

In vitro quality and hemostatic function of cold-stored CPDA-1 whole blood after repeated transient exposure to 28°C storage temperature

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Abstract

Background: Blood products are frequently exposed to room temperature or higher for longer periods than permitted by policy. We aimed to investigate if this resulted in a measurable effect on common quality parameters and viscoelastic hemostatic function of cold stored CPDA-1 whole blood.

Study Design and Methods: 450 ml of whole blood from 16 O Rh(D) positive donors was collected in 63 ml of CPDA-1 and stored cold. Eight bags were exposed to five weekly 4-h long transient temperature changes to 28°C. Eight bags were stored continuously at 4°C as a control. Samples were collected at baseline on day 1, after the first cycle on day 1 and weekly before each subsequent cycle (day 7, 14, 21, 28 and 35). Hemolysis, hematological parameters, pH, glucose, lactate, potassium, thromboelastography, INR, APTT, fibrinogen, and factor VIII were measured.

Results: CPDA-1 whole blood repeatedly exposed to 28°C did not show reduced quality compared to the control group on day 35. Two units in the test group had hemolysis of 1.1% and 1.2%, and two in the control group hemolysis of 0.8%. Remaining thromboelastography clot strength (MA) on day 35 was 51.7 mm (44.8, 58.6) in the test group and 46.1 (41.6, 50.6) in the control group ($p = .023$). Platelet count was better preserved in the test group (166.7 [137.8, 195.6] vs. 117.8 [90.3, 145.2], $p = .018$). One sample in the test group was positive for *Cutibacterium acnes* on day 35 + 6.

Conclusion: Hemolysis findings warrant further investigation. Other indicators of quality were not negatively affected.

KEYWORDS

CPDA-1, storage lesion, storage temperature, temperature violations, whole blood

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1 | INTRODUCTION

In recent years, whole blood has been widely adopted as an alternative to blood component-based resuscitation in massive hemorrhage, both prehospital and in-hospital.^{1–3} The drive to implement whole blood was largely initiated by the military, but the challenges faced by civilian emergency services are often comparable. For both civilian and military entities, there exists challenges not experienced by hospital-based medicine. For example, these groups are exposed to the extremes of temperature and extended periods away from hardened structures. Therefore, there is a need for blood that is readily available and has acceptable physiological and hemostatic function.

The required storage temperature of RBCs and whole blood is 2–6°C in Europe and 1–6°C in the US, with temperatures up to 10°C permitted during transport.^{4,5} Based on this, current practice in Norway is that blood should not be returned to inventory if it has been in room temperature for more than 30 min. Whole blood should be transfused within 4 h of removal from cold storage. The basis for the storage requirements appears to be studies on the time needed for the blood bag to reach a specific temperature, commonly 10°C. A much cited study by Pick et al. found that the surface temperature rose to 10.5°C 30 min after moving the blood from cold storage to room temperature.⁶ Similar results were reported from a study performed by Reiter et al.⁷

In reality, these time requirements are often exceeded, resulting in large wastage. In our center, 251 of 943 discarded RBC units (27%) and 12 of 129 discarded whole blood units (9%) in 2021 were related to temperature storage issues. Other centers experience even greater wastage. According to a report from the UK Blood Stocks Management Scheme, a fourth of red blood cell units (RBCs) discarded in the UK were because of storage temperature violations outside of the blood bank.

One potential way of reducing this wastage would be to increase the time allowed out of cold storage. The European guidelines were revised in 2020 to allow up to 60 min “subject to systems being in place to ensure this does not adversely impact the safety and quality of the components.”⁴ In the UK, this has been implemented for contingency use of WBC reduced RBCs in SAGM, if placed in quarantine for at least 6 h upon return to ensure that the temperature returns to 4°C.⁸

In a military setting, blood is a limited resource with supply chains that can easily be disrupted. Despite the use of cold transport cases, there is a risk of the blood being exposed to temperatures above the allowed limits

for longer than currently permitted. Discarding blood when the stock is limited and resupply difficult or impossible, can have fatal consequences.

Previous studies on RBCs have shown that various combinations of transient exposure to 22–30°C for durations of less than 24 h have limited effect on quality parameters such as hemolysis, glucose, lactate, potassium, and pH. Although there can be a reduction in ATP, it is still within acceptable levels.^{9–11}

Multiple studies have investigated whole blood storage at cold or warm storage separately. Pidcoke et al. found that there was little difference in hemolysis, hemoglobin, hemostatic function measured by thromboelastography, INR, APTT, fibrinogen, or factor VIII between CPD whole blood stored at 4°C and at 22°C for 5 days.¹² Glucose consumption was higher and pH fell at a higher rate when stored warm. Sivertsen et al. reported similar results in WBC reduced CPD whole blood after 3 days at 22°C or 2 h at 32°C.¹³

One study by Tzounakas et al. investigated CPDA-1 whole blood that had been gradually warmed to 17°C over a period of up to 8 h and then cooled back down to 4°C. They found hemolysis of 0.2% on day 35 and no bacterial contamination.¹⁴ They did not investigate other quality markers or consider hemostatic function. No studies have investigated the effect of exposing whole blood to repeated transient temperature changes on quality and hemostatic function.

In this study, we aimed to investigate the effect of exposing cold-stored CPDA-1 whole blood to weekly 4-h periods of 28°C over a 35-day storage period on hemolysis, hematological parameters, pH, glucose, lactate and potassium. In addition, we wanted to examine the potential effect on hemostatic function measured by thromboelastography and coagulation analyses.

2 | MATERIALS AND METHODS

2.1 | Study design and ethics

This laboratory study compared in vitro measurements of blood exposed to periodic changes in storage temperature (*test*) to blood stored continuously at 4°C (*control*). The study was carried out at the Department of Immunology and Transfusion Medicine at Haukeland University Hospital, Bergen, Norway. Sixteen donations were included, randomized to eight in each group. All blood was collected from volunteer donors after written informed consent. The Regional Committees for Medical and Health Research Ethics evaluated and approved the study (approval no. 2017/157).

2.2 | Blood collection

450 ml whole blood was collected from O Rh(D) positive donors of both sexes in bags containing citrate-phosphate-dextrose-adenine additive solution (CPDA-1) (PB-1CD456M5S, Terumo BCT, Lakewood, CO, USA). This is the same product as used by our military and likely to be used by us in civilian contingency scenarios. Donors were screened and approved for donation according to the standard local guidelines in our blood center. After collection, bags were kept at room temperature for 2 h before transfer to cold storage until the day after collection (day 1).

2.3 | Storage and temperature cycling

Blood in the test group was stored at 4°C in a temperature-monitored blood bank refrigerator and on days 1, 7, 14, 21, 28, and 35 transferred to a temperature-monitored incubator set to 28°C for 4 h before being returned to 4°C. Based on test measurements of core temperature done in blood bags, this would allow the blood to reach temperatures in excess of 8°C after the 30 minute limit for accepting the blood back in inventory and 26°C after the 4 h limit for storage outside the controlled storage area referred to in our transfusion guidelines. Blood in the control group was stored for a total of 35 days at 4°C in the same refrigerator (Figure 1).

2.4 | Sampling

Baseline sampling was performed before the first transient temperature cycle on day 1. An additional sample was taken 4 h after return to 4°C on day 1 to study the effect of a single cycle. Subsequent sampling was performed before each cycle on days 7, 14, 21, 28 and 35 to simulate a scenario where the blood is returned to the blood bank inventory and then used a week later (Figure 1).

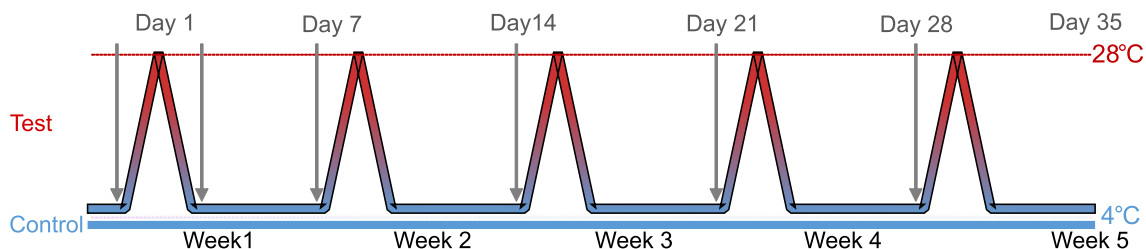


FIGURE 1 Cold-stored CPDA-1 whole blood was stored for 35 days and exposed to weekly 4-h temperature increases to 28°C followed by a return to 4°C. A control group was stored continuously at 4°C. Baseline samples were collected before cycling on day 1, followed by sampling 1 week after each cycle. An additional sample was taken 4 h after return to cold storage on day 1. Samples were taken from the control group at the same time points

Before sampling, bags were gently mixed by hand to ensure equal distribution. Sampling was performed by sterile welding a sample bag (BB*T015CM, Terumo BCT, Lakewood, CO) to the storage bag and transferring 15 ml of blood. An additional 20 ml was collected on day 1 and 35 for bacterial testing. Blood was centrifuged at 1800g for 10 min for analyses requiring plasma.

2.5 | Laboratory analyses

2.5.1 | Hematology

Hemoglobin in plasma was measured using a photometer (HemoCue Plasma/Low Hb System, Hemocue, Radiometer Medical). The percentage of hemolysis in the bag was then calculated as $10 \times \text{plasma_hgb} \times (1 - \text{hct}) / \text{hgb}$. Hemoglobin, hematocrit, platelet count and WBC count was analyzed using an automated hematology analyzer (Cell-Dyn Sapphire, Abbott Diagnostics). Platelet count was performed using the impedance channel of the analyzer according to established local procedures. Samples were analyzed in K₂EDTA.¹⁵

2.5.2 | Metabolic parameters

pH was measured on a blood gas analyzer (ABL825 FLEX, Radiometer Medical). Potassium, glucose, and lactate were analyzed on a chemistry analyzer (Cobas 8000/c702, Roche Diagnostics). The highest potassium concentration that could be quantitated was 25 mmol/L. Concentrations above this were recorded as 25 mmol/L for statistical purposes.

2.5.3 | Hemostatic function and coagulation

Hemostatic function was evaluated using a viscoelastic hemostatic assay with kaolin as activator (TEG 5000, Haemonetics). Time to first clot formation (R), clot kinetics (K),

rate of clot formation (angle) and maximum clot strength (maximum amplitude, MA) were recorded. An automated hemostasis analyzer was used to quantify fibrinogen and factor VIII concentrations and measure prothrombin time-international normalized ratio (INR) and activated partial thromboplastin time (APTT) (STA-R Evolution/STA-R Max, STA-Liquid Fib/STA-Fibrinogen 5, STA-Deficient VIII, STA-SPA+, STA-PTT Automate 5, STA-CaCl₂ and STA-Unicalibrator, Diagnostica Stago). Factor VIII samples were stored at -80°C prior to analysis.

2.5.4 | Bacterial growth

Bacterial testing was performed on days 1 and 35 to monitor potential bacterial growth. 10 ml blood was aseptically transferred to aerobic and anaerobic culture bottles (FA Plus/FN Plus) and incubated at 36°C for 7 days in the BacT/ALERT 3D 60 microbial detection system (bioMérieux). Positive samples were forwarded to a microbiological laboratory for verification and identification.

2.5.5 | Statistical analysis

All statistical analysis was performed using R version 4.1.1 (The R Foundation for Statistical Computing). Results were reported as means with 95% confidence intervals. To investigate the effect of storage time and temperature cycling, NLME package version 3.1–152 was used to fit a linear mixed effects model with treatment contrasts. Sample day, study group and their interaction were used as predictor for each outcome measure. The p -values given represent either the change from baseline measurements taken prior to the first cycle on day 1 to subsequent measurements on day 1, 7, 14, 21, 28 and 35 (p_{time}), or potential differences in this change between the test and control group (p_{test}). Mean values at each sample point were also compared using an unequal variances t -test (p_{mean}). A significance level of 5% was used for all analyses.

3 | RESULTS

3.1 | Hematology

As seen in Figure 2A, there was no difference in mean hemolysis in the test and control group. Irrespective of cycling, an increase in hemolysis was seen on day 14 and onwards. After three cycles and 21 days of storage, one

cycled unit had hemolysis above 0.8%. This increased to two units after five cycles and 35 days of storage (1.1% and 1.2%). The control group had two units with 0.8% hemolysis on day 35. Cycling did not negatively affect platelet count with a gradual decrease from day 7 to 28 in both groups ($p_{\text{time}} < .001$, $p_{\text{test}} > .05$). On day 28, blood in the test group had a higher platelet count than the control ($p_{\text{mean}} = .048$). After day 28, the control group flattened out while cycling resulted in a small increase on day 35 ($p_{\text{test}} = .018$) (Figure 2B).

Hemoglobin remained stable through cycling and storage, with a hemoglobin on day 35 of 12.2 g/dl (11.4, 12.9) in the test group and 12.4 g/dl (11.5, 13.2) in the control group ($p_{\text{time}} = .853$). Hematocrit increased from 39% (36, 41) to 40% (38, 43) in the test group and 39% (36, 42) to 41% (38, 43) in the control group ($p_{\text{time}} < .001$, $p_{\text{test}} > .05$). WBC counts decreased with storage, falling from $5.0 \times 10^9/\text{L}$ (4.1, 6.0) on day 1 to $2.0 \times 10^9/\text{L}$ (1.4, 2.6) on day 35 in the test group and $4.8 \times 10^9/\text{L}$ (4.3, 5.4) to $2.3 \times 10^9/\text{L}$ (1.6, 3.0) in the control group ($p_{\text{time}} < .001$, $p_{\text{test}} > .05$).

3.2 | Clinical chemistry

Cycling resulted in a minor increase in glucose consumption and lactate production compared to the control group ($p_{\text{time}} < .001$, $p_{\text{test}} < .05$) (Figure 3A,B). pH and potassium levels were slightly lower throughout storage with cycling (Figure 3C,D).

3.3 | Hemostatic function and coagulation

Initial cycling on day 1 did not result in a significant difference in hemostatic function measured by kaolin-activated thromboelastography. Cycling caused better preservation of angle on day 28 and 35, and better preserved K and MA on day 35 (MA 51.7 mm [44.8, 58.6] vs. 46.1 mm [41.6, 50.6], $p = .023$). A greater increase in R time was seen in the test group on day 7 ($p_{\text{test}} = .030$), but by day 14 no further significant difference was seen. Storage did not affect LY30. Mean MA remained within normal ranges for patient samples on day 35 in the test group, but not in the control group. Hemostatic function is shown in more detail in Figure 4. As shown in Table 1, both groups had a large decrease in factor VIII ($p_{\text{time}} < .001$), a minor reduction in fibrinogen ($p_{\text{time}} = .039$) and a minor increase in INR ($p_{\text{time}} < .001$) after 35 days of storage. There was no difference in how these parameters developed during storage in the two groups ($p_{\text{test}} = .486$ and $p_{\text{test}} = .059$, respectively).

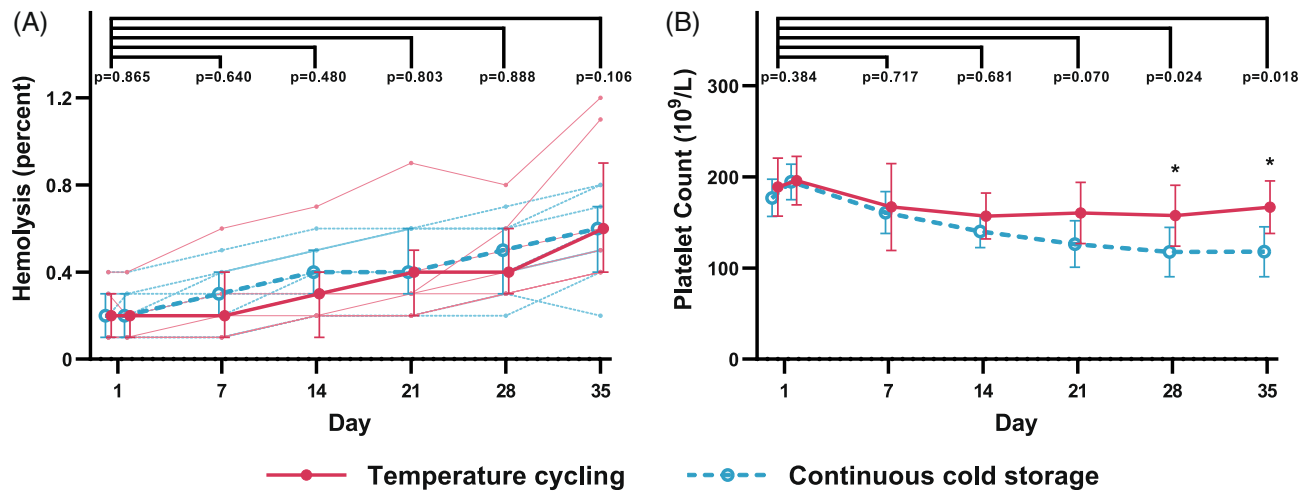


FIGURE 2 Hemolysis (A) and platelet count (B) in CPDA-1 whole blood stored continuously at 4°C (blue) or exposed to weekly 4-h temperature increases to 28°C (red). Points show mean values with 95% confidence intervals. *p*-values show significance for potential differences in change from day 1 to each time point in the test group compared to the control group (linear mixed effects model, R version 4.1.1 with NLME version 3.1–152, the R Foundation for Statistical Computing). * indicates $p < .05$. Thin lines represent individual blood bags

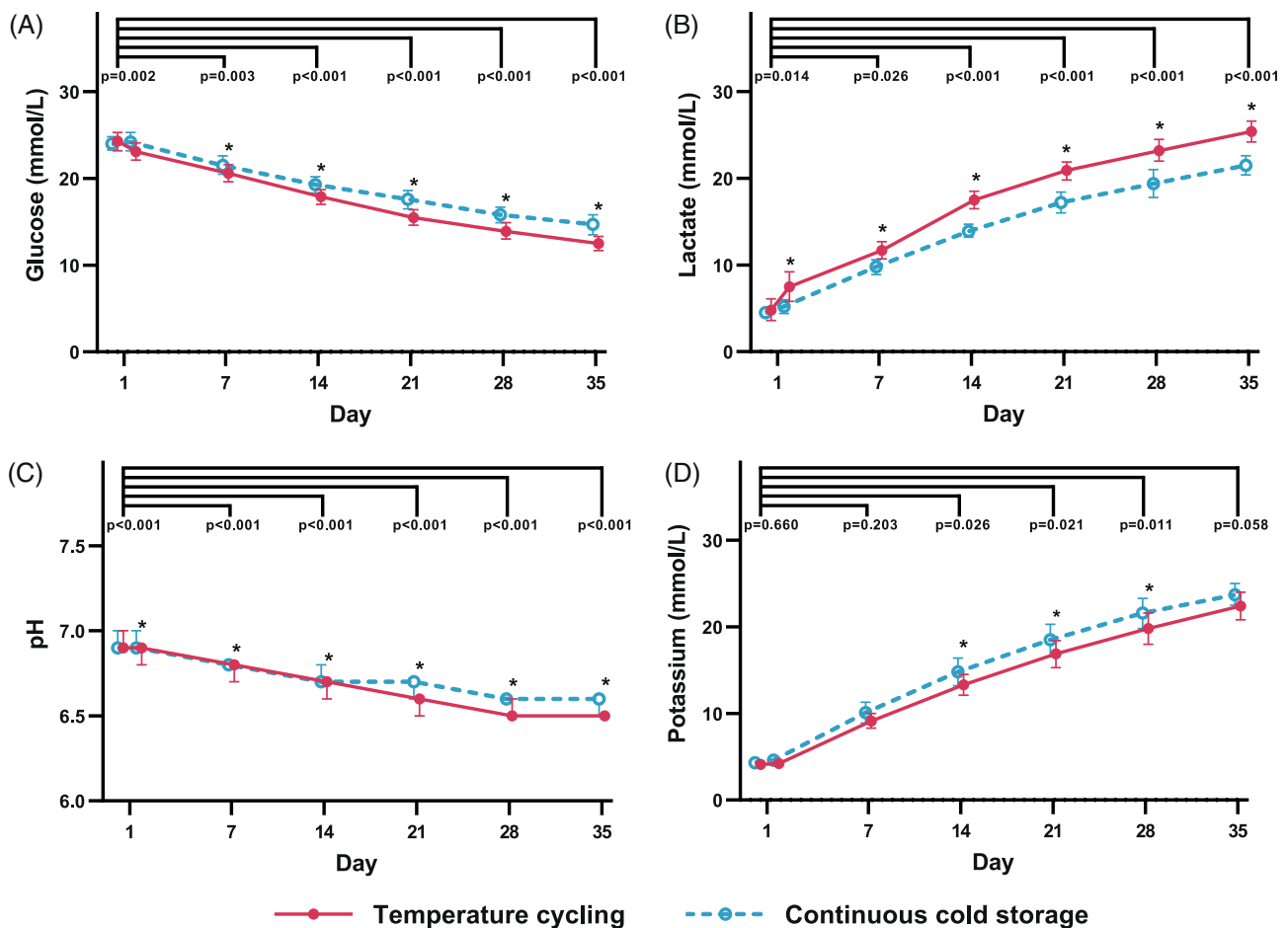


FIGURE 3 Glucose (A), lactate (B), pH (C), and potassium (D) in CPDA-1 whole blood stored continuously at 4°C (blue) or exposed to weekly 4-h temperature increases to 28°C (red). Points show mean values with 95% confidence intervals. *p*-values show significance for potential differences in change from day 1 to each time point in the test group compared to the control group (linear mixed effects model, R version 4.1.1 with NLME version 3.1–152, the R Foundation for Statistical Computing). * indicates $p < .05$

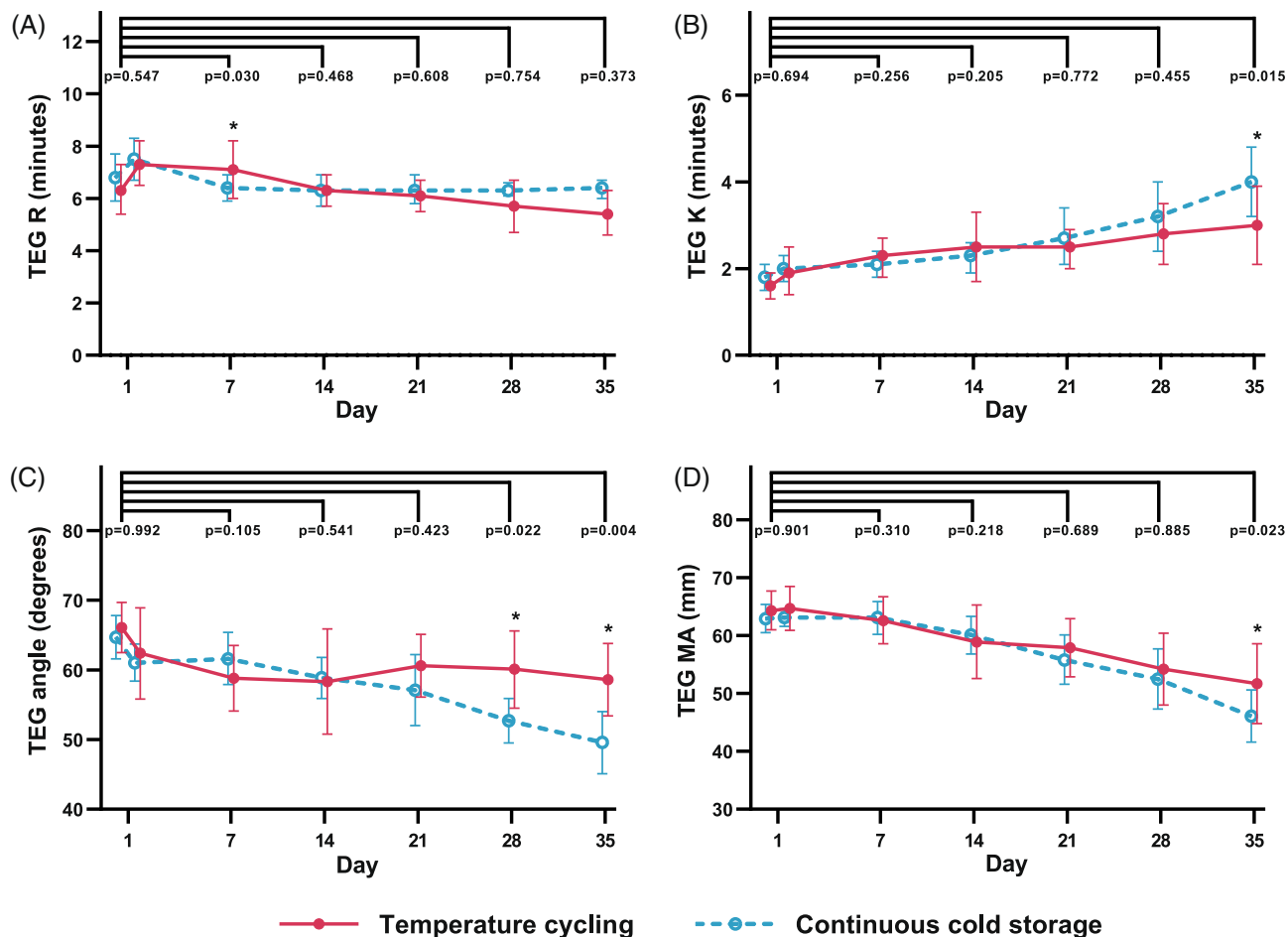


FIGURE 4 Thromboelastography parameters R (A), K (B), angle (C), and MA (D) in CPDA-1 whole blood stored continuously at 4°C (blue) or exposed to weekly 4-h temperature increases to 28°C (red). Points show mean values with 95% confidence intervals. *p*-values show significance for potential differences in change from day 1 to each time point in the test group compared to the control group (linear mixed effects model, R version 4.1.1 with NLME version 3.1–152, the R Foundation for Statistical Computing). Thin lines represent individual blood bags. * indicates $p < .05$

		Before 1st cycle	After 1st cycle	Day 35
Fibrinogen	Control	2.8 (2.4, 3.2)	2.8 (2.4, 3.2)	2.6 (2.3, 3.0) ^a
	Cycling	2.8 (2.4, 3.1)	2.8 (2.4, 3.2)	2.5 (2.1, 2.9)
Factor VIII	Control	80 (60, 100)	77 (57, 97)	18 (12, 25) ^a
	Cycling	78 (58, 97)	73 (58, 88)	23 (15, 32)
INR	Control	1.0 (1.0, 1.1)	1.1 (1.0, 1.1)	1.2 (1.1, 1.3) ^a
	Cycling	1.1 (1.0, 1.1)	1.0 (1.0, 1.1)	1.1 (1.0, 1.2)
APTT	Control	41 (38, 44)	41 (37, 44)	63 (47, 79) ^a
	Cycling	39 (36, 41)	39 (37, 42)	48 (44, 52)

Note: Values are means with 95% confidence intervals. There were no statistically significant differences between the control and cycling groups. (Linear mixed effects model, R version 4.1.1 with NLME version 3.1–152, The R Foundation for Statistical Computing).

^aSignificant change from before first cycle ($p < .05$).

TABLE 1 Coagulation parameters in CPDA-1 whole blood stored continuously at 4°C (control) or exposed to weekly 4-h temperature increases to 28°C (cycling)

3.4 | Bacterial growth

Bacterial testing was positive for *Cutibacterium acnes* in one bag in the test group on day 35 + 6. The bag was

negative on day 1 + 7. The bag had already been discarded and it was not possible to obtain a confirmatory sample. The bag did not differ in hemolysis, pH or other parameters from the other bags.

4 | DISCUSSION

In this study, we looked at the effect of exposing cold-stored CPDA-1 whole blood to weekly 4-h periods of 28°C over a 35-day storage period on hemolysis, hematological parameters, pH, glucose, lactate, potassium, thromboelastography and coagulation analyses. Our findings did not show any clear clinically significant differences in these parameters from exposing the blood to this scenario.

There was significant increase in hemolysis during storage in both groups, with no difference in mean hemolysis (day 35 $p_{\text{test}} = .106$). Two bags in the test group deviated from the rest, with a steep increase in hemolysis towards the end of the storage period. Additionally, two bags in the control presented with hemolysis of 0.8% on day 35, marginally exceeding the European standards of <0.8%.⁴ As seen in Figure 2A, there is variation in the initial hemolysis. It is not possible to state with confidence how this initial difference influenced the increasing hemolysis during storage, which may be affected by lipemia or donor-related variables like sex, age and donation interval.¹⁶

The effect of storage on hematological parameters confirmed the results from other studies on CPDA-1 whole blood,^{17–20} with stable hemoglobin and hematocrit, and a decrease in platelet count and WBC count. Aggregates prevented accurate counting of platelets in two of the test bags, one on day 7 and one on day 35. Platelet count was the only hematological parameter affected by cycling. The reduction in the test group was less than what was seen in the whole blood units that were stored continuously at 4°C.

Based on only minor significant differences in glucose, lactate, potassium and pH after multiple cycles, it did not seem that repeated transient exposure to 28°C resulted in greater storage lesion than continuous storage. We did not analyze ATP or 2,3-DPG in this study. However, a 2007 study by Hughes et al. found only a 10% reduction in ATP after 72 h of storage at 25°C in CPD.

Repeated transient exposure to 28°C resulted in better preservation of hemostatic function as measured by thromboelastography, showing faster clot formation (K, angle) and greater final clot strength (MA). The clot strength value reflects the effect of both platelets (approximately 80%) and fibrinogen (approximately 20%), and removal of platelets from samples has been shown to lead to a large reduction in MA.^{21,22} It has also been shown that cold stored platelets can retain hemostatic function measured with thromboelastography and aggregometry on day 21.²³ With no significant effect of cycling on coagulation measurements or hematology other than platelet count, it seems likely that the thromboelastography response is related to remaining platelet or platelet fragment function.

The *C. acnes* found in the sample from one of the test bags on day 35 + 6 could be due to a contamination during bacterial sampling on day 35, or it could have been introduced during blood collection. *C. acnes* is a slow-growing bacteria that grows best at temperatures of 30–37°C and can be difficult to detect. The period of time exposed to 28°C in our study was short at 4 h, reducing the likelihood of temperature induced increased risk of growth.²⁴ When venipuncture is performed, there is always a risk of skin fragments entering the donation bag and contaminating it.^{25,26} This risk can to an extent be abated by the use of proper disinfection procedures and performing routine bacterial testing of the products. The use of a sample diversion pouch can further reduce the risk,^{27–29} something the CPDA-1 collection bags we used did not have. Additionally, a recent study showed that the presence of WBCs might be beneficial if the blood is stored at higher temperatures.¹⁷

In conclusion, repeated transient exposure of cold-stored CPDA-1 whole blood to 28°C for a 4-h period followed by a return to 4°C once a week over a 35-day period did not have any notable negative impact on the measured quality parameters and hemostatic function. The effect on hemolysis beyond three cycles over 21 days was uncertain and warrants further investigation. In a military or contingency setting, the risks related to increased hemolysis should be weighed against the need for blood availability.

Our study did have some limitations. Our focus was on in vitro parameters that would give an indication of safety and hemostatic function. We did not investigate factors affecting RBC function such as ATP and 2,3-DPG, and we did not look at platelet function or activation isolated. Furthermore, because the study was entirely in vitro, we cannot definitely say if our findings translate to what we would expect in vivo without conducting such clinical trials.

The European guidelines state that the 30-minute limit on how long blood can be out of controlled storage can be extended to 60 min if this can be shown not to affect safety or quality.⁴ Our findings add to the existing evidence of this being safe, but further investigation is warranted before standard guidelines are changed. Taken together with existing research however, the extension to three periods of up to 60 min described for RBCs in the UK contingency guidelines⁸ should also be possible for CPDA-1 whole blood in local contingency scenarios.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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