Blood Doping

Effects of blood withdrawal and reinfusion on biomarkers of erythropoiesis in humans: Implications for anti-doping strategies

To discriminate autologous blood doping procedures from normal conditions, we examined the hematological response to blood withdrawal and reinfusion. We found that biomarkers of erythropoiesis are primarily affected in the anemic period. Therefore, individual variations in [Hb] exceeding 15% between samples obtained shortly before any major competition would be indicative of autologous blood manipulation.

Infusion of red blood cells in man to enhance oxygen carrying capacity of blood is a known practice since the 1947.[1] The release of recombinant human erythropoietin (rHu-EPO) most likely replaced blood transfusion practices since rHu-EPO was found to be more efficient and easy to manage by non-medical personnel. In year 2000, a urine test for rHu-EPO became available, which provided a means for detecting its illegal used.[2] Recently, athletes have been tested positive for allogeneic blood transfusion by means of flowcytometry[3] indicating a return to former blood transfusion practices. However, an unequivocal detection method revealing autologous transfusion is still not available.

Since accelerated or inhibited erythropoiesis leads to characteristic changes in peripheral blood parameters, irrespectively of the stimulating agent, an indirect detection method of rHu-EPO use based on changes in certain hematological parameters[4] has been implemented by some international sport federations. Despite its use, data

on the effect of blood withdrawal and reinfusion in these hematological parameters have never been reported in full. We hypothesize that autologous blood manipulations produce large variations in currently used biomarkers of erythropoiesis making individual hematological profiles an effective tool identifying this manipulation.

We measured hemoglobin concentration [Hb], hematocrit, reticulocytes, serum EPO and sTfR concentrations in 10 healthy, male subjects at baseline and after the withdrawal of 20±3% (1.3±0.2 L; mean±SEM) of the subjects' blood volume on day 0, 1, 3, 7 14, 21 and 28 and after reinfusion of 0.8±0.1 L of packed RBCs on day 0, 1, 3, 7, 14 and 21. To maintain blood volume after blood withdrawal, 1.3 L of hydroxyl-ethyl-starch (Voluven®) was infused. All subjects were given daily oral iron supplementation (100 mg). [Hb], hematocrit, reticulocytes was measured using an automatic hematology system (ADVIA 120, Bayer Diagnostics). Serum EPO and sTfR concentrations were determined by ELISA (R&D systems Inc.). The indirect OFF-hr blood models for rHu-EPO detection were applied.[4] A one-way ANOVA for repeated measurements was used to test for differences between control values and samples taken during the anemic and polycytemic phase.

We found that following blood withdrawal, [Hb] remained reduced on average by ~15% for 2 weeks (Table and Figure 1). Accordingly, s-EPO increased 4-fold within a day, declining exponentially thereafter. Reticulocyte count increased rapidly by 2.4-fold after 7 days, remaining elevated for another 7 days whereas sTfR increased by 60% by day 14 and remained elevated until 3 days after blood reinfusion.

Following blood reinfusion, [Hb] increased acutely by 8% returning to the initial baseline value after 2 days. s-EPO remained unchanged whereas reticulocyte count was reduced by 25%-37% from day 7 to 21. sTfR declined progressively after reinfusion. The highest OFF-hr score was 126 at day 2 during the polycytemic period corresponding to a 1 in 1000 cut-off threshold. Evidence

Table 1. Hematological parameters after blood withdrawal and reinfusion.

Days	Control C	Blood withdrawal 1	3	7	14	28	Reinfusion 0	1	3	7	14	21
Hb, g/dL	14.8±0.2	12.8±0.3*	12.4±0.2*	12.8±0.3*	13.2±0.2*	14.3±0.2	15.4±0.3*†	16.0±0.2*†	15.4±0.3*†	14.9±0.3	14.7±0.2	15.0±0.2
Hct, %	42.9±0.6	36.3±0.9*	35.5±0.7*	36.9±0.7*	38.9±0.6*	41.9±0.5	45.4±0.8†	47.1±0.5*†	45.2±0.8†	44.0±0.5	43.4±0.5	43.6±0.6
ret, %	1.1±0.1	1.6±0.1	1.9±0.1	3.0±0.2*	2.7±0.1*	1.3±0.1	1.3±0.1	1.1±0.1	1.1±0.1†	0.8±0.1*†	0.8±0.1*†	0.9±0.1†
s-EPO, mIU/mL	7.9±0.9	33.3±3.6*	28.5±3.6*	20.7±1.9*	14.8±1.4	10.5±0.9	NA	5.4±1.0	NA	9.0±0.9	6.6±0.9	7.8±0.9
sTfR, nmol/L	17.5±1.3	17.2±1.7	19.0±2.5	23.9±3.1	28.3±3.3*	28.4±3.0*	NA	33.3±3.7*	NA	28.1±2.9*	25.9±2.6	20.9±2.0
OFF-hr	85±3	53±5	43±5	24±4	33±4	75±3	87±4	96±3	87±3	95±3	90±2	92±3

*Values are means±SEM for 10 male subjects. Hb, hemoglobin concentration; Hct, hematocrit; ret, reticulocytes; s-EPO, serum erythropoietin; sTfR, soluble transferrin receptors; NA, Not Available. *Significantly different from control (p<0.05). †Significantly different preinfusion value(p<0.05).*
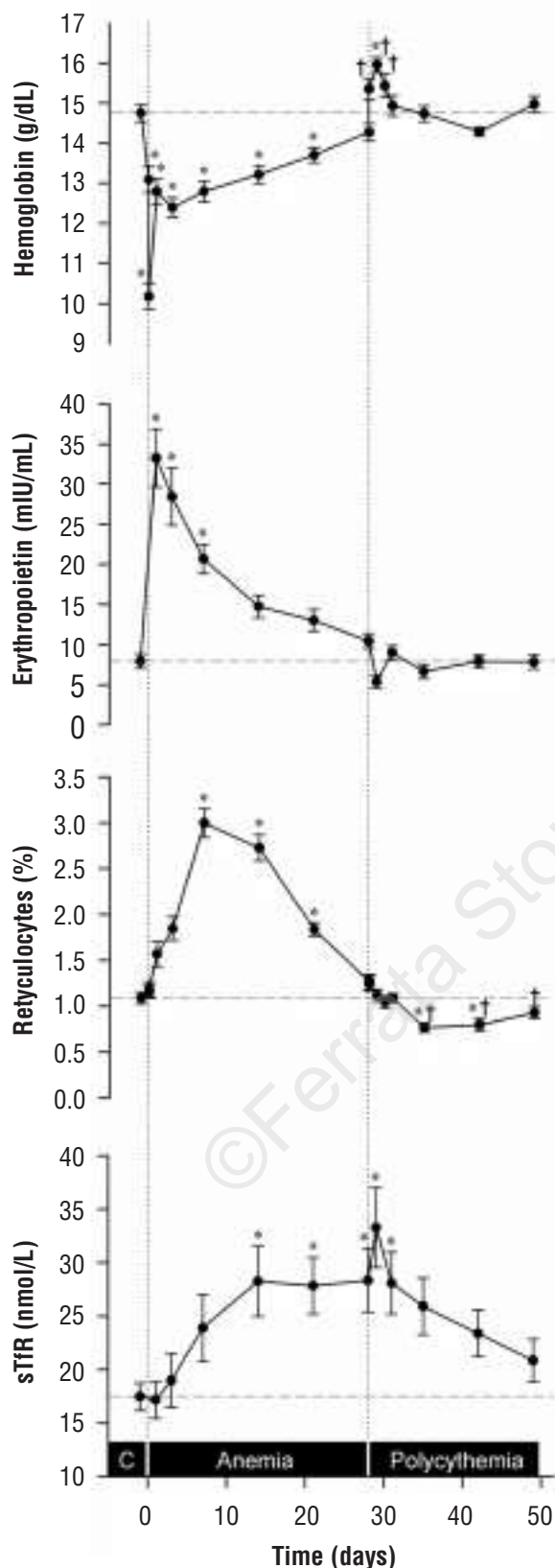
indicates that the hematocrit within-subject biological and seasonal variation in man shows a maximal relative change of 15% within a 95% confidence interval i.e. a change from 0.42-0.48.[5] Data of a wider range of sports also show within subject estimates of variance in [Hb] of 1.6 g/dL,[6] which agree with a10% fluctuation found in soccer players.[7] In the present study, however, all 10 subjects exceeded the normal variation, with [Hb] ranging from 19-39%. Even when the control [Hb] value was taken as the mean [Hb] value, 9 of 10 subjects exceeded their individual upper limit based on a 7.5% addition to their control [Hb] value (range 3.0-11.5%). Importantly, alterations in other hematological biomarkers (s-EPO, reticulocytes and sTfR) were observed at all times through this investigation, suggesting that determination of these biomarkers could be used as supportive evidence for erythropoietic manipulations with acute [Hb] increases of more than 7.5%.

At present, only two blood tests are available. To protect the health of the athlete, World Anti Doping Agency has implemented an upper limit of 17.0 g/dL, which – if exceeded – elicits a *no start* sanction. However, only one subject in this study showed [Hb] values higher than 17.0 g/dL. When using the OFF-hr model of Gore *et al*. constructed to convey the use of rHu-EPO4, we observed that none of the subjects demonstrated positive OFF-scores, rendering this model ineffective detecting blood doping.

In conclusion, autologous blood procedures induce a clear pattern of accelerated erythropoiesis during the anemic period. Within the limitations of the study, it is suggested that variations in [Hb] exceeding 15% between samples obtained in top ranked endurance athletes during the anticipated anemic period and shortly before any major competition would be indicative of autologous blood manipulation.

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Figure 1 (left).. Hemoglobin ([Hb]), serum erythropoietin, reticulocytes and serum transferrin receptors (sTfR) responses during the 4 weeks after blood withdrawal of 20% of subjects' blood volume (1.3±0.2 L) and during the 3 weeks after blood reinfusion of 0.8±2 L of packed RBCs. Blood samples were collected before the investigation (Control, C) and on day 0, 1, 3, 7 14, 21 and 28 after blood withdrawal and on day 0, 1, 3, 7, 14 and 21 after reinfusion. Horizontal lines illustrate control values. Data are means±SEM for 10 subjects. * Significantly different from control ($p<0.05$). † Significantly different from preinfusion values ($p<0.05$).

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