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TRANSFUSION

ORIGINAL RESEARCH

Blood Components

Exposure of cryopreserved red cell concentrates to real-world transient warming events has a negligible impact on quality

Jayme Kurach[1,2] | **Mackenzie Brandon-Coatham**[1] | **Carly Olafson**[1] | **Tracey R. Turner**[1] | **Celina Phan**[2] | **Mahsa Yazdanbakhsh**[2] | **Rafay Osmani**[2] | **Behrouz Ehsani-Moghaddam**[3] | **Gwen Clarke**[2] | **Jason P. Acker**[1,2]

[1]Innovation and Portfolio Management, Canadian Blood Services, Edmonton, Alberta, Canada

[2]Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada

[3]Epidemiology & Surveillance, Canadian Blood Services, Ottawa, Ontario, Canada

Correspondence
Jason P. Acker, Innovation and Portfolio Management, Canadian Blood Services, 8249-114 Street. Edmonton, AB T6G 2R8, Canada.
Email: jason.acker@blood.ca

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Abstract

Background: Red cell concentrates (RCCs) may be cryopreserved at Canadian Blood Services (CBS) for up to 10 years; however, inadvertent warming of these units over the prescribed storage temperature (≤ −65°C) may occur. These units may be discarded from inventory to avoid potential adverse transfusion outcomes. This study aimed to assess the quality of RCCs that experienced unintentional transient warming events (TWEs) related to freezer failures.

Study Design: Thirty cryopreserved RCCs with known TWEs were selected for this study and classified into three different experimental groups (Event 1 ($n = 5$) TWE > −65°C for 34 min; Event 2 ($n = 23$) TWE > −65°C for 48 h; and both Event 1 and Event 2 ($n = 2$) TWE > −65°C for 34 min and 48 h). Ten additional RCCs with no known TWEs, cryopreserved over the same period, were selected as controls. Thawed RCCs were deglycerolized using the Haemonetics ACP 215, and in vitro quality was assessed throughout hypothermic storage.

Results: RCCs from the control and all three experimental groups met the Canadian Standards Association (CSA) guidelines for hematocrit, total hemoglobin, and hemolysis at expiry. RCCs experiencing a singular TWE had similar in vitro quality to control RCCs.

Discussion: This study's findings revealed that single exposures to specific documented TWEs did not significantly impact the quality of RCCs

post-deglycerolization. While units should still be assessed on a case-by-case basis upon TWE, our work provides the first-ever evidence that supports a broader policy of unit retention by blood centers.

KEYWORDS

ACP 215, cryopreservation, glycerol, ice recrystallization, quality, red blood cells, transient warming

1 | INTRODUCTION

In Canada, blood centers maintain an inventory of red cell concentrates (RCCs) that include an array of red cell antigen phenotypes, a subset of which are rare (occurring in <1 in 1000 individuals) or very rare (occurring <1 in 3000).[1,2] To meet the transfusion needs of patients with rare or very rare phenotypes, blood centers often employ cryopreservation methods to keep a bank of frozen units.[1] Canadian Blood Services (CBS) operates a rare blood program wherein a frozen inventory of approximately 1400 units (as of July 2024) is maintained at four different sites across the country.[2–4] Rare donors are actively recruited and registered in the program, and their donations are collected, stored, and matched to phenotypically rare patients.[2] In 2021, 115 rare units were required for patients, and approximately 40 of these units came from frozen inventory.[2] At CBS, RCCs can be hypothermically stored between 1 and 6°C for up to 21 days prior to glycerolization and freezing.[4–6] Once cryopreserved using the high glycerol (40%) method; these inventories of rare phenotypes are stored at or below −65°C for 10 years with the possibility of extension.[6,7] Once a frozen RCC unit is matched to a potential recipient and identified as necessary for transfusion, it is thawed, deglycerolized, and resuspended in AS-3.[4] From this point, the RCC unit can be hypothermically stored for up to 14 days prior to transfusion if closed-system automated cell processors, like the ACP 215 (Haemonetics) are employed.[4] There are advantages to using frozen RCCs in some patient populations. Trauma patients who received cryopreserved RCCs exhibited increased tissue oxygenation and no elevation of proinflammatory, or anti-inflammatory cytokines 12 h posttransfusion.[8,9] A similar lack of cytokine production was identified when cryopreserved autologous blood donations were transfused into healthy human volunteers.[10] In cases of preoperative autologous blood donations, patients who received frozen blood had approximately 6% higher circulating hemoglobin mass than those who received refrigerated, unfrozen blood.[11]

Not only can maintaining cryopreserved blood products be expensive, requiring specialized equipment to store, manage, process, and monitor frozen inventory, but also logistical challenges can arise in the tracking, shipping, monitoring, and use of the cryopreserved units that requires staff to receive additional training. Due to the ultra-low temperatures utilized and varied lengths of storage time, there exists the potential for units to be inadvertently warmed above the recommended critical storage temperature (≤ −65°C for RCCs frozen using the high glycerol [40%] method) leading to what has been termed a "transient warming event" (TWE).[4,12] TWEs can occur for a few reasons including but not limited to routine inventory management practices, packing/shipping of units, and freezer/equipment failures. Current protocols at CBS indicate RCCs exposed to any identified TWE, regardless of maximum temperature reached and length of time outside the recommended critical storage temperature must be quarantined or removed from inventory to mitigate the risk of adverse transfusion outcomes in patients. The negative implications of TWEs have been studied in different cell types and tissues,[13–16] as well as red blood cells (RBCs) cryopreserved in 15% glycerol in conjunction with ice recrystallization inhibitors.[17] TWEs pose a risk to frozen inventories' product quality and post-thaw viability because they provide an opportunity for ice crystal growth both intra- and extracellularly.[18] Not all TWEs are equal though, and the extent of the risk associated with a given TWE is dependent on many factors, such as freezing container configuration, maximum temperature reached outside of the acceptable range, length of time outside of the acceptable range, as well as the cell concentration and cryoprotective agent (i.e., glycerol) used.

Currently, literature is scant on specific types of TWEs and the outcomes of these exposures on the quality of RBCs cryopreserved using a high glycerol method. Valeri et al. provided the first evidence indicating the minimal in vitro and in vivo impact of warming and subsequent refreezing of cryopreserved RBC units.[19] In the study by Valeri et al., they investigated the impact of warming cryopreserved units stored at −80°C to either −40°C for 4 weeks or −20°C for 2 weeks and found little impact on product quality.[19] The objective of the current study was to look at the quality of 30 RCC units which had been unintentionally exposed to three different types

of TWEs throughout their frozen storage period that were related to freezer failure events. In vitro quality was assessed by measuring the Canadian Standards Association (CSA) parameters for leukoreduced, frozen RBCs (hematocrit, hemoglobin and RBC hemolysis), as well as adenosine triphosphate (ATP) and extracellular K^+ concentrations, hematological indices, RBC morphology, osmotic fragility, osmotic hemolysis and RBC deformability. The quality of these units was assessed post-deglycerolization and throughout 14 days of hypothermic storage (HS) and compared with the quality of frozen units with no recorded TWE. Based on the specifics of these singular TWEs we hypothesized the effects on the RCCs quality would be minimal.

2 | MATERIALS AND METHODS

2.1 | Unit selection

Forty cryopreserved RCCs, excluding those with extremely rare blood types or known genetic RBC membrane changes, were obtained from frozen inventories across Canada. A variety of collection sites, collection methods, sex, and lengths of time frozen, were observed within both experimental and control groups (Table 1 and Supplementary Table 1). Thirty RCC units had undergone at least one "real-world" TWE and were divided into three different groups based on the type of TWE experienced: Event 1 units ($n = 5$) had a warming exposure of > -65°C for 34 min (maximum-recorded freezer temperature of −49°C); Event 2 units ($n = 23$) underwent a warming exposure of > -65°C for 48 h (maximum-recorded freezer temperature of −30°C); and finally the last group ($n = 2$) denoted as both Event 1 and Event 2 went through both warming exposures of > -65°C for 34 min and 48 h. These "real-world" TWEs were due to freezer failures and were not controlled warming events. The remaining 10 units designated "no TWE" were kept frozen, without any documented TWE incidents, for a similar length of time as the products with the recorded TWEs (Table 1).

2.2 | Unit glycerolization and deglycerolization

All details of the ACP 215 glycerolization, manual glycerolization, supernatant reduction, and deglycerolization processes used at CBS have been previously described.[4] Thirty-eight of the RCCs were manually glycerolized, and two were glycerolized on the ACP 215 (Haemonetics, MA, USA). Table 1 indicates the breakdown of which units within each experimental group were manually glycerolized and which were glycerolized using the ACP 215 (Haemonetics, MA, USA). Following glycerolization, units were placed in ≤ -65°C storage until they were rejected from CBS inventory due to age or TWE exposure. Once rejected, they remained in ≤ -65°C storage and were designated for extended quality testing. Thirty-eight of the selected units required a supernatant reduction step to remove excess glycerol, a step which was not completed prior to freezing with the manual glycerolization protocol. All units were deglycerolized on the ACP 215, resuspended in AS-3, and placed into HS for 14 days.

2.3 | RBC quality assessment

Thawed and deglycerolized RCC units were sampled aseptically on days 0, 1, 7, and 14 post-deglycerolization. A hematology analyzer (DxH 520, Beckman Coulter, Co. Clare, IE) was used to obtain hemoglobin (HGB) concentration and mean corpuscular volume (MCV) measurements. RCC samples were centrifuged (2200 × g, 10 min, 4°C), and extracellular potassium levels were measured from the supernatant by an accredited external laboratory (Alberta Precision Laboratories, Edmonton, Alberta, Canada) using an automated chemistry analyzer (Roche Cobas c503, Roche Diagnostics). Spun hematocrit and RBC hemolysis were determined using previously described methods.[20–22] Oxygen affinity was assessed using an automated blood-oxygen analyzer (Hemox-Analyzer Model B, TCS Scientific, New Hope, USA).[23–25] ATP concentration was measured spectrophotometrically using a previously described commercially available, enzyme-based assay (DiaSys Diagnostic Systems GmbH, Holzheim, Germany).[22] RBC morphology index scores were determined using fixed and stained blood smears.[22,26] Osmotic hemolysis was induced by incubating phosphate buffered saline washed RBCs in a hypotonic buffer.[27,28] Assessment of osmotic fragility was completed by exposing RBCs to a series of saline solutions (1.0–9.0 g/L) and determining the mean corpuscular fragility (MCF, the concentration which produces 50% hemolysis).[22]

2.4 | Assessment of RBCs' rheological properties

RBC deformability was examined, with and without exposure to an osmotic gradient, using an ektacytometer (LORRCA MaxSis, RR Mechatronics). RBCs were diluted 9:1000 in isotonic polyvinylpyrrolidone (PVP) solution, mixed and subjected to increasing shear stresses ranging from 0.95 to 30.0 Pa at 37°C. RBC deformability curves were transformed using an Eadie-Hofstee linearization

TABLE 1 Donor, relevant processing, and storage characteristics of the 40 cryopreserved units subjected to comprehensive quality testing.

	TWE 1	TWE 2	TWE 1 and 2	Control (No TWE)
Total number of RCCs (n)	5	23	2	10
Total number of recorded TWEs	1	1	2	0
Maximum freezer temperature reached during TWE(s)	−49°C	−30°C	−49°C (1st TWE) −30°C (2nd TWE)	N/A
Length of time above ≤ −65°C	34 min	48 h	34 min (1st TWE) 48 h (2nd TWE)	N/A
Sex (*n*)				
Female	2	12	1	5
Male	3	11	1	5
Donor age at donation (years)				
Mean ± SD[a]	64 ± 8	50 ± 14	45 ± 18	50 ± 9
Max	70	71	58	65
Min	51	23	32	35
Unit age at freezing (days)				
Mean ± SD	14 ± 2	10 ± 4	12 ± 2	12 ± 6
Max	15	17	13	22
Min	11	3	10	4
Manually glycerolized (*n*)[b]				
Yes	3	23	2	10
No	2	0	0	0
Length of time frozen (years)				
Mean ± SD	7.8 ± 2.7	12.2 ± 2.7	11.9 ± 0.1	11.6 ± 3.9
Max	11.7	17.4	11.9	11.6
Min	5.2	7.5	11.8	3.4

[a]SD, standard deviation.
[b]Manually glycerolized units were frozen in a 2 liter bag in the presence of glycerol and required supernatant reduction post-thaw prior to deglycerolization.

technique, to obtain the parameters: EI_{max} and K_{EI}.[29] Osmoscan curves were obtained using RBCs diluted 1:25 in isotonic PVP solution and subjected to an osmotic gradient ranging from 100 to 600 mOsm/kg at a constant shear stress (30 Pa) and temperature (37°C). The osmoscan indices (EI_{min}, EI_{max}, EI_{hyper}, O_{min}, OEI_{max}, and O_{hyper}) were extrapolated from the curves produced by plotting the elongation index (EI) of the RBCs against the osmolalities within the gradient. Additional parameters were evaluated using the Osmoscan indices: $\Delta Elongation = EI_{max}–EI_{min}$, and $\Delta Osmolality = OEI_{max}–O_{min}$.[30]

2.5 | Statistical analysis

Statistical analysis was performed using GraphPad Prism v.10.2.3 (GraphPad Software Inc., La Jolla, CA, US) and SAS 9.4 Maintenance 8 (SAS Institute Inc., Cary, NC, US). Mixed model analysis for repeated measurements with multiple comparisons was performed in GraphPad Prism software to estimate the effects of both TWE (Event 1, Event 2) and storage time (0, 1, 7, 14 days) and their interaction on several RBC quality parameters. In all analyses, a *p*-value less than .05 was considered statistically significant.

3 | RESULTS

Exposure to single "real-world" transient warming exposures does not significantly impact RBC hematological indices or RCCs ability to meet Canadian transfusion standard guidelines: The CSA requires 90% of tested leukoreduced, frozen deglycerolized blood products to be below

a maximum hematocrit (≤ 80%), above a minimum total HGB (> 35 g/unit), and below a maximum hemolysis (<0.8%).[6] For any experimental group, hematocrit and total HGB measurements was not significantly impacted by the presence of a TWE, and all met the prerequisite CSA standards for both parameters (Supplementary Figure 1A, Supplementary Figure 1B, and Table 2). No significant differences in RBC hemolysis were found among the study groups or time points (Figure 1). RCCs from all experimental groups met the CSA standards for RBC hemolysis up to their post-deglycerolization expiry deadlines (Table 2). No significant differences in MCV were reported between control and singular TWE experimental groups (Supplemental Figure 1C).

The in vitro *quality of the RBCs is not significantly impacted by a singular transient warming exposure but may be impacted by multiple exposures*: The ability of transiently warmed RCCs to withstand osmolality changes was assessed using osmotic hemolysis and osmotic fragility assays. At every testing day post-deglycerolization, RCCs exposed to two TWEs had lower osmotic hemolysis values when compared with RCCs exposed to a single or no TWE (Figure 2A). Throughout HS, control RCCs had statistically similar osmotic fragility values in comparison with the singular TWE groups ($p > 0.05$) (Figure 2B). ATP levels decreased with storage time regardless of the TWE (Figure 2C). On day 7 of storage, significantly lower ATP concentrations and p50 were recorded for units exposed to short TWEs (Event 1, exposure length = 34 min) compared with long TWEs (Event 2, exposure length = 48 h) ($p < .001$ and $p < .05$) (Figure 2C,D). Measurements of p50 decreased, thereby oxygen affinity increased throughout storage for all experimental groups (Figure 2D). The oxygen affinity of RBCs experiencing a singular or no TWE exposure was not statistically different (Figure 2D). All experimental groups showed similar increases in extracellular potassium concentration throughout the testing period (Figure 2E). The morphological index scores decreased throughout HS, with no significant differences being identified among the experimental groups (Figure 2F).

RCCs exposed to zero or one transient warming exposure (TWE) do not have altered deformability under an osmotic gradient: No significant differences in elongation (EI_{max}) or rigidity (K_{EI}) was observed among the experimental groups (Supplemental Figures 1D and 1E). Osmoscan revealed ΔElongation values were statistically similar among experimental groups; however, ΔElongation

TABLE 2 Percentage (%) of units in each experimental group that meet the CSA standards for hemoglobin, hematocrit and RBC hemolysis for deglycerolized RBCs.

Experimental group	Hemoglobin	Hematocrit	RBC hemolysis
TWE Event 1	100	100	100[a]
TWE Event 2	100	100	100
TWEs Event 1 and 2	100	100	100
Control (No TWE)	90	100	100

[a]3/5 units had a 24 h post-deglycerolization expiry because they were manually glycerolized, whereas 2/5 units had a 14 day post-deglycerolization expiry because they were glycerolized using the closed-system ACP-215. All units met the CSA standard for RBC hemolysis at their given expiries.

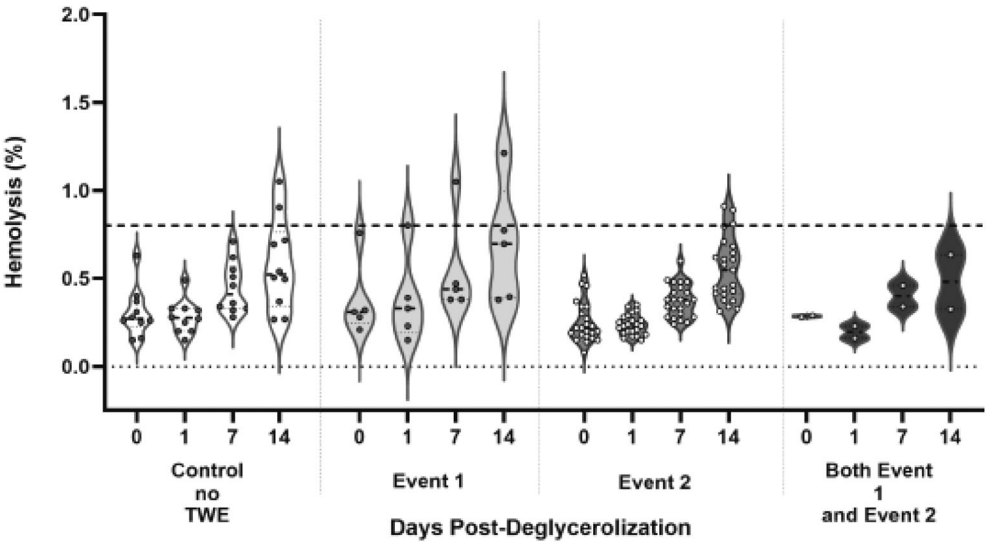

FIGURE 1 The effects of real-world transient warming events (TWEs) on the quality of RCCs at days 0, 1, 7, and 14 post-deglycerolization. Leukoreduced (LR)-RCCs exposed to no TWE, an Event 1, Event 2, or both TWEs were deglycerolized and stored for 2 weeks during which time hemolysis was measured. Median and interquartile ranges are represented by dashed and dotted horizontal lines on the violin plots, respectively. The CSA standard limit is indicated by a horizontal dashed line.

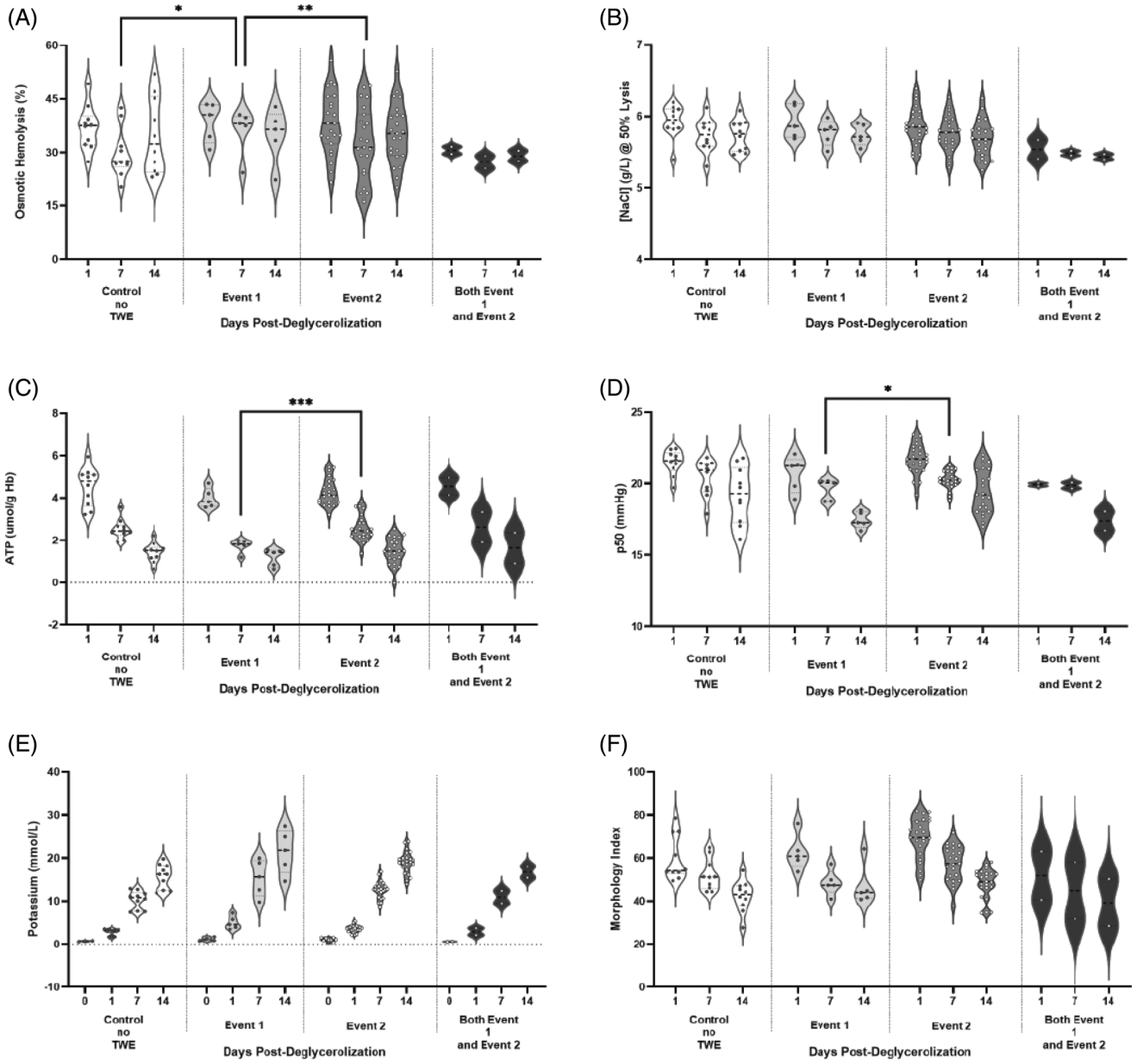

FIGURE 2 The effects of real-world transient warming events (TWEs) on the quality of RCCs at days 1, 7, and 14 post-deglycerolization. LR-RCCs exposed to no TWE, an Event 1, Event 2, or both TWEs were deglycerolized and stored for 2 weeks during which time (A) osmotic hemolysis, (B) osmotic fragility, (C) ATP concentration, (D) oxygen affinity, (E) potassium levels, and (F) morphology of RBCs were measured. Multiple comparisons tests were used to show statistical significance (***$p < .001$, **$p < .01$, *$p < .05$). Median and interquartile ranges are represented by dashed and dotted horizontal lines on the violin plots, respectively.

values for units exposed to multiple TWEs were lower at day 14 (Figure 3A–C). On day 1 and 14 of storage, the EI_{min} at hypotonic osmolality was higher for the units having undergone two TWEs compared with control units (Figure 3A,B,D). Throughout HS, the EI_{max} of units having experienced two TWEs was lower than all other groups (Figure 3A,B,E). The EI_{hyper} decreased when units were exposed to two TWEs in comparison with control and Event 2 TW exposures (Figure 3F). A significant decrease in the measured EI_{hyper} values were observed between the control and Event 1 TW exposures at day 1 post-deglycerolization ($p < .05$), while Event 2 TW exposures lead to an increase in measured EI_{hyper} values in comparison with Event 1 at the same testing point ($p < .0001$) (Figure 3F). Similar trends were observed for ΔOsmolality, OEI_{max}, and O_{hyper} as were showcased for their ΔElongation, EI_{max}, and EI_{hyper} counterparts (Supplementary Figure 2).

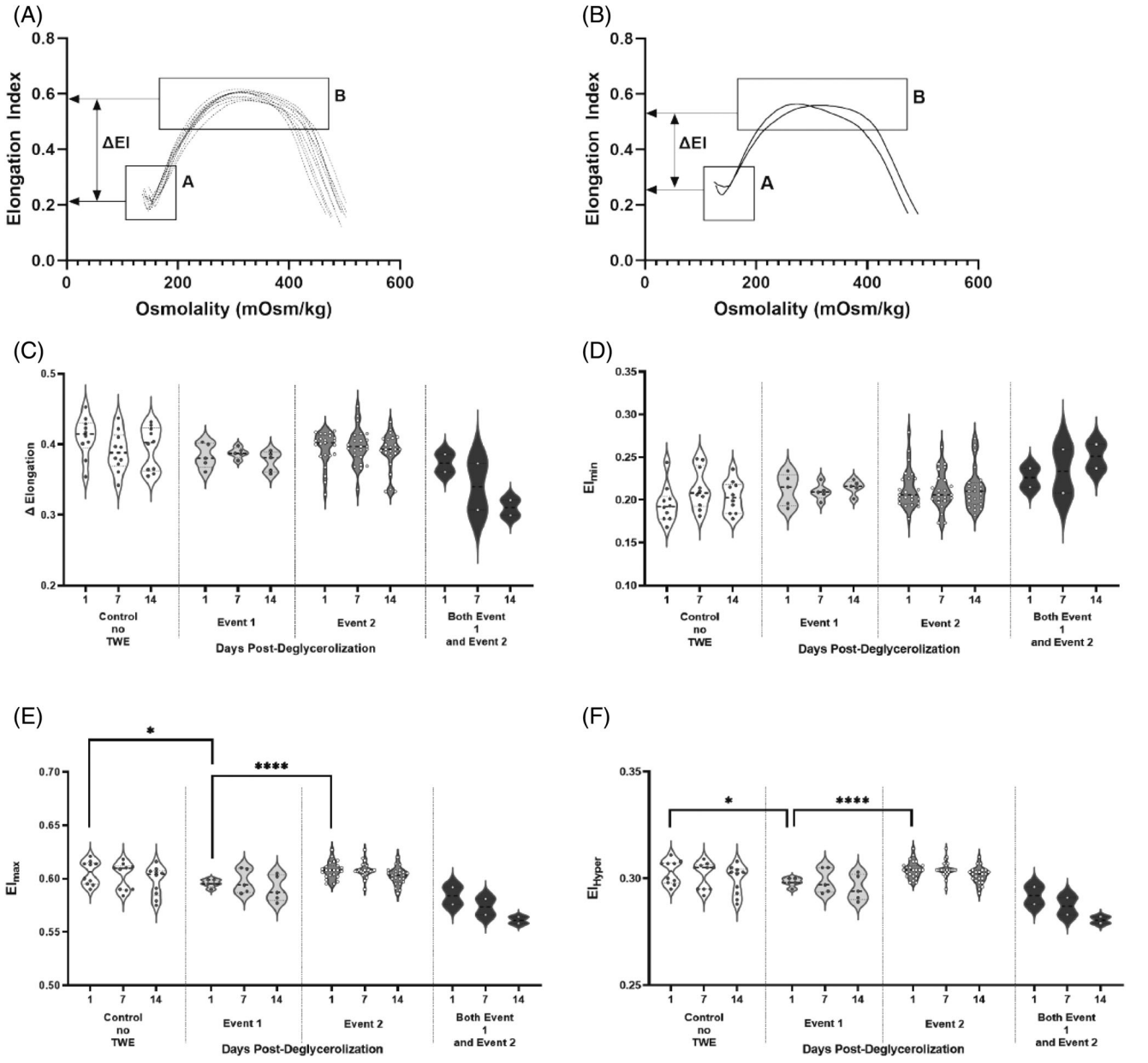

FIGURE 3 The effects of real-world transient warming events (TWEs) on the quality of RCCs at days 1, 7, and 14 post-deglycerolization. Osmoscan curves representing an individual unit within each experimental group: (A) control and (B) units experiencing multiple TWEs on day 14 of HS. Within the boxes labeled A on each graph the EI_{min} region is highlighted while the boxes labeled B indicate the regions wherein EI_{max} is found. LR-RCCs exposed to no TWE, an Event 1, Event 2, or both TWEs were deglycerolized and stored for 2 weeks during which time (C) delta elongation, (D) EI_{min}, (E) EI_{max}, and (F) EI_{hyper} were determined by ektacytometry. Multiple comparisons tests were used to show statistical significance (****$p < .0001$, *$p < .05$). Median and interquartile ranges are represented by dashed and dotted horizontal lines on the violin plots, respectively.

4 | DISCUSSION

This study is the first of its kind to show the potential negligible impact of certain TWEs on the post-thaw quality of cryopreserved RCCs'. The results indicate unintentional, shorter-term, single or multiple TWEs due to freezer failures while in blood bank inventory do not significantly impact the RCCs' ability to meet CSA Standards for hematocrit, total hemoglobin, or RBC hemolysis. All the units studied, regardless of TWE, met the CSA standards for total hemoglobin, hematocrit, and hemolysis up to their product specific expiry dates. Based on the CSA criteria alone, which provides an indication of the clinical suitability of the frozen, deglycerolized RCCs for transfusion, single or multiple TWEs did not significantly impact product quality.

Regardless of TWE exposure, all experimental groups followed similar trends attributable to the RBC storage lesion identified in the literature.[31–36] Both RBC hemolysis and extracellular potassium increased throughout HS. The oxygen affinity increased, as illustrated by a decrease in p50, as key metabolites such as ATP were also progressively depleted.[37–41] The ability of the RBCs to withstand extracellular osmotic fluctuations, as measured through osmotic fragility and osmotic hemolysis assays, appears to decrease throughout HS providing evidence of decreased cell structural integrity. RBC morphology index scores decreased as a larger proportion of RBCs progressed from a discoid shape to a spherocytic shape during HS storage.[39,41]

Although our deeper in vitro investigation revealed two TWEs could potentially compound damage to RBC quality, the experimental group showing these outcomes was underpowered. The Event 2 experimental group had the highest power with a sample size of $n = 23$, whereas the Event 1 experimental group and multiple TWE experimental group (experiencing both Event 1 and Event 2) had sample sizes of $n = 5$ and $n = 2$, respectively.

There were RBC quality parameters that changed based on exposure to the different singular TWEs. Lower ATP concentrations and p50 were recorded for the units exposed to short TWEs (Event 1, exposure length = 34 min) compared with long TWEs (Event 2, exposure length = 48 h). Besides experimental group size differences, units comprising the Event 1 testing group were known to be a mix of manual and closed-system glycerolized RCCs, whereas the products within the Event 2 group consisted of only manually glycerolized RCCs. Variables such as collection bag type/manufacturer, anti-coagulant, storage solution, and the time the unit was stored in HS prior to cryopreservation, in addition to the glycerolization method and open- versus closed-system processing approach used are all notable in interpreting the downstream quality of TWE RCCs. Good documentation practices should not only include the specifics related to the TWE exposures (i.e., maximum time outside of the critical temperature range and maximum-recorded freezer temperature) but also provide adequate insight into all the parameters associated with collection/processing and cryopreservation.

Closed-system glycerolization on the ACP 215 (Haemonetics) (two units in the Event 1 group) compared with the manual glycerolization method (three units within Event 1, and all units within the Event 2, multiple TWEs and No TWEs groups) introduces a multitude of procedural differences, which were not fully investigated. Different sized freezing bags, increased handling time due to bag transfers and additional centrifugations, and restricted volume transfers because of ACP 215 centrifuge bowl size limitations all have the potential to influence both the levels of hemoglobin, RBC hemolysis, and other in vitro parameters within frozen RCC products.[5] The majority of the frozen products ($n = 28/30$) analyzed in this study were manually glycerolized; thus, further investigation using the closed-system ACP 215 is required to determine its impact on these variables in relation to TWE exposures. The different glycerolization methods included within the Event 1 group may provide an explanation for some of the significant differences in quality parameters in comparison with the Event 2 group.

Overall, negligible differences in the majority of the in vitro quality assays were identified between the controls and single TW exposure groups; however, multiple TWE ($n = 2$) exposures appeared to potentially produce more osmotically fragile RBCs with increased oxygen affinity.[19] As mentioned previously due to limited sample size, caution must be used in claiming that multiple TWEs are more damaging to RBC quality than the singular TWEs. Nevertheless, it is critical to note that the osmotic hemolysis values were highly variable within the experimental groups. Mykhailova et al., found teenage males exhibit increased osmotic hemolysis throughout HS in comparison with their female and senior counterparts, while Kanias et al. demonstrated significantly higher osmotic hemolysis values in males, especially those with African American racial background.[27,42] These potential additional sources of variation within our experimental groups that were unaccounted for within the original study design highlights the need in the future to investigate the interplay of intrinsic donor factors with TWEs.

While minimal deformability changes were seen over a range of shear stresses, in agreement with our other in vitro measures of the RCCs, the units experiencing multiple TWEs showed impairments in deformability when exposed to varying osmolalities. Our results indicate a potential inability by RBCs exposed to these two TWEs to regulate their cell volume and an impaired ability to deform over the physiologic range of osmolalities; however, additional testing on RCCs experiencing similar multiple TWEs is needed.[30] Decreased deformability can lead to increased blood viscosity, and interruptions in bulk and microcirculatory blood flow.[43] The in vivo implications of decreased deformability have been shown to include decreased capillary flow, decreased wound healing, impaired blood flow, and disruption in oxygen homeostasis.[44,45] Multiple TWEs then, like the ones examined in this study, may provide the opportunity for cumulative rheological damage not seen from a singular TWE, which in turn may have implications posttransfusion.

The introduction of more than one freeze–thaw cycle may provide the perfect conditions for progressive damage caused by ice recrystallization. Studies have shown that ice recrystallization during freeze–thaw cycles leads

to an increase in ice crystal size at the expense of the structural integrity of the cell.[46] Although glycerol provides some protection against ice recrystallization, its protective ability at a concentration of 40% is limited to its use with a slow cooling rate (1°C/minute) and sustained storage at ≤ −65°C. Further research is required to determine the range of specifications of the protective effects for 40% glycerol during different types of TWEs, including different cooling and warming rates and exposure lengths at various temperatures.

The objective of the current study was to assess the quality of RCCs that had been cryopreserved for an extended period and had been exposed to unintentional "real-world" TWEs. Determining the significance, or lack thereof, of TWEs impact on RCC quality can assist in evidence-based decision practices regarding inventory management of units for patients with rare phenotypes, which is particularly critical as these products may be the only transfusable unit available to these patients. The novel findings from this study revealed single exposures to these specific TWEs, which occurred at Canadian blood centers, did not significantly impact the quality of RCCs post-deglycerolization. The application of these conclusions to any unit experiencing a TWE is cautioned as multiple factors may impact the quality outcome. These include but are not limited to how the RCCs were cryopreserved, the maximum temperatures reached outside of the critical range, and the length of time spent outside of that temperature range. Our work emphasizes that to elucidate the effect of any given TWE, comprehensive documentation of all potential variables should be included in any post-event investigation. While TW units should still be managed on a case-by-case basis, our work provides evidence to support a broader policy of unit retention by inventory management centers.

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CONFLICT OF INTEREST STATEMENT

The authors disclose no conflicts of interest relevant to this study.

ORCID

Celina Phan https://orcid.org/0009-0009-1077-4863
Mahsa Yazdanbakhsh https://orcid.org/0000-0002-2677-4747
Behrouz Ehsani-Moghaddam https://orcid.org/0000-0002-5038-1118
Jason P. Acker https://orcid.org/0000-0002-1445-827X

REFERENCES

1. Blake JT, Clarke G. Modeling rare blood in Canada. Transfusion. 2019;59(2):582–92. https://doi.org/10.1111/trf.15027
2. Laureano M, Clarke G, Yan MTS. How do I provide rare red cells to patients? Transfusion. 2022;63(4):670–8.
3. Goldman M, St CL. Rare donor program: Canadian Blood Services. Immunohematology. 2016;32(1):15–6.
4. Turner TR, Acker JP. A Canadian perspective on the use and preparation of cryopreserved red cell concentrates. Transfus Apher Sci. 2020;59(4):102853. https://doi.org/10.1016/j.transci.2020.102853
5. Howell A, Turner TR, Hansen A, Lautner LJ, Yi Q, Acker JP. Closed system processing variables affect post-thaw quality characteristics of cryopreserved red cell concentrates. Transfusion. 2022;62(12):2577–86. https://doi.org/10.1111/trf.17138
6. Blood and blood components. CAN/CSA-Z902:20: CSA Group. 2020.
7. Valeri CR, Ragno G, Pivacek LE, Cassidy GP, Srey R, Hansson-Wicher M, et al. An experiment with glycerol-frozen red blood cells stored at −80 degrees C for up to 37 years. Vox Sang. 2000;79(3):168–74. https://doi.org/10.1159/000031236
8. Fabricant L, Kiraly L, Willes C, Differding J, Underwood S, Deloughery T, et al. Cryopreserved deglycerolized blood is safe and achieves superior tissue oxygenation compared with refrigerated red blood cells: a prospective randomized pilot study. J Trauma Acute Care Surg. 2013;74(2):371–7.
9. Hampton DA, Wiles C, Fabricant LJ, Kiraly L, Differding J, Underwood S, et al. Cryopreserved red blood cells are superior to standard liquid red blood cells. J Trauma Acute Care Surg. 2014;77(1):20–7; discussion 26–7. https://doi.org/10.1097/TA.0000000000000268
10. Hult A, Malm C, Oldenborg PA. Transfusion of cryopreserved human red blood cells into healthy humans is associated with rapid extravascular hemolysis without a proinflammatory cytokine response. Transfusion. 2013;53(1):28–33. https://doi.org/10.1111/j.1537-2995.2012.03710.x
11. Ashenden M, Morkeberg J. Net haemoglobin increase from reinfusion of refrigerated vs. frozen red blood cells after autologous blood transfusions. Vox Sang. 2011;101(4):320–6. https://doi.org/10.1111/j.1423-0410.2011.01493.x
12. Jahan S, Kaushal R, Pasha R, Pineault N. Current and future perspectives for the cryopreservation of cord blood stem cells. Transfus Med Rev. 2021;35(2):95–102. https://doi.org/10.1016/j.tmrv.2021.01.003
13. Pasha R, Howell A, Turner TR, Halpenny M, Elmoazzen H, Acker JP, et al. Transient warming affects potency of cryopreserved cord blood units. Cytotherapy. 2020;22(11):690–7. https://doi.org/10.1016/j.jcyt.2020.04.039
14. Quintana AB, Coda Zabetta CD, Baumgartner NO, Biancardi ME, Bessone V, Rodriguez JV, et al. Morphological and biochemical analysis of human cardiac valve allografts

after an increment of the cryostorage temperature. Cryobiology. 2009;59(1):96–101. https://doi.org/10.1016/j.cryobiol.2009.05.003
15. Germann A, Oh YJ, Schmidt T, Schon U, Zimmermann H, von Briesen H. Temperature fluctuations during deep temperature cryopreservation reduce PBMC recovery, viability and T-cell function. Cryobiology. 2013;67(2):193–200. https://doi.org/10.1016/j.cryobiol.2013.06.012
16. Chabot D, Tremblay T, Pare I, Bazin R, Loubaki L. Transient warming events occurring after freezing impairs umbilical cord-derived mesenchymal stromal cells functionality. Cytotherapy. 2017;19(8):978–89. https://doi.org/10.1016/j.jcyt.2017.04.005
17. Briard JG, Poisson JS, Turner TR, Capicciotti CJ, Acker JP, Ben RN. Small molecule ice recrystallization inhibitors mitigate red blood cell lysis during freezing, transient warming and thawing. Sci Rep. 2016;6:23619. https://doi.org/10.1038/srep23619
18. Mazur P. Freezing of living cells: mechanisms and implications. Am J Physiol. 1984;247(3 Pt 1):C125–42. https://doi.org/10.1152/ajpcell.1984.247.3.C125
19. Valeri CR, Pivacek LE, Cassidy GP, Ragno G. In vitro and in vivo measurements of human RBCs frozen with glycerol and subjected to various storage temperatures before deglycerolization and storage at 4 degrees C for 3 days. Transfusion. 2001;41(3):401–5. https://doi.org/10.1046/j.1537-2995.2001.41030401.x
20. Acker JP, Hansen AL, Kurach JD, Turner TR, Croteau I, Jenkins C. A quality monitoring program for red blood cell components: in vitro quality indicators before and after implementation of semiautomated processing. Transfusion. 2014;54(10):2534–43. https://doi.org/10.1111/trf.12679
21. Zwart A, van Assendelft OW, Bull BS, England JM, Lewis SM, Zijlstra WG. Recommendations for reference method for haemoglobinometry in human blood (ICSH standard 1995) and specifications for international haemiglobinocyanide standard (4th edition). J Clin Pathol. 1996;49(4):271–4. https://doi.org/10.1136/jcp.49.4.271
22. Almizraq R, Tchir JD, Holovati JL, Acker JP. Storage of red blood cells affects membrane composition, microvesiculation, and in vitro quality. Transfusion. 2013;53(10):2258–67. https://doi.org/10.1111/trf.12080
23. Mykhailova O, Olafson C, Turner TR, D'Alessandro A, Acker JP. Donor-dependent aging of young and old red blood cell subpopulations: metabolic and functional heterogeneity. Transfusion. 2020;60(11):2633–46. https://doi.org/10.1111/trf.16017
24. Kister J, Wajcman H. Oxygen equilibrium measurements of human red blood cells. Methods Mol Med. 2003;82:49–64. https://doi.org/10.1385/1–59,259–373-9:049
25. Guarnone R, Centenara E, Barosi G. Performance characteristics of Hemox-analyzer for assessment of the hemoglobin dissociation curve. Haematologica. 1995;80(5):426–30.
26. Usry RT, Moore GL, Manalo FW. Morphology of stored, rejuvenated human erythrocytes. Vox Sang. 1975;28(3):176–83. https://doi.org/10.1111/j.1423-0410.1975.tb02756.x
27. Kanias T, Lanteri MC, Page GP, Guo Y, Endres SM, Stone M, et al. Ethnicity, sex, and age are determinants of red blood cell storage and stress hemolysis: results of the REDS-III RBC-omics study. Blood Adv. 2017;1(15):1132–41. https://doi.org/10.1182/bloodadvances.2017004820
28. Vettore L, Zanella A, Molaro GL, de Matteis MC, Pavesi M, Mariani M. A new test for the laboratory diagnosis of spherocytosis. Acta Haematol. 1984;72(4):258–63. https://doi.org/10.1159/000206398
29. Stadnick H, Onell R, Acker JP, Holovati JL. Eadie-Hofstee analysis of red blood cell deformability. Clin Hemorheol Microcirc. 2011;47(3):229–39. https://doi.org/10.3233/CH-2010-1384
30. Nemeth N, Kiss F, Miszti-Blasius K. Interpretation of osmotic gradient ektacytometry (osmoscan) data: a comparative study for methodological standards. Scand J Clin Lab Invest. 2015;75(3):213–22. https://doi.org/10.3109/00365513.2014.993695
31. Valeri CR, Ragno G, Pivacek LE, Cassidy GP, Srey R, Hansson-Wicher M, et al. A multicenter study of in vitro and in vivo values in human RBCs frozen with 40-percent (wt/vol) glycerol and stored after deglycerolization for 15 days at 4 degrees C in AS-3: assessment of RBC processing in the ACP 215. Transfusion. 2001;41(7):933–9. https://doi.org/10.1046/j.1537-2995.2001.41070933.x
32. Phan C, Kurach J, Foxcroft M, Xu D, Olafson C, Clarke G, et al. Modification of deglycerolization procedure improves processing and post-thaw quality of cryopreserved sickle trait red cell concentrates. Cryobiology. 2024;115:104903. https://doi.org/10.1016/j.cryobiol.2024.104903
33. Turner TR, Clarke G, Denomme GA, Skeate R, Acker JP. Effect of cryopreservation on a rare McLeod donor red blood cell concentrate. Immunohematology. 2021;37(2):78–83. https://doi.org/10.21307/immunohematology-2021-012
34. Holovati JL, Wong KA, Webster JM, Acker JP. The effects of cryopreservation on red blood cell microvesiculation, phosphatidylserine externalization, and CD47 expression. Transfusion. 2008;48(8):1658–68. https://doi.org/10.1111/j.1537-2995.2008.01735.x
35. Lelkens CC, Noorman F, Koning JG, Truijens-de Lange R, Stekkinger PS, Bakker JC, et al. Stability after thawing of RBCs frozen with the high- and low-glycerol method. Transfusion. 2003;43(2):157–64. https://doi.org/10.1046/j.1537-2995.2003.00293.x
36. Valeri CR, Srey R, Tilahun D, Ragno G. The in vitro quality of red blood cells frozen with 40 percent (wt/vol) glycerol at −80 degrees C for 14 years, deglycerolized with the Haemonetics ACP 215, and stored at 4 degrees C in additive solution-1 or additive solution-3 for up to 3 weeks. Transfusion. 2004;44(7):990–5. https://doi.org/10.1111/j.1537-2995.2004.04001.x
37. Yoshida T, Prudent M, D'Alessandro A. Red blood cell storage lesion: causes and potential clinical consequences. Blood Transfus. 2019;17(1):27–52. https://doi.org/10.2450/2019.0217-18
38. D'Alessandro A, Kriebardis AG, Rinalducci S, Antonelou MH, Hansen KC, Papassideri IS, et al. An update on red blood cell storage lesions, as gleaned through biochemistry and omics technologies. Transfusion. 2015;55(1):205–19. https://doi.org/10.1111/trf.12804
39. Tinmouth A, Chin-Yee I. The clinical consequences of the red cell storage lesion. Transfus Med Rev. 2001;15(2):91–107. https://doi.org/10.1053/tmrv.2001.22613
40. Beutler E, Meul A, Wood LA. Depletion and regeneration of 2,3-diphosphoglyceric acid in stored red blood cells. Transfusion. 1969;9(3):109–15. https://doi.org/10.1111/j.1537-2995.1969.tb05527.x
41. Karger R, Lukow C, Kretschmer V. Deformability of red blood cells and correlation with ATP content during storage as leukocyte-depleted whole blood. Transfus Med Hemother. 2012;39(4):277–82. https://doi.org/10.1159/000339809

42. Mykhailova O, Brandon-Coatham M, Phan C, Yazdanbakhsh M, Olafson C, Yi QL, et al. Red cell concentrates from teen male donors contain poor-quality biologically older cells. Vox Sang. 2024;119:417–27. https://doi.org/10.1111/vox.13602
43. Alexy T, Detterich J, Connes P, Toth K, Nader E, Kenyeres P, et al. Physical properties of blood and their relationship to clinical conditions. Front Physiol. 2022;13:906768. https://doi.org/10.3389/fphys.2022.906768
44. Sutera SP, Gardner RA, Boylan CW, Carroll GL, Chang KC, Marvel JS, et al. Age-related changes in deformability of human erythrocytes. Blood. 1985;65(2):275–82.
45. Rogers SC, Brummet M, Safari Z, Wang Q, Rowden T, Boyer T, et al. COVID-19 impairs oxygen delivery by altering red blood cell hematological, hemorheological, and oxygen transport properties. Front Physiol. 2023;14:1320697. https://doi.org/10.3389/fphys.2023.1320697
46. Mazur P, Leibo SP, Chu EH. A two-factor hypothesis of freezing injury. Evidence from Chinese hamster tissue-culture cells. Exp Cell Res. 1972;71(2):345–55. https://doi.org/10.1016/0014-4827(72)90303-5

SUPPORTING INFORMATION

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