens perform their hematopoietic effects, regardless of their effect on erythropoietin secretion. In mice, androgens increase not only hemoglobin levels but also the number of granulocytes<sup>3</sup>. *In vitro* androgens enhance hematopoiesis in bone marrow cultures and stimulate the erythroid as well as the myeloid lines in semi-solid cell cultures<sup>4,5</sup>. Subsequently clinical evidence accumulates that androgens do not affect erythropoietin synthesis and secretion:

Notwithstanding the differences in serum levels of testosterone and hemoglobin in men and women, the erythropoietin concentration in the plasma is the same<sup>6</sup>.

In artificially-induced androgen deficiency with analogues of the releasing factor of luteinizing hormone (luteinizing hormone-releasing factor, LHRH) no changes in serum erythropoietin levels are observed<sup>7</sup>.

Dickerman et al (1999) found that in the majority of cases of androgen-induced erythrocytosis erythropoietin levels are more often low than high<sup>8</sup>.

Hara N et al (2010) found no differences in the levels of erythropoietin in patients before and after anti-androgen therapy<sup>9</sup>.

Testosterone dose-dependently increases the levels of hemoglobin and hematocrit without this being linked to an increase in erythropoietin levels<sup>10</sup>.

Ballal SH et al (1991) found that in patients with chronic renal failure undergoing dialysis, administration of androgens (nandrolone decanoate) enhances the effect of erythropoietin, leading to reduction of the dose necessary for achieving target hemoglobin levels<sup>11</sup>. The authors suggest that this is due to increased sensitivity by androgens of erythroid progenitors to erythropoietin.

In patients with diabetes mellitus type 2 the low level of testosterone is a risk factor for the development of anemia irrespective of the erythropoietin level and the presence or absence of accompanying renal failure<sup>12,13</sup>.

It is likely that androgen-mediated increase in hemoglobin and hematocrit is not carried out through their influence on erythropoietin. More recent studies (Bachman E et al,

2014) again found that testosterone-induced increase in hematocrit and hemoglobin is associated with elevated erythropoietin levels, but this is also accompanied by other mechanisms, such as reduced hepcidin and ferritin. The studies were conducted before the start of TRT, at the first, third and sixth month after the beginning of TRT and at the third month after its discontinuation. Patients were treated with 100 mg of testosterone per day in the form of a transdermal gel. One month after the start of therapy serum erythropoietin levels rise above normal values. The interesting in this study is that 6 months after therapy initiation the levels of erythropoietin and hepcidin return to pre-treatment levels regardless that testosterone administration continues. Erythropoietin however remains non-suppressed and its levels are disproportionately high for the elevated hemoglobin and hematocrit. This suggests that a new erythropoietin/hemoglobin "set point" is established with a lack of negative feedback inhibition. Respective to the increased utilization of iron elevated values of soluble transferrin receptor (sTfR) and ratio sTfR/log ferritin are identified<sup>14</sup>.

#### *Androgens and Transferrin Receptor*

In plasma iron is transported bound to serum transferrin. Through interaction with a specific membrane receptor (transferrin receptor, TfR) the iron enters the cells. In humans and animals, a soluble serum form of this receptor has been identified - sTfR. sTfR is a monomer of the tissue receptor that lacks the first 100 amino acids. It circulates in the form of a complex between transferrin and its receptor. Erythroblasts are the main source of serum sTfR. Bone marrow erythropoietic activity is the major determinant of serum sTfR levels. Its levels increase when erythropoiesis is stimulated and decrease when erythropoietic activity of the bone marrow is suppressed. Determination of sTfR in serum is useful for clarifying the pathophysiology of anemias; for quantitative analysis of erythropoietic activity; for the proliferative capacity of bone marrow, and for monitoring the response of erythropoietic tissue in the treatment of anemia before there have been changes in hemoglobin. sTfR is also an indicator of iron deficiency, since its levels increase in this case<sup>15</sup>. T'Sjoen G et al in a clinical study on the effects of testosterone and estrogens on the level of sTfR found that prior to therapy plasma levels of sTfR are the same in men and women, regardless of the significant differences in their serum testosterone, hemoglobin and hematocrit. Probably in spite of gender-dependent differences in hemoglobin and hematocrit values, established stable erythropoietic activity is characterized by comparable levels of sTfR in both sexes. In the said study the application of testosterone in women increases plasma testosterone levels above normal, as well as the levels of hemoglobin, hematocrit, sTfR and IGF-1 after 4 months of treatment with no clear correlation between IGF-1 and sTfR. Probably testosterone directly stimulates erythropoiesis and its effect is partially mediated by IGF-1. In men treated with the androgen receptor antagonist cyproterone acetate no changes in the level of sTfR are found, and the increase in IGF-1 is insignificant and again does not correlate with sTfR. The administration of oral or

transdermal estrogens in men receiving cyproterone acetate sharply lowers the levels of testosterone, hemoglobin and hematocrit, but only oral estrogens decrease sTfR as well. In this case, the authors found a correlation between the levels of sTfR and IGF-1. The latter is increased by transdermal application of estrogens and decreases upon their oral administration. The differences in sTfR levels in both estrogen regimens cannot be explained by their effects on serum testosterone, since it is comparable in both cases. These data show that the levels of sTfR cannot be an indicator of the effect of testosterone on erythropoiesis, since not in all cases of its decrease reduced serum sTfR is also observed<sup>16</sup>.

#### *Androgens, IGF-1 and Erythropoiesis*

Insulin-like growth factor-1 (IGF-1) is a peptide that participates in the regulation of cell proliferation and differentiation. It carries out numerous effects on carbohydrate, fat, protein metabolism, bones and hematopoiesis<sup>17</sup>. IGF-1 is secreted by many tissues, but over 90% of serum IGF-1 is synthesized in the liver, wherein the production is dependent on the secretion of growth hormone (GF). The latter decreases with age and during the so called somatopause decreased levels of IGF-1 accelerate age-related physiological changes<sup>18,19</sup>. It is known that the GF-IGF-1 axis plays a role in the regulation of erythropoiesis. Although anemia is not a key symptom of growth hormone deficiency, it is associated with abnormal erythropoiesis, both in children and in adults. Exogenous administration of GF increases the level of hemoglobin<sup>20</sup>. There are some age-related differences in this respect. Treatment of adults with growth hormone deficiency increases plasma volume and red blood cell mass, but not hemoglobin. The latter increases in children treated with growth hormone. The mechanism by which IGF-1 affects erythropoiesis is not sufficiently understood. It is likely the effects of GF and IGF-1 on erythropoiesis are synergistic. In *in vitro* experiments it has been found that physiological concentrations of IGF-1 stimulate human and murine erythroid precursors, regardless of the presence or absence of erythropoietin. To achieve the same effect much higher doses of GF are necessary. In experiments with murine erythroid colony-forming units the effect of IGF-1 is more pronounced in the absence of erythropoietin and less so in its presence. The administration of IGF-1 in newborn rats accelerates the production of erythrocytes. Human GF and recombinant IGF-1 stimulate erythropoiesis and increase serum erythropoietin in hypophysectomised rats. Moreover, the effect on erythropoiesis precedes the rise in erythropoietin. Sometimes normal erythropoietin levels can be found in patients with CRF, but the levels of IGF-1 are elevated<sup>21</sup>. On the other hand, exogenous administration of erythropoietin in humans has no significant effect on the level of IGF-1<sup>22</sup>. These data indicate that most likely there is no positive or negative feedback of erythropoietin to IGF-1. Of interest are the studies on serum IGF-1 levels in patients undergoing anti-androgen therapy due to prostate carcinoma. Hara N et al found that exogenous androgen suppression does not affect the level of serum GF, but elevates that of IGF-1. The pre-therapy existing correlation

between serum IGF-1 concentration on the one hand, and erythrocyte count and hemoglobin, on the other, does not occur after the beginning of anti-androgen treatment. Said authors found that the increased levels of IGF-1 correlate with that of luteinizing hormone. It is probable that the increased IGF-1 due to anti-androgen therapy is bioactive, as it is known that gonadotrophic pituitary gland cells express IGF-1 receptors. On the other hand, the effect of IGF-1 in bone marrow is unsatisfactory, since androgen-depriving therapy is accompanied with low hemoglobin levels. This suggests that IGF-1 or its bone marrow receptor are functionally inactive against a background of anti-androgen therapy<sup>9</sup>.

#### *Effect of Androgens on Hepcidin*

Another hypothesis for the mechanism of action of testosterone on erythropoiesis is by influencing the iron-regulatory peptide hepcidin. The assumption of a peripheral mechanism of testosterone is based on the fact that it has a minimal proliferative effect on purified erythroid progenitors (CD34+) *ex vivo*<sup>24</sup>. Hepcidin is a peptide synthesized in the liver and its physiological role is to bind and destroy the ion channels for iron, referred to as ferroportins. Increased levels of hepcidin, in infection or aseptic inflammation for example, reduce systemic bioavailability of iron, which manifests with moderately expressed anemia. Conversely, low levels of this peptide are associated with increased iron absorption, increased bioavailability and stimulation of erythropoiesis. Bachman E et al (2010) in a clinical study on young (19-35 years) and older (59-75 years) men, treated with testosterone enanthate, found that testosterone dose-dependently and age-dependently lowers serum hepcidin. Serum testosterone levels above the physiological suppress hepcidin with over 50% and this process is dose dependent in patient follow-up for a period of 20 weeks. In comparison to young men, the group aged 59-75 years has higher levels of hepcidin before testosterone administration. The study shows that in this age group hepcidin suppression is more pronounced. This explains the mentioned age-related differences in erythropoiesis stimulation by testosterone. Of course, a number of other mechanisms also contribute to the stronger response in older compared to younger men, such as age-related differences in life expectancy of erythrocytes, limited intravascular space with aging and others<sup>25</sup>. The mechanism by which testosterone affects hepcidin is not clarified. There are various assumptions including direct inhibition of this peptide's transcription. It is not clear whether the regulatory role of testosterone on hepcidin influences other life processes, such as iron-dependent synthesis of myoglobin, oxidative phosphorylation (cytochromes) and other iron-dependent enzymes.

#### *Interaction of androgens with cytokines and growth factors, influencing erythropoiesis*

Interferon  $\gamma$  is a cytokine with an inhibitory effect on hematopoiesis. It is involved in the pathogenesis of bone marrow failure. In experiments with cell cultures the androgen preparation oxymetholone does not affect IFN- $\gamma$ -induced suppression of colony-stimulating cells in the bone marrow. This suggests that the direct effect of

androgens on hematopoietic progenitor cells is minimal<sup>26</sup>. Possibly the myelo-stimulating effect is due to other mechanisms. The stromal cell-derived factor (SDF-1) is produced constitutively in the bone marrow stroma. It plays a central role in the settling of hematopoietic stem cells, an action mediated by its receptor - CXCR4. This factor is involved in the positive regulation of hematopoiesis. It stimulates survival and proliferation of hematopoietic progenitor cells by demonstrating synergy with other hematopoietic growth factors. On the other hand, SDF-1 inhibits the cell cycle in the most primitive hematopoietic cells, *in vitro* as well as *in vivo*. Down regulation of bone marrow stromal production of SDF-1 leads to inhibition of the survival and growth of hematopoietic cells. *In vitro* experiments have shown that the active metabolite of testosterone - 5-dihydrotestosterone, but not testosterone itself, as well as some synthetic androgens (oxymetholone) inhibit SDF-1 production and increase apoptosis. This effect is completely reversible by adding SDF-1 to the cell cultures. This paradoxical interaction between androgens and SDF-1 is probably a regulatory mechanism for maintaining hemostasis of hematopoiesis<sup>26</sup>. Androgens suppress B-lymphocytes. This suppression is mediated by the increased TGF- $\beta$  production in the bone marrow stroma. Presumably, the down regulation of SDF-1 contributes to this as well, since the latter is also involved in the maturation of B-lymphocytes.

#### *Role of Estrogens, Produced by Testosterone Aromatization in Hematopoiesis Stimulation*

It is known that testosterone converts into estrogens by aromatization, which leads to the formation of estradiol. Calado et al (2009) found that androgens regulate telomerase expression and activity by their aromatization into estrogens. Estradiol stimulates the alpha estrogen receptor (ER $\alpha$ ), which leads to increased production of telomerase. It is an enzyme that participates in cell replication by protecting telomeres from shortening during mitosis. The TERT gene (telomerase reverse transcriptase) encodes a critical component of this enzyme. When activated by estradiol, ER $\alpha$  binds to a TERT gene promoter and enhances its transcription. This increases telomerase stability and stimulates proliferation and survival of hematopoietic stem cells<sup>27</sup>. This is a possible direct mechanism for hematopoiesis stimulation by sex hormones. On the other hand, Rochira et al (2009) in a clinical study on men with aromatase deficiency found that the effect of testosterone on erythropoiesis does not require its conversion into estrogens<sup>28</sup>.

#### REFERENCES

1. Fried W, Gurney CW. The erythropoietic-stimulating effects of androgens. *Ann N Y Acad Sci.* 1968 Mar 29;149(1):356-65.
2. Rencricca NJ, Solomon J, Fimian WJ Jr, Howard D, Rizzoli V, Stohlman F Jr. The effect of testosterone on erythropoiesis. *Scand J Haematol.* 1969;6(6):431-6.
3. Udupa KB, Reissmann KR. Stimulation of granulopoiesis by androgens without concomitant

- increase in the serum level of colony stimulating factor. *Exp Hematol.* 1975 Jan;3(1):26-31.
4. Francis GE, Berney JJ, Bateman SM, Hoffbrand AV. The effect of androstanes on granulopoiesis in vitro and in vivo. *Br J Haematol.* 1977 Aug;36(4):501-10.
  5. Beran M, Spitzer G, Verma DS. Testosterone and synthetic androgens improve the in vitro survival of human marrow progenitor cells in serum-free suspension cultures. *J Lab Clin Med.* 1982 Feb;99(2):247-53.
  6. Miller ME, Chandra M, Garcia JF. Clinical applications of measurement of serum immunoreactive levels of erythropoietin. *Ann N Y Acad Sci.* 1985;459:375-81.
  7. Weber JP, Walsh PC, Peters CA, Spivak JL. Effect of reversible androgen deprivation on hemoglobin and serum immunoreactive erythropoietin in men. *Am J Hematol.* 1991 Mar;36(3):190-4.
  8. Dickerman RD, Pertusi R, Miller J, Zachariah NY. Androgen-induced erythrocytosis: is it erythropoietin? *Am J Hematol.* 1999 Jun;61(2):154-5.
  9. Hara N, Nishiyama T, Takizawa I, Saito T, Kitamura Y, Takahashi K. Decline of the red blood cell count in patients receiving androgen deprivation therapy for localized prostate cancer: impact of ADT on insulin-like growth factor-1 and erythropoiesis. *Urology.* 2010 Jun;75(6):1441-5. doi: 10.1016/j.urology.2009.11.021.
  10. Coviello AD, Kaplan B, Lakshman KM, Chen T, Singh AB, Bhasin S Effects of graded doses of testosterone on erythropoiesis in healthy young and older men. *J Clin Endocrinol Metab.* 2008 Mar;93(3):914-9.
  11. Ballal SH, Domoto DT, Polack DC, Marciulonis P, Martin KJ Androgens potentiate the effects of erythropoietin in the treatment of anemia of end-stage renal disease. *Am J Kidney Dis.* 1991 Jan;17(1):29-33.
  12. Grossmann M, Panagiotopoulos S, Sharpe K, MacIsaac RJ, Clarke S, Zajac JD, Jerums G, Thomas MC Low testosterone and anaemia in men with type 2 diabetes. *Clin Endocrinol (Oxf).* 2009 Apr;70(4):547-53. doi: 10.1111/j.1365-2265.2008.03357.x.
  13. Bhatia V, Chaudhuri A, Tomar R, Dhindsa S, Ghanim H, Dandona P Low testosterone and high C-reactive protein concentrations predict low hematocrit in type 2 diabetes. *Diabetes Care.* 2006 Oct;29(10):2289-94.
  14. Bachman E, Travison TG, Basaria S, Davda MN, Guo W, Li M, Connor Westfall J, Bae H, Gordeuk V, Bhasin S. Testosterone induces erythrocytosis via increased erythropoietin and suppressed hepcidin: evidence for a new erythropoietin/hemoglobin set point. *J Gerontol A Biol Sci Med Sci.* 2014 Jun;69(6):725-35. doi: 10.1093/geron/glt154.
  15. Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. *Clin Chim Acta.* 2003 Mar;329(1-2):9-22.
  16. T'Sjoen GG, Beguin Y, Feyen E, Rubens R, Kaufman JM, Gooren L. Influence of exogenous oestrogen or (anti-) androgen administration on soluble transferrin receptor in human plasma. *J Endocrinol.* 2005 Jul;186(1):61-7.
  17. Daughaday WH, Rotwein P. Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. *Endocr Rev.* 1989 Feb;10(1):68-91.
  18. Chahal HS, Drake WM. The endocrine system and ageing. *J Pathol.* 2007 Jan;211(2):173-80.
  19. Moromisato DY, Roberts C Jr, Brasel JA, Mohan S, Cowles E, King SM, Cooper DM. Erythrocyte insulin-like growth factor-L binding in younger and older males. *Clin Endocrinol (Oxf).* 1998 Mar;48(3):339-45.
  20. Sohmiya M, Kato Y. Effect of long-term administration of recombinant human growth hormone (rhGH) on plasma erythropoietin (EPO) and haemoglobin levels in anaemic patients with adult GH deficiency. *Clin Endocrinol (Oxf).* 2001 Dec;55(6):749-54.
  21. Vihervuori E, Virtanen M, Koistinen H, Koistinen R, Seppälä M, Siimes MA. Hemoglobin level is linked to growth hormone-dependent proteins in short children. *Blood.* 1996 Mar 1;87(5):2075-81.
  22. Nelson AE, Howe CJ, Nguyen TV, Seibel MJ, Baxter RC, Handelsman DJ, Kazlauskas R, Ho KK. Erythropoietin administration does not influence the GH-IGF axis or makers of bone turnover in recreational athletes. *Clin Endocrinol (Oxf).* 2005 Sep;63(3):305-9.
  23. Hero M, Wickman S, Hanhijärvi R, Siimes MA, Dunkel L. Pubertal upregulation of erythropoiesis in boys is determined primarily by androgen. *J Pediatr.* 2005 Feb;146(2):245-52.
  24. Kim SW, Hwang JH, Cheon JM, Park NS, Park SE, Park SJ, Yun HJ, Kim S, Jo DY. 2005 Direct and indirect effects of androgens on hematopoietic cells in vitro. *J Korean Med Sci* 20:409-416
  25. Bachman E, Feng R, Travison T, Li M, Olbina G, Ostland V, Ulloor J, Zhang A, Basaria S, Ganz T, Westerman M, Bhasin S. Testosterone suppresses hepcidin in men: a potential mechanism for testosterone-induced erythrocytosis. *J Clin Endocrinol Metab.* 2010 Oct;95(10):4743-7. doi: 10.1210/jc.2010-0864.
  26. Kim SW, Hwang JH, Cheon JM, Park NS, Park SE, Park SJ, Yun HJ, Kim S, Jo DY. Direct and indirect effects of androgens on survival of hematopoietic progenitor cells in vitro. *J Korean Med Sci.* 2005 Jun;20(3):409-16.
  27. Calado RT<sup>1</sup>, Yewdell WT, Wilkerson KL, Regal JA, Kajigaya S, Stratakis CA, Young NS. Sex hormones, acting on the TERT gene, increase telomerase activity in human primary hematopoietic cells. *Blood.* 2009 Sep 10;114(11):2236-43. doi: 10.1182/blood-2008-09-178871. Epub 2009 Jun 26.
  28. Rochira V, Zirilli L, Madeo B, Maffei L, Carani C Testosterone action on erythropoiesis does not require its aromatization to estrogen: Insights from the testosterone and estrogen treatment of two aromatase-deficient men. *J Steroid Biochem Mol Biol.* 2009 Feb;113(3-5):189-94. doi: 10.1016/j.jsbmb.2008.12.007. Epub 2008.