Evaluation of Sex on RBC Storage Hemolysis: Considerations for Transfusion Medicine

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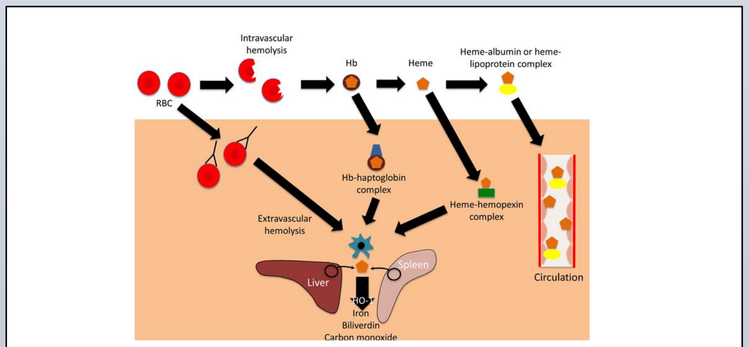
ABSTRACT

RBC transfusion is the most common inpatient medical procedure, offering life-saving therapy to ~5 million Americans every year. Storage within the blood bank is a logistic necessity to enable blood transfusion as a commodity. However, it comes at the cost of the progressive accumulation of a series of biochemical and morphological alterations to the stored erythrocyte. These alterations are collectively referred to as the "storage lesion". Clinically, the storage lesion has been implicated with increased risk of pulmonary complications such as transfusion-related acute lung injury (TRALI), multi-organ failure (MOF), and mortality [1-2]. The progression and severity of the storage lesion is impacted by several donor-specific biological factors such as age, ethnicity, and sex[3-4]. Most notably, the male hormone testosterone was identified as an etiological mechanistic contributor to the storage lesion[3,4]. Testosterone has been shown to increase store RBCs propensity to hemolyze creating heterogeneity of regularly issued blood products [3-4]. However, despite this recent evidence, little is known about the mechanism by which donor sex and testosterone levels affect RBC propensity to hemolyze. To investigate potential mechanisms mediated by testosterone, omics-based technology was employed to determine systemic and red cell-specific metabolic signatures of testosterone. These signatures were later validated and compared to subjects with sub and supra-physiological levels of testosterone. Metabolites of the arginine pathway, as well as acyl-carnitines and fatty acids, were found to be mediated by testosterone. However, further investigation is still needed to identify how testosterone mediation directly affects these pathways and ultimately RBC hemolyze.



Orf, K., & Cunnington, A. J. (2015). Infection-related hemolysis and susceptibility to Gram-negative bacterial co-infection. *Frontiers in microbiology*, *6*, 666. doi:10.3389/fmicb.2015.00666

Figure 1 – Schematic of intravascular and extravascular blood hemolysis.

OBJECTIVES

- Identification of metabolic shifts in RBCs under supra-physiological testosterone levels.
- Effect of elevated testosterone on blood storage capacity and quality in the blood bank.
- Investigation of hormone (gender-defining hormones) mediation on RBCs metabolic pathways

METHODS AND MATERIALS

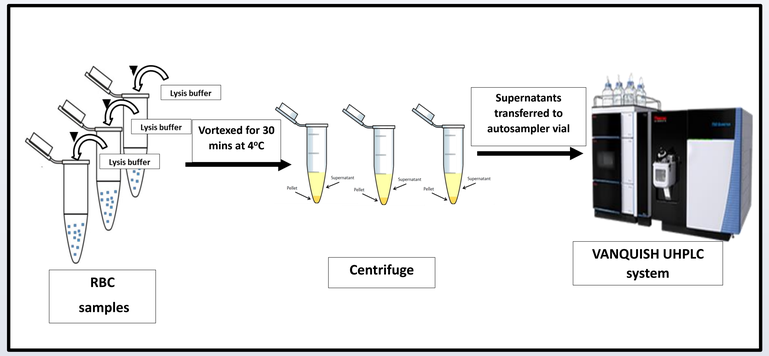


Figure 2 – Metabolomic work flow of RBC samples. Samples were lysed with lysis buffer or HPLC grade MeOH and precipitated to remove the protein contaminants. Supernatants were carefully removed from precipitant and analyzed using a Thermo Fisher VANQUISH UHPLC system.

RESULTS

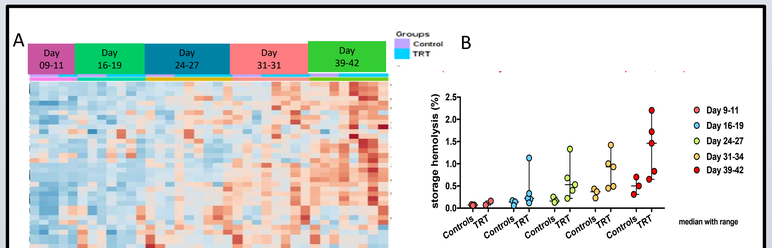
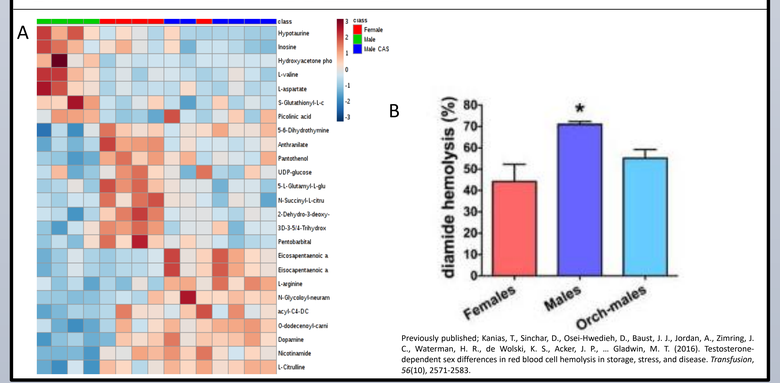


Figure 3 – Metabolomic profile of human RBC's in male donors vs donors on TRT over 42 days of cold storage. Identification of RBC metabolites was completed using MAVEN software. **(A)** Metabolites discovered via MAVEN is graphed represented in the heatmap using MetaboAnalyst 4.0 software. Levels of expression shift from high expression (dark red) for an individual subject/metabolite combination to low expression (dark blue) for an individual subject/metabolite combination. **(B)** Scatter plot showing the percentage of storage hemolysis from day 9-42 in healthy male donors vs donors on TRT .



Previously published; Kanias, T., Sinchar, D., Osei-Hwedieh, D., Baust, J. J., Jordan, A., Zimring, J. C., Waterman, H. R., de Wolski, K. S., Acker, J. P., ... Gladwin, M. T. (2016). Testosterone-dependent sex differences in red blood cell hemolysis in storage, stress, and disease. *Transfusion*, *56*(10), 2571-2583.

Figure 4 – Metabolomic profile of mice RBC's. Identification of RBC metabolites was completed using MAVEN software. **(A)** Metabolites discovered via MAVEN is graphed represented in the heatmap using MetaboAnalyst 4.0 software. **(B)** Scatter plot showing the percentage of storage hemolysis from day 9-42 in healthy male donors vs donors on TRT .

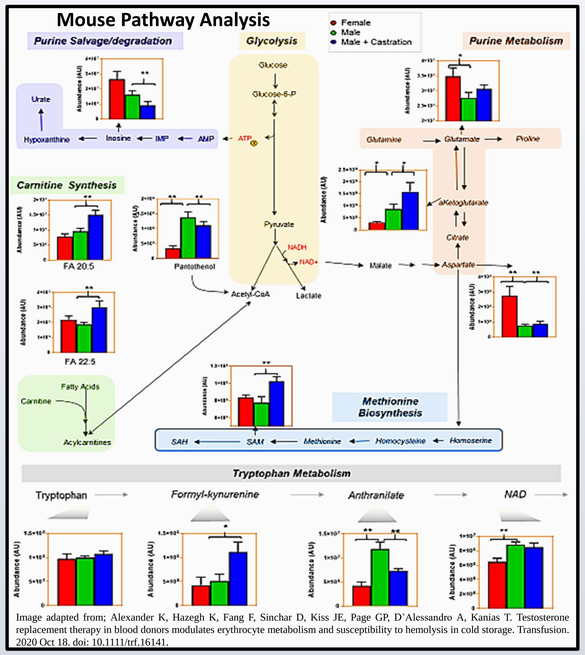


Image adapted from; Alexander K, Hazegh K, Fang F, Sinchar D, Kiss JE, Page GP, D'Alessandro A, Kanias T. Testosterone replacement therapy in blood donors modulates erythrocyte metabolism and susceptibility to hemolysis in cold storage. Transfusion. 2020 Oct 18. doi: 10.1111/trf.16141.

Figure 5 – Metabolic analysis of impacted pathways. Identification of RBC metabolic pathways affected by mouse castration. All metabolomic analysis was completed using MAVEN software. Bar graphs showing the peak area (abundance). All graph's were made using Prism GraphPad 8.0.

CONCLUSIONS

- Our analysis reveal a predisposition of male-RBC to hemolysis more than female RBC's under cold storage.
- Metabolites of the arginine pathway, as well as acyl-carnitines and fatty acids, were found to be mediated by testosterone.
- Future direction: investigate the mechanism that gender-defining hormones use to mediate blood hemolysis using a longitudinal transgender cohort.

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