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Testosterone action on erythropoiesis does not require its aromatization to estrogen: Insights from the testosterone and estrogen treatment of two aromatase-deficient men☆

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ABSTRACT

Androgens act on erythropoiesis, but the relative role of testosterone (T) and estradiol (E_2) on erythropoietic parameters in men is a poorly investigated issue. In order to evaluate separately the effects on erythropoiesis of high-dose T administration alone and of physiological dose of E_2 administration alone two adult men with aromatase deficiency were assessed before and during each treatment. Blood cell count, hemoglobin (Hb), hematocrit (Hct), erythrocyte mean cell volume (MCV), erythrocyte mean corpuscular hemoglobin (MCH), erythrocyte mean corpuscular hemoglobin concentration (MCHC), serum ferritin, iron and total iron-binding capacity (TIBC), serum erythropoietin, serum total testosterone and estradiol were evaluated. Hb, Hct and red cell count rose during testosterone treatment, consistently with the increase in circulating testosterone, but failed to increase during estradiol treatment. A decrease in Hb, Hct and red cell count was recorded in one of the two subjects during estradiol treatment, with a concomitant decrease in serum testosterone. Circulating T alone is capable of and sufficient to influence erythropoiesis, especially at supraphysiological dosage, while circulating E_2 have not the same effect on erythropoietic parameters, suggesting the hypothesis that the erythropoietic changes induced by androgens are not mediated via its aromatization to estrogens.

1. Introduction

The effects of androgens on erythropoiesis have been well understood since 1940s [1,2] and androgens were the main pharmacologic agents used for erythropoietic stimulation before the availability of recombinant hematopoietic growth factors [3]. On the other hand, estrogens action on erythropoiesis has not received the same attention, leaving a lack of knowledge about the respective role of each sex steroid on the erythropoietic parameters.

Androgen action on erythropoiesis [4] is highlighted by the gender differences in blood hemoglobin [5–7] and by the changes in circulating hemoglobin that occur only in boys at puberty [8,9]. Consistently, hemoglobin concentration (Hb) and hematocrit (Hct) are higher in men than in women [5–7], with this gender difference absent before puberty. In particular, pubertal increase of Hb occurs only in boys at puberty, with an approx five-month delay with respect to the increase in circulating testosterone levels [8,9]. This gender differences in erythropoiesis have been traditionally ascribed to the amount of androgens that is greater in men than in women, but they could be also related to menstrual blood loss in women [10], even though evidence on this latter mechanism are controversial [11]. Furthermore, the androgen deprivation in men reduces erythropoiesis [12–16] and hypogonadism is frequently associated with reduced Hct [17]. Both conditions are reversible with androgen replacement treatment [18] or by removing androgen deprivation [16,19]. Finally, age-related anemia is at least partially due to the decline in circulating androgens levels in older men [20] and polycythemia represents an undesired risk for testosterone replacement treatment that needs monitoring [21]. All these findings suggest that sex hormones regulate erythropoiesis, that androgens may trigger pubertal up-regulation of erythropoiesis in boys [22] and that they may play a role in the maintenance of Hb levels typical of adult males [4,18].

Although the mechanisms by which androgens exert their influence on erythropoiesis have not been completely clarified yet, it seems that testosterone may act directly on bone marrow stem cells [3,4,16,23–25] rather than indirectly by increasing erythropoietin

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(Epo) [26], the latter being a potent and effective agent for red cells synthesis stimulation [27,28].

The possibility that androgens may act on erythrocytosis via their conversion to estrogens, as it happens in other biological actions in men [29,30], is still a poorly investigated issue. Nevertheless, gender differences in erythropoiesis at puberty [8,9,22] and during adulthood [4,7], and the erythropoietic effects of non-aromatizable androgens [31], all indirectly argue against this hypothesis. Even several pathophysiological models suggest that androgens directly modulate erythropoiesis and that their conversion to estrogens does not play a role in this biological function. Consistently, androgen excess in women leads to a red cell count higher than normal, such as in females with congenital adrenal hyperplasia [32] or those receiving large doses of androgens [33]. On the other hand, a possible effect of estrogen on erythropoiesis has been postulated on the basis of the results of several studies. Recently, high doses of estrogen administered to male-to-female transsexuals resulted in a decrease of both Hct and Hb concentrations, but the concomitant administration of an antiandrogen drug did not permit to establish if the reduction of erythropoiesis was due to the direct action of estrogen or if it was related to androgen deficiency or both [34]. The most important evidence that rose the hypothesis of a possible role of estrogens in male erythropoiesis comes from a recent study showing a direct association between hematocrit and serum estradiol in men treated with testosterone [35]. A direct action of estrogen on erythropoiesis was previously shown also in women [36] as well as in animal studies [37]. However, all these studies suffer either from the presence of an intact aromatase enzyme, meaning that exogenous androgens may still be converted into estrogens in women, or from an intact hypothalamic feedback, causing exogenous estrogens to lead to a concomitant but not complete androgen suppression in men. Thus, the relative effect of each sex steroid on erythropoiesis remains an as yet unsolved issue in men. Thus, to check if androgens may act on erythropoiesis via their conversion to estrogen is of importance on a physiological viewpoint, especially if recent data on this issue are considered [34,35].

The aim of this study was to evaluate separately the effects of supraphysiological testosterone treatment and those of physiological estradiol treatment on some parameters of erythropoiesis in two adult men with aromatase deficiency. This investigation model is complementary to the study protocols in which estrogen suppression obtained by using aromatase inhibitors has been limited by the potency of the drugs administered [22,38]. Indeed, human congenital complete aromatase deficiency is a more useful model for testing separately the androgen and estrogens effects [30,39,40], since in these patients the conversion pathway of androgen to estrogen is not functioning.

2. Materials and methods

The study was carried out on two male subjects affected by aromatase deficiency; their detailed clinical and genetic studies have already been published [39,40]. The subject here referred to as Subject 1 is the aromatase-deficient man described by Carani et al. in 1997 [39,41], while Subject 2 is the aromatase-deficient man described by Maffei et al. in 2004 [40], who was also affected by concomitant mild hypogonadism [40,41]. Both subjects were treated with supraphysiological doses of testosterone at the beginning of their clinical management, and thereafter, once estrogen deficiency was diagnosed, transdermal estradiol treatment was started.

The effects of a supraphysiological dose of testosterone administration alone [testosterone enanthate 250 mg i.m. every 10 days (Subject 1) and every 15 days (Subject 2) lasting at least 6 months), and a physiological dose of estradiol administration alone (transdermal estradiol 25 μg twice weekly for 6 months) on erythropoiesis were evaluated before and during each treatment. Both testosterone treatment and estrogen treatment lasted a minimum of six months, and the period of washout between the two treatments was longer than twelve months. Testosterone treatment was performed until the diagnosis of aromatase deficiency had been made in order to attain bone maturation [39,40]. The study design is summarized in detail in Fig. 1 and in particular it is illustrated the timing of biochemical evaluation before and after at least six months of each treatment (Fig. 1).

2.1. Biochemical analyses

The routine laboratory investigations included a complete blood count (erythrocyte, leukocyte and platelet count) hemoglobin, hematocrit, erythrocyte mean cell volume (MCV), erythrocyte mean corpuscular hemoglobin (MCH), erythrocyte mean corpuscular hemoglobin concentration (MCHC), serum ferritin, iron and total iron-binding capacity (TIBC). The following parameters were also subsequently evaluated on stored samples: serum Epo, serum total testosterone and serum estradiol. All these parameters were evaluated during each phase of the study. The study was performed, for Subject 1, at the Endocrine Unit of the University of Modena and Reggio Emilia, and for, Subject 2, at the Consultorios Asociados de Endocrinologia of Buenos Aires, Argentina. Serum samples of both men were stored separately, but serum measurements of Epo, testosterone and estradiol performed on stored samples were subsequently all assayed at the Endocrine Unit of the University of Modena and Reggio Emilia. Blood count and the other hematological parameters except Epo were performed at the time of blood collection and the data were obtained from the patient's record charts.

Blood samples for the analysis of both blood and serum were collected after an overnight fast, at 08:00 h, by venipuncture of the antecubital vein. Serum samples obtained by centrifugation were stored at −80 °C, until assayed. Blood sample was collected at the second week of the 6th month of therapy for both treatments and after 7 days from the administration of testosterone enanthate during testosterone treatment.

Hematologic parameters (Hb, Hct, MCV, MCH, MCHC, and blood cell count) were analyzed using an automated cell counter [Cell-Dyn 3500 (Abbott Laboratories, Abbott Park, IL, USA)] [42,43].

Serum ferritin, iron and TIBC were measured by commercially available kits.

Serum Epo was quantified using an immunochemiluminometric assay on an IMMULITE analyzer (DPC, Los Angeles, Calif). The detection limit of the assay is 0.2 U/L. The within-run coefficient of variation (CV) was <9% and the total CV <9% in the concentration ranged from 7 to 148 U/L.

Serum total testosterone was measured using commercial RIA (Diagnostic product corp., Los Angeles, CA). The inter-assay and the intra-assay coefficients of variation for testosterone were 11% and 5% respectively. The cross-reactivity with 5α-dihydrotestosterone was 2.8%; antibody cross-reactivity against less potent androgens (androstenedione, 3β-androstanediol, dehidroepiandrosterone) and other possible interfering steroids was less than 1%.

Serum estradiol levels were determined using a commercially available double antibody RIA (Third-Generation DSL-39100, Diagnostic Systems Laboratories, Inc., Webster, TX) with a sensitivity of 0.6 pg/mL (2.2 pmol/L), the lowest standard at 1.5 pg/mL (5.5 pmol/L), linearity to 150 pg/mL (550 pmol/L), and an ED50 of 20 pg/mL (73 pmol/L). The cross-reactivity with estrone and with less potent estrogens was less than 7 and 0.45%, respectively. The inter-assay and the intra-assay coefficients of variation for estradiol were 4.1–9.9 and 3.4–3.9%, respectively.

Both subjects gave written and informed consent for both treatments and for the publication of the data.

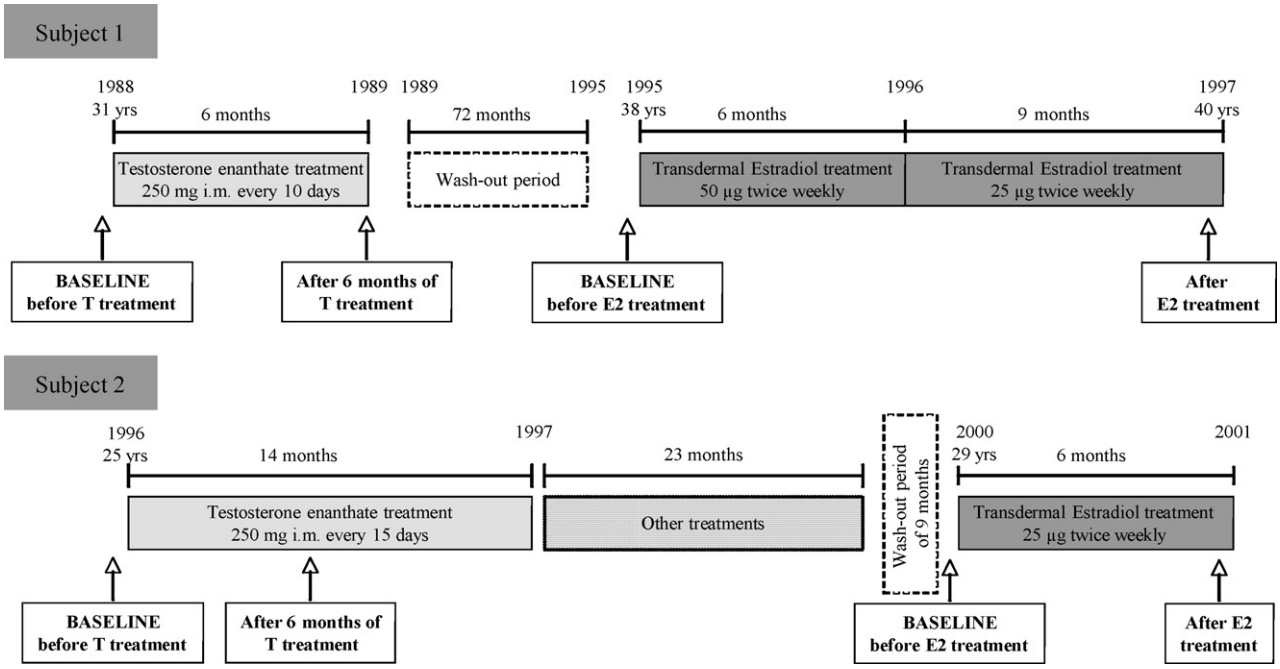

Fig. 1. Study design of the two subjects and treatments schedules according with the clinical time course. The timing of biochemical evaluation before and after at least six months of each treatment is indicated by the arrows. T: testosterone; E2: estradiol.

3. Results

3.1. Sex steroids levels

Serum testosterone rose during testosterone treatment in both subjects, reaching values above the normal range (Table 1). Serum testosterone during transdermal estradiol treatment fell below the normal range in Subject 2, remaining unchanged in Subject 1 (Table 1) [39,40,42].

Serum estradiol was within the normal range only during estradiol treatment, while being undetectable before testosterone and estradiol treatment as well as during testosterone treatment (Table 1) [39,40,42].

3.2. Erythropoiesis parameters

In both subjects Hb, Hct and red cell count increased during testosterone treatment (Table 1). During estradiol treatment, Hb, Hct and red cell count remained unchanged in Subject 1, while decreased slightly in Subject 2 (Table 1).

Serum iron and ferritin decreased during testosterone treatment in both subjects, while no change occurred during transdermal estradiol treatment.

MCV, MCH, MCHC, leukocyte and platelet count, TIBC and Epo did not change throughout the entire protocol.

4. Discussion

Although based on two subjects, the investigation model here illustrated allows a detailed characterization of the action of each sex steroid on erythropoiesis in men, since in these patients the conversion pathway of androgen to estrogen is not functioning.

Studies based on different but complementary settings have shown that restoring normal circulating testosterone and decreasing estradiol in elderly men by means of aromatase inhibitors changed neither hematocrit nor hemoglobin [38]. Furthermore, in adolescent boys, even in the presence of a reduction in circulating estrogens, the androgen-dependent stimulation of erythropoiesis may be achieved by the concomitant administration of both testosterone and an aromatase inhibitor [22]. Recently, a study of the changes in erythropoietic parameters in male-to-female transsexuals treated with high doses of estrogens, provided further indirect evidence of the absence of estrogen effects on erythropoiesis [34]. T'Sjoen and colleagues have shown that high-dose estrogen therapy combined with antiandrogen treatment leads to a decrease in Hb and Hct levels, probably because the therapy induces a very significant reduction in serum testosterone in male-to-female transsexuals [34]. Data from the present setting did not confirm neither a possible relationship between serum estradiol and hematocrit in men [35], nor a direct suppressive modulation of erythropoiesis by estrogens, as it has been previously suggested in women [36,37].

Since we used a human model of congenital estrogen deficiency characterized by the lack of both circulating and locally produced estrogens, this study does not suffer from the limits due either to the potency of the aromatase inhibitors used for distinguish androgen from estrogen actions [22,38], or to the concomitant presence of a functioning aromatase enzyme able to convert part of the androgens administered [18,19], or to incomplete hypothalamic–pituitary and androgen suppression during estrogen administration to male-to-female transsexuals [34].

This study demonstrates that aromatization of testosterone to estradiol is not required in order to stimulate erythropoiesis in adult men. Indeed, treatment with a supraphysiological amount of testosterone alone led to a marked increase in red blood count, Hct and Hb, while the restoration of normal circulating estrogens by means of transdermal estradiol treatment alone did not stimulate erythropoiesis in these two aromatase-deficient men. Furthermore, transdermal estradiol treatment resulted in a decrease in red blood count, Hct and Hb in Subject 2, in all likelihood as a consequence of the concomitant fall in serum testosterone. Also, the lack of changes in erythropoietic parameters during transdermal estradiol treatment alone in these two aromatase-deficient men confirms that in adult men androgens act directly on erythropoiesis ensuring thus adequate Hb and Hct levels. Therefore, these findings are consistent with the results previously established merely on the basis

Table 1
Biochemical and hormonal parameters of two aromatase-deficient men before and during testosterone (T) treatment at a supraphysiological dosage (testosterone enanthate 250 mg i.m. every 2 weeks), and before and during transdermal estradiol (E_2) replacement treatment (25 μg twice weekly). Both testosterone treatment and estrogen treatment lasted a minimum of six months, and the period of washout between the two treatments was longer than twelve months. All data are expressed in Conventional Units; SI values are in parentheses.

Parameter	Normal range	Subject	Before T	During T	Washout period (>12 months)	
					Before E_2	During E_2
LH mIU/mL (IU/L)	3.7–15.2 (*3.7–15.2*)	Subject 1	8.9 (*8.9*)*	7.3 (*7.3*)*	5.6 (*5.6*)#	3.1 (*3.1*)#
		Subject 2	3.7 (*3.7*)§	n.a.	7 (*7*)§	5.9 (*5.9*)§
FSH mIU/mL (IU/L)	3.7–15.2 (*3.7–15.2*)	Subject 1	13.6 (*13.6*)*	11 (*11*)*	17.1 (*17.1*)#	8.1 (*8.1*)#
		Subject 2	23 (*23*)§	n.a.	20 (*20*)§	10.5 (*10.5*)§
Serum Testosterone ng/mL (nmol/L)	3–10 (*10–35*)	Subject 1	3.9 (*13.5*)*	11.86 (*41*)*	5.2 (*18*)#	4.2 (*14.5*)#
		Subject 2	3.8 (*13.1*)§	9.1 (*31.5*)§	2.7 (*9.3*)§#	1.45 (*5*)§#
Serum Estradiol pg/mL (pmol/L)	10–40 (*36–146*)	Subject 1	<1.5 (*<5.5*)*	<1.5 (*<5.5*)*	<1.5 (*<5.5*)#	24 (*88*)#
		Subject 2	<1.5 (*<5.5*)§	<1.5 (*<5.5*)§	<1.5 (*<5.5*)§#	23.5 (*86*)§#
Blood Hemoglobin g/dL (g/L)	14–17.5 (*140–175*)	Subject 1	14.2 (*142*)	16.3 (*163*)	14.5 (*145*)	14.7 (*147*)
		Subject 2	14.8 (*148*)	16.9 (*169*)	14.5 (*145*)	13.9 (*139*)
Hematocrit % (proportion of 1.0)	42–54 (*0.42–0.54*)	Subject 1	40.5 (*0.405*)	44.9 (*0.449*)	40.7 (*0.407*)	40.9 (*0.409*)
		Subject 2	42.0 (*0.420*)	49.2 (*0.492*)	42.9 (*0.492*)	39.8 (*0.398*)
MCV fL (μ^3)	82–98 (*82–98*)	Subject 1	84 (*84*)	90 (*90*)	86.2 (*86.2*)	87.2 (*87.2*)
		Subject 2	88 (*88*)	89 (*89*)	93.0 (*93.0*)	90 (*90*)
MCH pg (μμg)	27–32 (*27–32*)	Subject 1	28 (*28*)	28.5 (*28.5*)	30.7 (*30.7*)	31.3 (*31.3*)
		Subject 2	31 (*31*)	31.2 (*31.2*)	31.4 (*31.4*)	30.6 (*30.6*)
MCHC g/dL (g/L)	32–38 (*320–380*)	Subject 1	33.4 (*334*)	31.7 (*317*)	35.6 (*356*)	35.9 (*359*)
		Subject 2	35.2 (*352*)	35.0 (*350*)	33.8 (*338*)	34.1 (*341*)
Red Blood Cell Count $\times 10^6/\mu L$ ($x10^{12}/L$)	4.5–6 (*4.5–6*)	Subject 1	4.07 (*4.07*)	5.01 (*5.01*)	4.72 (*4.72*)	4.69 (*4.69*)
		Subject 2	4.78 (*4.78*)	5.2 (*5.2*)	4.61 (*4.61*)	4.1 (*4.1*)
Blood Leukocyte Count $\times 10^3/\mu L$ ($x10^9/L$)	4–10 (*0.004–0.01*)	Subject 1	5.8 (*0.0058*)	7.5 (*0.0075*)	5.7 (*0.0057*)	6.3 (*0.0063*)
		Subject 2	8.6 (*0.0086*)	8.0 (*0.008*)	9.0 (*0.009*)	8.8 (*0.0088*)
Blood Platelet Count $\times 10^3/\mu L$ ($x10^9/L$)	150–400 (*150–400*)	Subject 1	243 (*243*)	298 (*298*)	237 (*237*)	234 (*234*)
		Subject 2	239 (*239*)	236 (*236*)	241 (*241*)	246 (*246*)
Serum Iron μg/dL (μmol/L)	60–160 (*11–29*)	Subject 1	122 (*22*)	76 (*13.6*)	66 (*12*)	89 (*16*)
		Subject 2	110 (*20*)	81 (*14.5*)	78 (*14*)	80 (*14.3*)
Serum Ferritin ng/mL (pmol/L)	25–495 (*56–1112*)	Subject 1	312 (*701*)	263 (*590*)	279 (*626*)	285 (*640*)
		Subject 2	315 (*707*)	278 (*624*)	290 (*651*)	301 (*676*)
TIBC μg/dL (μmol/L)	250–420 (*45–75*)	Subject 1	293 (*52*)	311 (*55*)	380 (*68*)	376 (*58*)
		Subject 2	332 (*59*)	354 (*63*)	365 (*65*)	342 (*61*)
Serum Erythropoietin mIU/mL (IU/L)	3.7–15.2 (*3.7–15.2*)	Subject 1	13.6 (*13.6*)	14.9 (*14.9*)	13.9 (*13.9*)	14.2 (*14.2*)
		Subject 2	11.5 (*11.5*)	12.8 (*12.8*)	11.2 (*11.2*)	11.8 (*11.8*)
Total Cholesterol mmol/L (mg/dL)	3.5–5 (*140–200*)	Subject 1	6.9 (*279*)*	6.2 (*248*)*	7.6 (*306*)*	3.9 (*158*)
		Subject 2	4.5 (180)§	4.9 (*199*)§	4.4 (*177*)§	4.5 (*182*)§
HDL-Cholesterol mmol/L (mg/dL)	>1.1 (*>45*)	Subject 1	1.05 (*42*)*	0.7 (*29*)*	1.07 (*43*)*	1.3 (*53*)
		Subject 2	n.a.	n.a.	0.7 (*31*)§	0.9 (*37*)§
Tryglicerides mmol/L (mg/dL)	<1.9 (<175)	Subject 1	2.8 (*257*)*	3.1 (*287*)*	3.3 (*305*)*	1.3 (*119*)
		Subject 2	n.a.	n.a.	2.1 (*199*)§	1.6 (*148*)§

Hb: hemoglobin; MCV: erythrocyte mean corpuscular volume; MCH: erythrocyte mean corpuscular hemoglobin; MCHC: erythrocyte mean corpuscular hemoglobin concentration; TIBC: total iron-binding capacity; n.a.: not available.
Data already published by *Carani et al. (1997), §Maffei et al. (2004), and #Rochira et al. (2006).

of indirect evidence, provided by gender differences in erythropoiesis [4–7], which become manifest at puberty in boys [8,9,22,25], and by the preservation of the erythropoietic effects by molecules, such as dihydrotestosterone (DHT) having a purely androgenic action [31]. As expected, the effect on erythropoiesis was so evident in this setting since supraphysiological doses of testosterone were used, the effect of testosterone being dose-dependent [26], as reinforced by the restoration of normal parameters of erythropoiesis after the cessation of testosterone treatment in these two patients.

The lack of change in Epo levels during testosterone treatment suggests once again that androgens probably act directly on erythropoiesis and the hypothesis of an action on bone marrow cells through their interaction with a nuclear androgen receptor [44] remains plausible. The direct action of androgen on erythropoiesis is also supported by the absence of differences in serum Epo levels between men and women [7], between hypogonadal and normal men [16], and between testosterone treated and untreated men [23,24]. Consistently, the hematopoietic effect of androgen in patients with chronic renal failure has been supposed to be independent from related changes in serum Epo levels, the latter being considered as an epiphenomenon [45], and a recent study did not show any difference in Epo by using graded doses of testosterone in healthy young and older men [26].

In conclusion, even though not novel, these findings lead to a better understanding of the physiology of androgen action and the underlying pathophysiological and clinical implications related to both androgen excess and androgen deficiency. Moreover, they

improve our knowledge of the respective roles of androgens and estrogens, showing once more that both are essential for a wide spectrum of physiological actions, but that androgens, not estrogens, can stimulate directly erythropoiesis in men. Testosterone and its metabolite DHT do not have higher affinity for the estrogen receptors, but evidence suggests that some testosterone metabolites, such as 3α-diol and 3β-diol, may exert their action through the binding to the estrogen receptors [46–47]. Whether or not these mechanisms of androgen actions operate in vivo in humans remains still to be elucidated. Thus, the possibility that androgens may influence several biological functions, as hematopoiesis, through the interaction between androgens metabolites and the estrogen receptors remains a challenging issue.

Finally, improving our knowledge on the specific effects of each sex steroid may be of help for the development of drugs with a precise and restricted targeting in order to avoid undesired effects, as it is advisable for compound with androgenic properties and positive effect on bone, lipids, etc., but that does not affect erythropoiesis [48].

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References

[1] E.P. McCullagh, T.R. Jones, A note on the effect of certain androgens upon the red blood cell count and upon the glucose tolerance, Cleveland Clin. Quart. 8 (1941) 79.
[2] P. Steinglass, A.S. Gordon, H.A. Charripper, Effect of castration and sex hormones on blood of rat, Proc. Soc. Exp. Biol. Med. 48 (1941) 169.
[3] N.T. Shahidi, Androgens and erythropoiesis, N. Engl. J. Med. 289 (1973) 72–80.
[4] A.D. Mooradian, J.E. Morley, S.G. Korenman, Biological actions of androgens, Endocr. Rev. 8 (1987) 1–28.
[5] S.M. Garn, A.S. Ryan, S. Abraham, G. Owen, Suggested sex and age appropriate values for "low" and "deficient" hemoglobin levels, Am. J. Clin. Nutr. 34 (1981) 1648–1651.
[6] V.E. Loy, F.K. Morris, R.F. Schilling, L.L. Schloesser, K.M. Strutz, Hemoglobin concentrations as determined by a methemoglobin method, Am. J. Clin. Pathol. 26 (1956) 1450–1455.
[7] M.E. Miller, M. Chandra, J.F. Garcia, Clinical applications of measurement of serum immunoreactive levels of erythropoietin, Ann. N. Y. Acad. Sci. 459 (1985) 375–381.
[8] W.A. Daniel Jr., Hematocrit: maturity relationship in adolescence, Pediatrics 52 (1973) 388–394.
[9] F.H. Gardner, E.C. Besa, Physiologic mechanisms and the hematopoietic effects of the androstanes and their derivatives, Curr. Top. Hematol. 4 (1983) 123–195.
[10] S.K. Cole, A.M. Thomson, W.Z. Billewicz, A.E. Black, Haematological characteristics and menstrual blood losses, J. Obstet. Gynaecol. Br. Commonw. 79 (1972) 994–1001.
[11] J.T. Reeves, S. Zamudio, T.E. Dahms, I. Asmus, B. Braun, G.E. Butterfield, R.G. McCullough, S.R. Muza, P.B. Rock, L.G. Moore, Erythropoiesis in women during 11 days at 4,300 m is not affected by menstrual cycle phase, J. Appl. Physiol. 91 (2001) 2579–2586.
[12] S.O. Asbell, S.A. Leon, W.J. Tester, H.D. Brereton, C.T. Ago, M. Rotman, Development of anemia and recovery in prostate cancer patients treated with combined androgen blockade and radiotherapy, Prostate 29 (1996) 243–248.
[13] S. Chander, R. Choo, C. Danjoux, G. Morton, A. Pearse, G. Deboer, E. Szumacher, A. Loblaw, P. Cheung, T. Woo, Effect of androgen suppression on hemoglobin in prostate cancer patients undergoing salvage radiotherapy plus 2-year buserelin acetate for rising PSA after surgery, Int. J. Radiat. Oncol. Biol. Phys. 62 (2005) 719–724.
[14] L.M. Eri, K.J. Tveter, Safety, side effects and patient acceptance of the luteinizing hormone releasing hormone agonist leuprolide in treatment of benign prostatic hyperplasia, J. Urol. 152 (1994) 448–452.
[15] R. Fonseca, S.v. Rajkumar, W.L. White, A. Tefferi, H.C. Hoagland, Anemia after orchiectomy, Am. J. Hematol. 59 (1998) 230–233.
[16] J.P. Weber, P.C. Walsh, C.A. Peters, J.L. Spivak, Effect of reversible androgen deprivation on hemoglobin and serum immunoreactive erythropoietin in men, Am. J. Hemat. 36 (1991) 190–194.
[17] J.B. Hamilton, L.D. Bunch, G.E. Mestler, R. Imagawa, Effect of castration in man upon blood sedimentation rate, hematocrit and hemoglobin, J. Clin. Endocrinol. Metab. 24 (1964) 506–511.
[18] P.J. Snyder, H. Peachey, J.A. Berlin, P. Hannoush, G. Haddad, A. Dlewati, J. Santanna, L. Loh, D.A. Lenrow, J.H. Holmes, S.C. Kapoor, L.E. Atkinson, B.L. Strom, Effects of testosterone replacement in hypogonadal men, J. Clin. Endocrinol. Metab. 85 (2000) 2670–2677.
[19] S. Bhasin, L. Woodhouse, R. Casaburi, A.B. Singh, D. Bhasin, N. Berman, X. Chen, K.E. Yarasheski, L. Magliano, C. Dzekov, J. Dzekov, R. Bross, J. Phillips, I. Sinha-Hikim, R. Shen, T.W. Storer, Testosterone dose–response relationships in healthy young men, Am. J. Physiol. Endocrinol. Metab. 281 (2001) E1172–E1181.
[20] L. Ferrucci, M. Maggio, S. Bandinelli, S. Basaria, F. Lauretani, A. Ble, G. Valenti, W.B. Ershler, J.M. Guralnik, D.L. Longo, Low testosterone levels and the risk of anemia in older men and women, Arch. Intern. Med. 166 (2006) 1380–1388.
[21] E.L. Rhoden, A. Morgentaler, Risks of testosterone-replacement therapy and recommendations for monitoring, N. Engl. J. Med. 350 (2004) 482–492.
[22] M. Hero, S. Wickman, R. Hanhijärvi, M.A. Siimes, L. Dunkel, Pubertal upregulation of erythropoiesis in boys is determined primarily by androgen, J. Pediatr. 146 (2005) 245–252.
[23] R.D. Dickerman, R. Pertusi, N.Y. Zachariah, F. Schaller, Androgen-induced erythrocytosis, Am. J. Hematol. 59 (1998) 263–264.
[24] R.D. Dickerman, R. Pertusi, N.Y. Zachariah, F. Schaller, Androgen-induced erythrocytosis: is it erythropoietin? Am. J. Hematol. 61 (1999) 154–155.
[25] S. Krabbe, T. Christensen, J. Worm, C. Christiansen, I. Transbøl, Relationship between haemoglobin and serum testosterone in normal children and adolescents and in boys with delayed puberty, Acta Paediatr. Scand. 67 (1978) 655–658.
[26] A.D. Coviello, B. Kaplan, K.M. Lakshman, T. Chen, A.B. Singh, S. Bhasin, Effects of graded doses of testosterone on erythropoiesis in healthy young and older men, J. Clin. Endocrinol. Metab. (2007) (Epub ahead of print).
[27] R. Alexanian, Erythropoietin excretion in man following androgens, Blood 28 (1966) 1007–1009.
[28] N. Rishpon-Meyerstein, T. Kilbridge, J. Simone, W. Fried, The effect of testosterone on erythropoietin levels in anemic patients, Blood 31 (1968) 453–460.
[29] B.J. Deroo, K.S. Korach, Estrogen receptors and human disease, J. Clin. Invest. 116 (2006) 561–570.
[30] M. Faustini-Fustini, V. Rochira, C. Carani, Oestrogen deficiency in men: where are we today? Eur. J. Endocrinol. 140 (1999) 111–129.
[31] P. Kunelius, O. Lukkarinen, M.L. Hannuksela, O. Itkonen, J.S. Tapanainen, The effects of transdermal dihydrotestosterone in the aging male: a prospective, randomized, double blind study, J. Clin. Endocrinol. Metab. 87 (2002) 1467–1472.
[32] A.P. Gold, A.F. Michael Jr., Congenital adrenal hyperplasia associated with polycythemia, Pediatrics 23 (1959) 727–730.
[33] B.J. Kennedy, A.S. Gilbertsen, Increased erythropoiesis induced by androgenic-hormone therapy, N. Engl. J. Med. 256 (1957) 719–726.
[34] G.G. T'Sjoen, Y. Beguin, E. Feyen, R. Rubens, J.M. Kaufman, L. Gooren, Influence of exogenous oestrogen or (anti-) androgen administration on soluble transferrin receptor in human plasma, J. Endocrinol. 186 (2005) 61–67.
[35] A.S. Dobs, A.W. Meikle, S. Arver, S.W. Sanders, K.E. Caramelli, N.A. Mazer, Pharmacokinetics, efficacy, and safety of a permeation-enhanced testosterone transdermal system in comparison with bi-weekly injections of testosterone enanthate for the treatment of hypogonadal men, J. Clin. Endocrinol. Metab. 84 (1999) 3469–3478.
[36] E.A. Mirand, A.S. Gordon, Mechanism of estrogen action in erythropoiesis, Endocrinol. 78 (1965) 325–332.
[37] H. Horiguchi, E. Oguma, F. Kayama, The effects of iron deficiency on estradiol-induced suppression of erythropoietin induction in rats: implications of pregnancy-related anemia, Blood 106 (2005) 67–74.
[38] B.Z. Leder, J.L. Rohrer, S.D. Rubin, J. Gallo, C. Longcope, Effects of aromatase inhibition in elderly men with low or borderline-low serum testosterone levels, J. Clin. Endocrinol. Metab. 89 (2004) 1174–1180.
[39] C. Carani, K. Qin, M. Simoni, M. Faustini-Fustini, S. Serpente, J. Boyd, K.S. Korach, E.R. Simpson, Effect of testosterone and estradiol in a man with aromatase deficiency, N. Engl. J. Med. 337 (1997) 91–95.
[40] L. Maffei, Y. Murata, V. Rochira, G. Tubert, C. Aranda, M. Vazquez, C.D. Clyne, S. Davis, E.R. Simpson, C. Carani, Dysmetabolic syndrome in a man with a novel mutation of the aromatase gene: effects of testosterone, alendronate, and estradiol treatment, J. Clin. Endocrinol. Metab. 89 (2004) 61–70.
[41] V. Rochira, L. Zirilli, A.D. Genazzani, A. Balestrieri, C. Aranda, B. Fabre, P. Antunez, C. Diazzi, C. Carani, L. Maffei, Hypothalamic–pituitary–gonadal axis in two men with aromatase deficiency: evidence that circulating estrogens are required at the hypothalamic level for the integrity of gonadotropin negative feedback, Eur. J. Endocrinol. 155 (2006) 513–522.
[42] J.W. Williams, M.W. Morris, D.A. Nelson, Examination of the blood, in: E. Beutler, M.A. Lichtman, B.S. Coller, T.J. Kipps (Eds.), Williams Hematology, 5th ed., McGraw-Hill Inc., New York, 1995, pp. 8–15.
[43] J.W. Williams, M.W. Morris, D.A. Nelson, Automated blood cell counting, in: E. Beutler, M.A. Lichtman, B.S. Coller, T.J. Kipps (Eds.), Williams Hematology, 5th ed., McGraw-Hill Inc., New York, 1995, pp. L1–L3.
[44] M. Claustres, C. Sultan, Androgen and erythropoiesis: evidence for an androgen receptor in erythroblasts from human bone marrow cultures, Horm. Res. 29 (1988) 17–22.

[45] J.L. Teruel, R. Marcén, J.F. Navarro, J.J. Villafruela, M. Fernández Lucas, F. Liaño, J. Ortuño, Evolution of serum erythropoietin after androgen administration to hemodialysis patients: a prospective study, Neprhron 70 (1995) 282–286.
[46] R.J. Handa, T.R. Pak, A.E. Kudwa, T.D. Lund, L. Hinds, An alternate pathway for androgen regulation of brain function: activation of estrogen receptor beta by the metabolite of dihydrotestosterone, 5alpha-androstane-3beta, 17beta-diol, Horm. Behav. 53 (2008) 741–752.
[47] T.R. Pak, W.C. Chung, L.R. Hinds, R.J. Handa, Estrogen receptor-beta mediates dihydrotestosterone-induced stimulation of the arginine vasopressin promoter in neuronal cells, Endocrinology 48 (2007) 3371–3382.
[48] C. Wang, R.S. Swerdloff, Editorial: should the nonaromatizable androgen dihydrotestosterone be considered as an alternative to testosterone in the treatment of the andropause? J. Clin. Endocrinol. Metab. 87 (2002) 1462–1466.