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ORIGINAL RESEARCH

TRANSFUSION

Toxic masculinity in red blood cell units? Testosterone therapy in blood donors revisited

Kelsey Hazegh[1] | **Bradley D. Anawalt**[2] | **Larry J. Dumont**[1,3] | **Tamir Kanias**[1,3]

[1]Vitalant Research Institute, Denver, Colorado, USA

[2]Division of General Internal Medicine, University of Washington Medical Center, Seattle, Washington, USA

[3]Department of Pathology, University of Colorado Denver Anschutz Medical Campus, Aurora, Colorado, USA

Correspondence
Tamir Kanias, Vitalant Research Institute, 717 Yosemite St, Denver, CO 80230, USA.
Email: tkanias@vitalant.org

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Abstract

Background: FDA guidelines limit the use of blood from donors taking testosterone replacement therapy (TRT) to red blood cell (RBC) concentrates, whereas plasma and platelets are discarded. The purpose of this study is to bring awareness to above-average free testosterone concentrations in RBC units from TRT donors.

Study design: We quantified the concentrations of free (bioavailable; pg/ml) and total (protein bound and free; ng/dl) testosterone in plasma (frozen within 24 h) and supernatants from 42-day stored leukocyte-reduced RBC units from 17 TRT male donors and 17 matched controls (no TRT). Total testosterone concentrations were determined by liquid chromatography with tandem mass spectrometry (LC–MS/MS). Free testosterone concentrations were quantified in the same samples using equilibrium dialysis/LC–MS/MS.

Results: Plasmafree and total testosterone concentrations in TRT donors were 2.9 and 1.8 times higher than that of controls. Total testosterone concentrations in RBC supernatants were about 30% of that of plasma. In contrast, free testosterone concentrations in RBC supernatants were 80%–100% of that of plasma and were significantly ($p = .005$) higher in TRT compared with controls (252.3 ± 245.3 vs. 103.4 ± 88.2 pg/ml). Supraphysiological free testosterone concentrations (>244 pg/ml) in RBC supernatants were observed in five TRT donors and two control donors.

Conclusions: RBC units from TRT donors may contain supraphysiological concentrations of free testosterone. This may be resolved by avoiding blood collections soon after testosterone dosing and by enhanced screening of TRT donors. These data establish a rationale for new studies and reexamination of the current guidelines concerning the utilization of blood components from TRT donors.

KEYWORDS

blood donors, blood quality, red blood cells, testosterone

Abbreviations: FDA, U.S Food and Drug Administration; LR-RBC, leukocyte-reduced RBCs; LC-MS/MS, liquid chromatography with tandem mass spectrometry; ns, not statistically significant; RBC, Red blood cells; SHBG, sex hormone binding globulin; TRT, Testosterone replacement therapy.

1 | INTRODUCTION

The clinical use of sex hormones has been historically associated with younger women who may use contraceptive drugs for birth control. In recent years, a different type of sex hormone therapy has gained significant popularity among middle-aged men in the form of testosterone replacement therapy (TRT).[1] Soon after, concern has been raised regarding the abuse and health risks associated with exposure to exogenous testosterone.[2,3] The rise in TRT popularity has resulted in the appearance of two new populations of prospective blood donors: individuals who presented at blood centers for therapeutic phlebotomy due to testosterone-induced erythrocytosis, and new or returning allogeneic blood donors on testosterone therapy. In response to a growing number of prospective blood donors on prescription testosterone, the U.S Food and Drug Administration (FDA) and the American Association of Blood Banks (AABB) developed policies and procedures to address the safety of blood components collected through therapeutic phlebotomy of individuals with testosterone-induced erythrocytosis. Minutes from a 2014 meeting recommended that only the red blood cells (RBCs) may be distributed for transfusion, whereas the plasma and platelets should be discarded. In addition, donation interval for such individuals may be shorter than 8 weeks.[4] This meeting also addressed the need for studies that will define the concentrations of plasma testosterone that would be acceptable for distribution.

We recently reported that 2.2% of leukocyte-reduced (LR)-RBC units in a regional division of a large blood service organization (Vitalant) were donated by individuals taking testosterone prescription, who were characterized by high prevalence of obesity, high-frequency blood donations (up to 29 in 24 months compared with 12 in non-TRT control donors), higher blood pressure, and increased hemoglobin concentrations as compared with matched control donors.[5] Our evaluations of the quality of stored RBCs from TRT donors (therapeutic and allogeneic blood donations) and matched controls suggested that TRT was associated with changes in RBC metabolism and predisposition to osmotic hemolysis.[6] Specifically, we observed significant changes in metabolic pathways related to oxidative stress, free fatty acids, and acyl-carnitines that could partially explain the mechanisms, by which testosterone modulates RBC susceptibility to hemolysis.[7] These observations have prompted us to determine the concentrations of testosterone in plasma and LR-RBC units from the same samples characterized in our aforementioned studies. We hypothesized that testosterone concentration in plasma from TRT donors would be higher than that of controls, and that most donor testosterone is removed during the production of LR-RBC units. To our surprise, we found that free testosterone (bioavailable form) concentrations in supernatants from 42-day stored RBCs were nearly the same as measured in plasma. Thus, the aim of this study is to bring awareness to this phenomenon that may prompt a reevaluation of current TRT donors' selection criteria and blood utilization, and to highlight the need for new studies that will determine the clinical implications of testosterone administered in blood components.

2 | MATERIALS AND METHODS

2.1 | Human subjects

This study was conducted under regulations applicable to all human subject research supported by federal agencies including institutional review board approval. The data were obtained from plasma and LR-RBC units collected from 17 middle age (50.7 ± 8.8 years) male individuals on testosterone therapy, 8 of whom were registered as allogeneic blood donors and the rest were recruited for this project. The type of testosterone medications (e.g., SottoPelle, Androgel, testosterone cypionate), dose, and routes of administrations (topical, subcutaneous pellets, injections) varied among those individuals. Control donors ($n = 17$) were matched to the TRT individuals for sex, age (51.8 ± 10.9 years, $p = .74$), and ethnicity. Whole blood was processed using standard methods to plasma and leukocyte-reduced RBCs on additive solution-3.

2.2 | Quantification of free and total testosterone

The majority (about 98%) of testosterone in the circulation is in its inactive form and is bound to androgen binding proteins, such as sex hormone binding globulin (SHBG), whereas free testosterone (about 2%) is the bioavailable form.[8] The concentrations of total (protein-bound) testosterone in plasma (frozen within 24 h after blood collection) and supernatants from 7 and 42 days old RBC units (collected after centrifugation of RBC concentrates at 1500× g, 10 min, 18°C) were determined by liquid chromatography with tandem mass spectrometry (LC–MS/MS). Free testosterone concentrations were quantified in the same samples using equilibrium dialysis/LC–MS/MS. All tests were performed in a clinical lab (University of Colorado Hospital). The reference ranges are 47–244 pg/ml for free testosterone and 300–890 ng/dl for total testosterone.

2.3 | Statistical analysis

Free and total testosterone values were not normally distributed as determined by Shapiro–Wilk test. Therefore, nonparametric two-tailed tests were used to test for differences in free and total testosterone concentrations between TRT and control donors (Mann–Whitney test) or between plasma and RBC supernatants (Wilcoxon paired test). A nonparametric ANOVA (Friedman test) tested for differences in free testosterone concentrations in plasma and RBCs stored for 7 and 42 days. Correlation analyses were conducted using Spearman's r tests. All data were analyzed using GraphPad Prism 9 (GraphPad Software, Inc, La Jolla, CA).

3 | RESULTS

3.1 | RBC units from TRT donors may contain supraphysiological concentrations of free testosterone

To support the study's hypothesis, we found higher concentrations of free and total testosterone in plasma collected from TRT individuals compared with matched controls. As summarized in Table 1, average plasmafree and total testosterone concentrations were about 2.9 and 1.8 times higher in TRT than in controls, respectively. Average plasmafree testosterone in TRT individuals (305.0 ± 320.7 pg/ml) was above the upper limit of the reference range (244 pg/ml). The large standard deviations observed in plasma free and total testosterone concentrations reflected the variation in testosterone levels among all individuals and in TRT in particular. In fact, 5 of the 17 tested TRT individuals (about 30%) had supraphysiological concentrations of free and total testosterone levels. In contrast, two individuals (about 12%) from the control group had above-average concentrations of plasma free and total testosterone.

Evaluation of free testosterone concentrations in supernatants from stored RBCs produced from the same whole blood bags suggested that average free testosterone in the TRT group was about 83% of that of plasma, whereas no change was observed in the control group. In addition, the average free testosterone concentration in RBC supernatants from TRT donors was about 2.5 times higher ($p = .005$) than that of controls (252.3 ± 245.3 pg/ml vs. 103.4 ± 88.2 pg/ml; Table 1). In comparison, lower free testosterone concentrations were measured in RBC supernatants from two female donors (5.4 and 8.1 pg/ml), suggesting that the concentrations observed in RBCs from male donors were not due to an assay artifact. Average total testosterone concentrations in RBC supernatants from all donors were about 30% of that of plasma, suggesting that the majority (about 70%) of total testosterone was removed during the manufacturing of RBC concentrates.

As shown in Figure 1A, the difference between plasma and RBC supernatant concentrations of free testosterone was larger in individuals with supraphysiological testosterone than those with reference range testosterone levels. Correlation analysis (Spearman's r) revealed significant ($p < .0001$) and strong ($r = .9832$) association between plasma and RBC supernatant free testosterone concentrations (Figure 1B). A decrease (51%–83%) in total testosterone concentrations between plasma and RBC supernatant was observed in all samples (Figure 1C). Similar to free testosterone, we observed a good correlation (Spearman's $r = .8655$, $p < .0001$) in total testosterone concentrations between plasma and RBC supernatant (Figure 1D). However, none of the total testosterone concentrations found in the RBC supernatants were above the reference range.

Considering these observations, we asked whether free testosterone concentrations in RBC supernatants vary throughout cold storage. To answer this question, we quantified the concentrations of free testosterone in 7-day-old stored RBCs in a subset of the donors (two TRT and two matched controls) and found that these concentrations (61.3 ± 23.1 pg/ml) were comparable, although somewhat lower ($p = .04$), to those measured at day 42 of storage (69.3 ± 25.7 pg/ml) or in plasma (66.9 ± 29.8 pg/ml) (Figure 2).

TABLE 1 Free and total testosterone concentrations in plasma bags and supernatants from 42-day-old leukocyte-reduced RBC units donated by male donors who received testosterone replacement therapy (TRT) or matched controls

			TRT		Control		
Testosterone	**Component**	**Reference range**	**Mean ± SD (range)**	***n***	**Mean ± SD (range)**	***n***	***p* (TRT vs. control)**
Free (pg/ml)	Plasma	47–244 pg/ml	305.0 ± 320.7 (37.3–1101)	17	104.4 ± 93.8 (42.5–412.3)	17	*.005
Free (pg/ml)	RBC supernatant	47–244 pg/ml	252.3 ± 245.3 (40.7–870)	17	103.4 ± 88.2 (45.0–365.4)	17	*.005
Total (ng/dl)	Plasma	300–890 ng/dl	886.2 ± 722.5 (91.3–2436)	17	484.5 ± 307.9 (157.2–1358)	17	.053
Total (ng/dl)	RBC supernatant	300–890 ng/dl	252.6 ± 228.0 (44.4–807.4)	17	141.6 ± 115.2 (41.4–462)	17	*.034

*Designates significant ($p < .05$; Mann–Whitney test) differences in testosterone concentrations between TRT and controls.

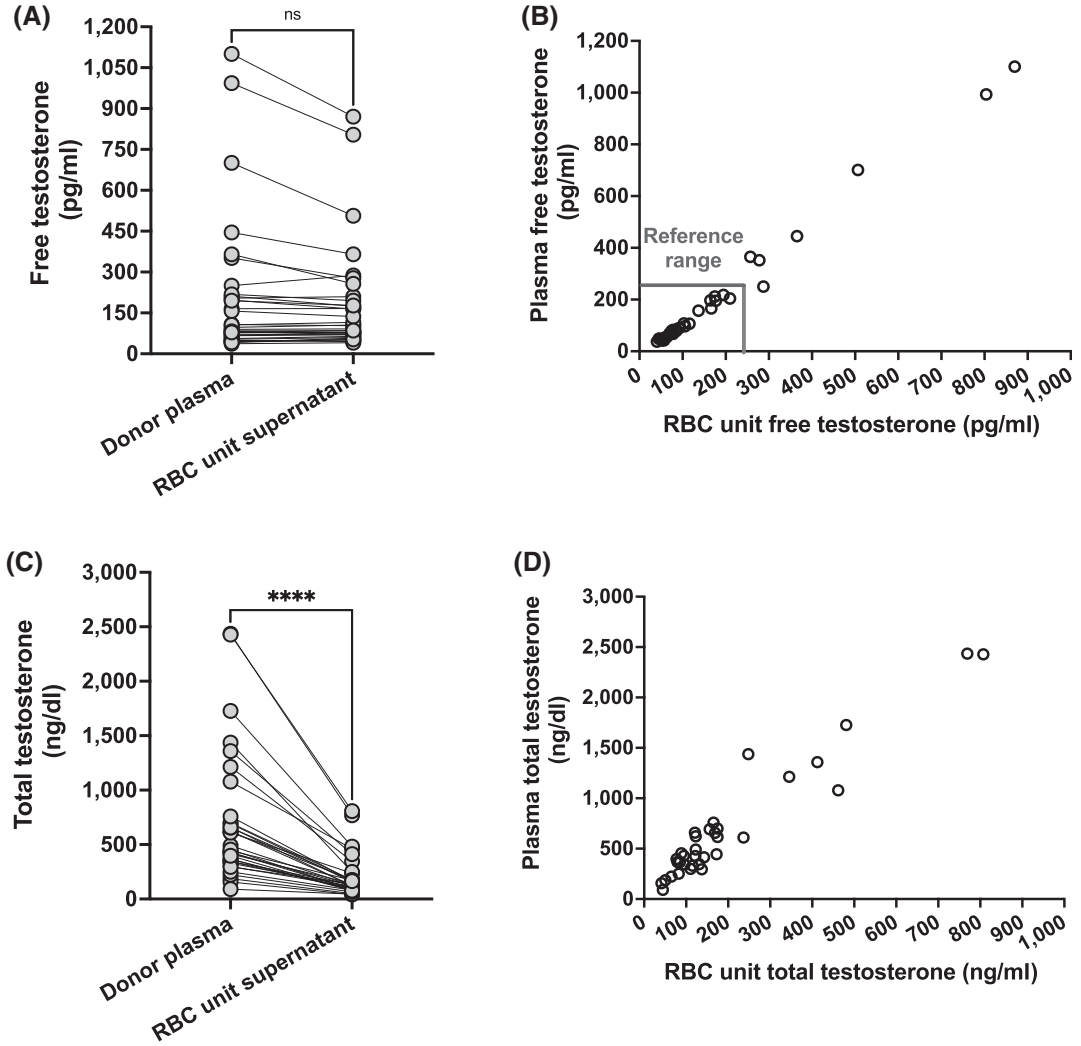

FIGURE 1 Comparison and correlation analyses of testosterone concentrations in plasma bags and supernatants from 42-day-old leukocyte-reduced RBC units. Data were derived from 34 male blood donors (17 on testosterone replacement therapy (TRT) and 17 matched controls). (A) Free testosterone concentrations (pg/ml) in donor plasma bags versus RBC unit supernatant ($p = .291$); ns, not significant. (B) Correlation (Spearman's r) between plasma and RBC supernatant free testosterone concentrations. $r = .9832$, $p < .0001$. Note that seven individuals (five TRT and two controls) had free testosterone concentrations above the reference range. (C) Total testosterone concentrations (ng/dl) in donor plasma bags versus RBC unit supernatant. ****$p < .0001$. (D) Correlation (Spearman's r) between plasma and RBC supernatant total testosterone concentrations. $r = .8655$, $p < .0001$

4 | DISCUSSION

4.1 | RBCs are possible carriers of free testosterone

The unanticipated presence of free testosterone in RBC unit supernatants is puzzling and raises questions about its distribution in whole blood. If the majority of free testosterone is localized in plasma (as opposed to blood cells), then the removal of plasma during the manufacturing of LR-RBC units would significantly reduce its concentration in RBC units as in the case of total testosterone. This assumption may have driven current blood banking policies to restrict blood utilization from TRT donors to LR-RBCs and to discard plasma and platelets. Furthermore, the low concentration of residual donor plasma (about 5%)[9] found in LR-RBC units cannot explain the high concentrations of free testosterone, which are similar to what we observed in the plasma bags. One explanation relies on the unique characteristic of RBCs to transport and deliver a variety of drug formulations including nanoparticles.[10] This phenomenon was given the term "red blood cell hitchhiking."[11] A study that evaluated the permeability of RBCs to radio-labeled sex hormones including testosterone suggested that RBCs may contribute to 5%–15% of sex hormone transport in the circulation.[12] Similar to other sex steroids, (free) testosterone is lipophilic in nature and capable of diffusing into target cells.[13] Thus, it is possible that the free testosterone

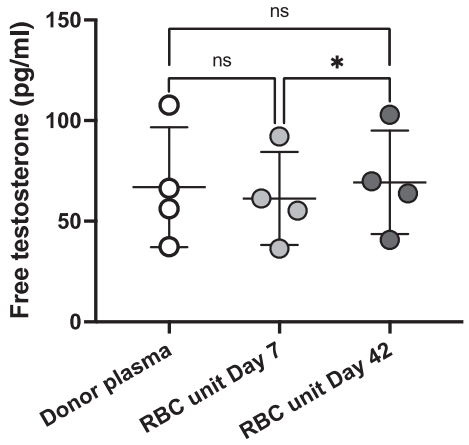

FIGURE 2 Comparison of free testosterone concentrations (pg/ml) in donor plasma and RBC supernatants collected on days 7 and 42 of cold storage. Data were derived from four male donors (two on testosterone replacement therapy and two matched controls). Each dot of the graph corresponds to data from an individual and mean ± standard deviation is reported. *$p = .04$; ns, not significant

concentrations found in our tested RBC units reflect a mechanism by which testosterone is transiently "hosted" in RBCs that may contribute to its transport to targeted tissues. In contrast, protein-bound testosterone is incapable of freely diffusing across cell membranes, which may explain why 70% of it were removed during the production of LR-RBC units. The remaining protein-bound testosterone may be present in the residual plasma or enclosed by RBCs. These, of course, are only assumptions that require further validation.

4.2 | Do supraphysiological concentrations of free testosterone in RBC units pose a risk to transfusion recipients?

There is no definitive answer to this question given the absence of controlled clinical trials that would determine the impact of supraphysiological testosterone in RBC units on transfusion outcomes. However, current knowledge suggests that the supraphysiological levels of free testosterone measured in our study are not likely to promote adverse events in most transfused patients. It is important to note that the estimated testosterone dose that might be given to a transfused patient is more critical than the concentration of free testosterone measured in a blood component. Assuming that free testosterone is equally distributed between RBCs and supernatant (worst case scenario) and a volume of 350 ml blood bag, the total dose in a bag with the highest free testosterone concentration measured (870 pg/ml) is about 0.3 μg. In comparison, testosterone prescriptions may be given orally (testosterone undecanoate) at 40 mg/day or via injections (testosterone cypionate) at 200 mg every 2 weeks.[14] Furthermore, the average daily testosterone production rate in women was estimated at 0.3 mg.[15] Circulating free testosterone is likely to be metabolized (glucuronated) rapidly in the liver into inactive metabolites that are excreted in the urine.[8] In addition, the chances of transfusing a patient with more than one RBC unit from a TRT donor are slim given their low proportion in the overall donor population.[5] Although the transfusion efficacy (TRT RBC survival in the patient circulation) has not been determined in humans, to our knowledge, there have not been any reports concerning the safety of blood components derived from TRT donors.

While likely innocuous to most patients, there are cases in which patient exposure to testosterone may be unfavorable. Such cases include androgen-sensitive patients or patients with liver failure whose body may not be able to efficiently convert free testosterone to its inactive metabolites. One example, which was discussed during the 2014 FDA and AABB meeting,[4] is neonates who may be subjected to multiple transfusions from a dedicated RBC unit.

4.3 | Recommended actions for reevaluation of policies concerning blood donors with prescription testosterone

Recently, Nemkov et al. provided a fascinating and intriguing overview of how the exposure of blood donors to the environment (exposome) or certain medications impacts the metabolism and function of stored RBCs.[16] This study emphasized the growing need to advance the field of precision transfusion medicine through donor, blood component, and recipient linkage studies. To this end, we have focused our research on TRT-mediated changes in RBC characteristics linked to hemolysis during cold storage and after transfusion in mice.[6,7] This study presented a different route by which TRT in blood donors may introduce a risk to certain transfusion recipients. Thus, our first recommendation is to conduct new basic and translational studies to rule out any safety issues that may stem from excessive presence of free testosterone in blood components. Such studies may evaluate the kinetics of testosterone uptake and release by RBCs or use animal models of transfusion to track the fate of selected doses of radio/fluorescent-labeled testosterone co-infused with stored RBCs. Identifying and limiting RBC units from testosterone-treated men to vulnerable patient populations may be premature until additional studies identify potential safety issues.

The second recommendation is to expand the screening of blood donors with prescription testosterone to include more information about the type of testosterone medication and dose, side effects (erythrocytosis), and timing of the last dose taken prior to blood collection. A significant spike in blood testosterone levels is likely to occur within the first 24 h after dosing; therefore, avoiding blood collection soon after testosterone therapy may reduce the chance of issuing blood products with supraphysiological testosterone. Our data indicate that three of the five TRT donors with supraphysiological testosterone received testosterone injections (testosterone cypionate) 0, 3, and 5 days before blood donation. Thus, it is important to understand the interactions between the route of testosterone administration, dose, and the half-life of each medication. Last, it should be noted that blood donor screening for prescription testosterone is not uniform across all countries and jurisdictions, meaning that plasma and platelets may be issued for transfusion. To our knowledge, no adverse events have been reported that could be linked to such components. Therefore, evaluating the quality and safety of stored platelets and plasma from TRT donors in the United States may negate the wastage of these viable components.

One limitation of our study was the relatively small number of participants (n = 17/group) that limited our ability to provide an estimate of the proportion of TRT donors with above reference range of plasma free and total testosterone concentrations. In conclusion, our findings established a rationale for reexamination of the current FDA and AABB guidelines concerning the safety and utilization of blood components from TRT donors. If concerns about supraphysiological testosterone have driven the decision to reject platelets and plasma derived from TRT blood donations, then LR-RBC units are no exception with regard to free testosterone. If deemed safe, blood utilization from such individuals can be expanded to include platelets and plasma products. This is important in times of blood shortage as recently experienced during the outbreak of COVID-19 and in light of the rise in TRT popularity among men, which is expected to further increase the number of blood donors on this therapy.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

ORCID

Larry J. Dumont https://orcid.org/0000-0002-1715-0748
Tamir Kanias https://orcid.org/0000-0001-6558-0913

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