

## Making whole blood available in austere medical environments: donor performance and safety

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**BACKGROUND:** To provide whole blood on the battlefield can be a challenge, but a buddy system protocol is both an elegant and the only currently available means to supply blood to a Special Forces team in far-forward locations. Our aim was to investigate donor-safety associated with such a protocol.

**METHODS:** This study was a randomized, double-blinded, controlled trial that aimed to evaluate the immediate effects of a 450 cc blood donation on physical performance in fatigued and dehydrated Special Forces soldiers. The primary outcome variables were absolute and relative maximal oxygen uptake ( $VO_{2max}$ ), exercise tolerance time (ETT) and heart rate (HR).

**RESULTS:** Relative  $VO_{2max}$  decreased by 7.1% in the donation group between pre and posttest, compared to no change in the control group. Absolute  $VO_{2max}$  decreased by 11.2 and 3.6% between pre and posttest in the donation and control groups, respectively. Mean ETT in the donation group was on average 92 seconds shorter compared to baseline, which represents a decrease of 9.5%.

**CONCLUSION:** Donating blood after a week of strenuous physical activity is feasible for Special Forces personnel. While the donation results in some diminishment of  $VO_{2max}$ , a 3.6%-11.2% decrease in relative  $VO_{2max}$ , and in elevation of submaximal HR levels highly trained personnel continue to perform well both at both sub-maximal and maximal effort levels.

### INTRODUCTION

Knowledge gained by medical practitioners in military conflicts over the last decade has contributed to significant changes in transfusion strategies addressing traumatic hemorrhagic shock.<sup>1</sup> The transition from a crystalloid and/or colloid-based resuscitation approach to one that is blood-based is reflected in the fluid preferences for resuscitation of hemorrhagic shock recommended in the recent Tactical Combat Casualty Care (TCCC) guidelines. TCCC guidelines now recommend that whole blood (WB) be used as the primary resuscitation fluid.<sup>2</sup> This is also a current trend in the civilian EMS services in several countries.<sup>3</sup> And an increasing number of NATO and other allied nations are implementing TCCC guidelines in recognition of the published improvements in the survival of severely injured combat wounded over the course of the decade-long conflict. The guidelines have undergone continuous

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reevaluation, resulting in optimized treatment strategies that are associated with lower mortality and morbidity of wounded service personnel.<sup>4</sup>

Before the TCCC guidelines were changed in 2014,<sup>2</sup> some military units had already revised their protocols in favor of WB-based resuscitation strategies. The U.S. 75<sup>th</sup> Ranger Regiment and the Norwegian Naval Special Operations Commando (NORNAVSOC) have implemented protocols that now include the use of freeze-dried plasma, cold-stored whole blood, and warm fresh whole blood (WFWB). Successful WFWB implementation relies on the establishment of the so-called “buddy transfusion” system, in which military personnel are paired by compatible blood types so that donors are available for each other in the smallest of service units. It is based on a far-forward “walking blood bank” concept in which service personnel at the scene of action donate blood for immediate transfusion of a severely bleeding casualty. A buddy system protocol is not only an elegant way of providing an optimal, equal ratio blood product without the functional loss and logistical challenges associated with blood storage, it is the only currently available means to supply blood to military units operating in far-forward locations. We believe this could be equally valid in civilian settings like small, geographically remote communities, or even in cruise liners operating far away from medical treatment facilities. As an example, Royal Caribbean Cruiseliners (RCCL) has successfully implemented a “buddy transfusion” protocol on board their 32 cruise liners with excellent results. Some questions remain unanswered; however, such as whether the known reduction in exercise performance immediately after donation of 450 mL of blood causes significant impairment, and whether civilian proscriptions against post-donation exercise can be disregarded. The blood bank at Haukeland University Hospital in Bergen blood donors are instructed to avoid rigorous physical activity for 24 hours after donation of a unit of blood.

Execution of blood donation in military settings is not only a medical decision, but also a tactical one, and the potential for a “walking blood bank” to put the rest of the team in harms’ way must be fully evaluated. A literature search was conducted, and while several reports of change in exercise performance 24 hours post-donation were found these studies were insufficient to fully address the impact on overall performance.<sup>5-12</sup>

Physical performance after blood donation is poorly described in the literature before this study, and the effect of the extreme conditions encountered in a Special Forces field exercise on donor performance have not previously been evaluated. The main difference from other trials is the extent of preconditioning our subjects underwent before blood donation and retesting. In 2012 we postulated that donation would not have immediate post-donation effects, and to test the hypothesis we conducted

an exercise performance evaluation on physically fit members of a Special Forces unit and found that donor performance was not adversely affected immediately after donation of a full unit (450 mL).<sup>13</sup> This study was performed on rested service personnel under optimal conditions, however, which is unlikely to simulate realistic field conditions. During an actual mission, donors are likely to be suffering from fatigue, hunger, and dehydration, which could adversely affect the donor’s ability to maintain optimal performance after donation. A thorough literature search did not uncover relevant publications on this subject, and thus a follow-up study was designed to address this question. Such a study is necessary to adequately test whether “buddy transfusion” protocols can be implemented without incurring unacceptable risks to the donor. A far-forward WFWB resuscitation protocol in an austere environment with possible prolonged evacuation times is potentially life-saving.

Combat readiness skills are difficult to measure, and universally adopted, validated metrics to assess performance have not been established. In our previous study, we focused on two skills which are indispensable on most field missions and tested them independently; these were maximal oxygen consumption ( $VO_{2max}$ ) and hiking as a measure of endurance and shooting skills as a measure of cognitive performance. Prioritizing these skills would be difficult in a deployed environment, but given that endurance is a precondition of cognitive metrics of performance, this study focused on  $VO_{2max}$  assessment. We hypothesized that a 450 mL blood donation does not affect  $VO_{2max}$  performance under conditions of severe fatigue.

## MATERIALS AND METHODS

This study was a randomized, double-blinded, controlled trial that aimed to evaluate the immediate effects of a 450 mL blood donation on physical performance in fatigued and dehydrated subjects, approved by The Regional Ethics Committee in Bergen – Norway. The primary outcome variables were absolute and relative maximal oxygen uptake ( $VO_{2max}$ ), exercise tolerance time (ETT) and heart rate (HR). Direct measurement of  $VO_{2max}$  was selected as a marker of endurance capacity since it is often considered the “gold standard” method for determination of aerobic fitness.<sup>14</sup> ETT is a validated measure of physical performance.<sup>15</sup> Similarly, submaximal HR correlates with performance capacity,<sup>16</sup> and results were compared to those of the control population.

### Subjects and sampling

All NORNAVSOC soldiers participating in the 2013 spring exercise “Quick Feet” were invited to participate in this study (n = 13), all of who were male. No females were

available for invitation. Successful completion of the exercise was the inclusion criterion, and pre-exercise data collected from excluded subjects were not included in the final analysis. All participants provided written consent after full disclosure of risks and benefits were presented in both oral and written form. As expected, not all participants were able to finish the exercise, one due to arthritis and one due to general illness, and thus were excluded from the final analysis. After randomization, the study groups consisted of six donors and five controls.

The mission consisted of a 6-day field exercise in which subjects endured 16-18 hours/day of marching through rough terrain carrying all necessary supplies in a backpack weighing 16-18 kg. The exercise simulates conditions in which participants are caught behind enemy lines and must evade capture while traveling to safety. Randomization using the coin flip technique was performed after completion of exercise. Power analysis was not possible to perform due to lack of similar work done in the past.

### Measurements

All required personnel and equipment to perform the study, including a deployable test-lab for  $VO_{2max}$  testing, was forwarded to the Naval Special Forces facility in northern Norway. Pre-exercise testing and post-exercise and blood donation testing was performed according to the same protocol, under standardized conditions, and test technicians remained unchanged.

During exercise preparations at the base all candidates underwent baseline testing one day before they were deployed. Candidates returned to the base via helicopter at the end of the exercise and reported for retesting within 20 minutes of arrival. Blood samples (10 mL total) were collected in glass tubes (Vacuette EDTA and GEL) before blood donation and  $VO_{2max}$  testing. The GEL glass tubes underwent centrifugation within 30-120 minutes after blood withdrawal, at 3000 rpm for 10 minutes and then stored at room temperature for 6 hours. The EDTA glass tubes were stored at room temperature for 6 hours. Room temperature storage and centrifugation was done at the test site before blood samples were sent to the University Hospital of Northern Norway – Harstad for analysis, a 1-hour drive away. Body weight was measured using a calibrated, digital scale, and stadiometer (model 708; Seca Corp. Hamburg, Germany) to the nearest 0.1 kg and 0.5 cm, respectively. Subjects wore light sport clothing and removed their shoes before being weighed. Before analysis, 0.2-0.5 kg was subtracted from the weight to adjust for clothing weight.

All candidates underwent venipuncture of the middle antecubital vein with a 14-gauge needle (Terumo BCT, Lakewood, CO) regardless of study assignment, and received blacked-out goggles and Peltor Optime II Ear-muffs to prevent them from discovering whether a unit of

blood was being taken or not. Blood units were collected in standard CPDA-containing blood bags (Terumo BCT, Lakewood, CO) and a blood collection mixer scale (Baxter, Deerfield, IL) was programmed to collect 450 mL of blood, following current standards for blood collection in Norwegian blood banks. For subjects in the control arm, the collection bag tubing was occluded to prevent blood collection. Investigators involved in data analysis were blinded to donation allocation by physically separating the  $VO_{2max}$  test laboratory from the blood donation site.

$VO_{2max}$  was measured according to the manufacturer's instructions through a three-way directional valve (model 2700, Hans Rudolf Inc, Kansas City, MO) according to the Bruce Protocol in the mobile test laboratory, starting at 2.7 km/hour and 10% inclination.<sup>17</sup> The test was performed on a treadmill (PPS 55 Sport, Woodway GmbH, Weil am Rhein, Germany) using an automatic predefined stepwise protocol with increasing speed and incline. The treadmill was calibrated on elevation and speed before both tests. The subjects ran until exhaustion, and ETT to the nearest second was registered. HR was monitored in all subjects during the test (S610, Polar Electro OY, Kempele, Finland) and the highest HR attained was defined as peak heart rate (HR-peak). Oxygen uptake was measured continuously with an automatic metabolic system (Oxygon Pro, Erich Jaeger GmbH, Hoechberg, Germany) using the mixing chamber mode setting at 30-second sampling intervals. This system has been verified to be accurate for measuring oxygen uptake.<sup>17</sup> Testing was performed in a temperature-controlled environment (17°C-20°C) with adequate ventilation. The online system was gas-calibrated with room air and certified calibration gases, and volume was calibrated manually with a 3 L syringe (Hans Rudolf Inc., Kansas City, MI) before every second test. The mean of the two highest consecutive measurements was defined as  $VO_{2max}$ . The determination of whether maximal effort had been reached during the  $VO_{2max}$  test was a subjective assessment made by the test technician. In cases when there was doubt as to whether  $VO_{2max}$  was reached, the test was accepted if peak respiratory exchange ratio was above or equal to 1.05.

### Statistics

The normal distribution of primary outcome variables was assessed with the Shapiro-Wilk test and visual inspection. Differences within groups (pre to posttest) were analyzed with a paired-sample t test, and an unpaired t tests was used to check for differences between groups (BD vs. CON group). Data are shown as mean values, including 95% confidence intervals (CI) or 1 standard deviation (SD). Statistical analyses were performed in SPSS (version 21, IBM Corp., Armonk, NY). A p-value <0.05 was considered statistically significant.

TABLE 1. ●●●

	BD (n = 6)			CONT (n = 5)		
	Pre	Post	Change	Pre	Post	Change
BW (kg)	87.7 (83.7, 91.7)	83.9 (80.1, 87.6)	-3.8 (-4.7, -3.0)*	87.5 (68.5, 106.5)	84.6 (66.8, 102.4)	-2.9 (-4.5, -1.3)*
ETT (sec)	968 (933, 1002)	876 (813, 939)	-92 (-144, -40)*	970 (921, 1019)	937 (849, 1025)	-33 (-80, 14)
VO <sub>2max</sub> (L/min)	4.91 (4.59, 5.23)	4.36 (3.98, 4.73)	-0.55 (-0.70, -0.41)*†	5.03 (4.22, 5.84)	4.85 (4.18, 5.51)	-0.18 (-0.45, 0.08)
VO <sub>2max</sub> (mL/kg/min)	56.0 (52.9, 59.2)	52.0 (48.4, 55.6)	-4.0 (-5.6, -2.5)*†	57.8 (53.4, 62.3)	57.8 (51.3, 64.4)	0 (-2.8, 2.8)
RER	1.19 (1.17, 1.21)	1.13 (1.09, 1.17)	-0.06 (-0.10, -0.02)*	1.19 (1.15, 1.23)	1.13 (1.09, 1.18)	-0.06 (-0.12, 0)
Ventilation (L/min)	177 (164, 191)	162 (150, 174)	-15 (-39, 8)	175 (150, 201)	168 (149, 186)	-8 (-23, 8)

NB.  $p = 0.06$  for difference in change in ETT between BD and CONT group.

\*  $p < 0.05$  between pre and posttest within group.

†  $p < 0.05$  between BD and CONT group.

BW = body weight; BF = body fat; ETT = exercise tolerance time; VO<sub>2max</sub> = maximal oxygen uptake; RER = respiratory exchange ratio.

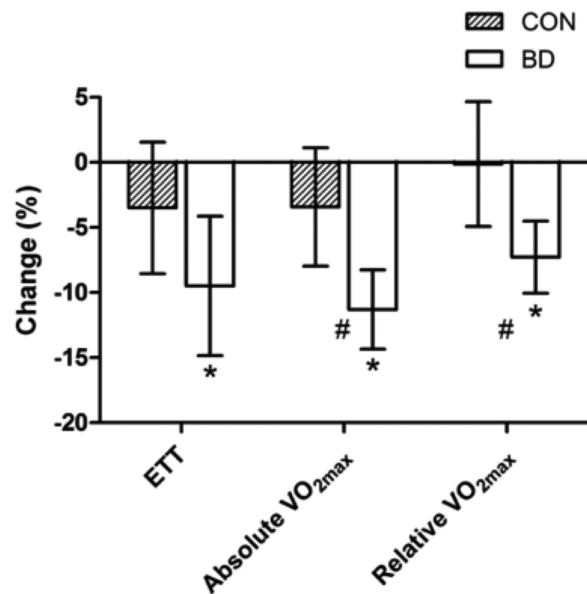


Fig. 1. Figure shows percentage difference in exercise tolerance time (ETT), relative and absolute oxygen uptake between the control group (CON) and the donation group (BD), and inbetween the same group. \* indicates  $p < 0.05$  between pre and posttest within group. # indicates  $p < 0.05$  between BD and CONT group.

## RESULTS

There were no significant differences in age or weight between the two groups. Mean bodyweight in the test and control groups varied before and after the exercise, but not between groups (loss of 3.8 and 2.9 kg, respectively; Table 1).

The Shapiro-Wilk test confirmed that primary outcome variables (VO<sub>2max</sub>, ETT, and HR) were normally distributed. Relative VO<sub>2max</sub> calculations showed a 7.1% decrease in mean VO<sub>2max</sub> in the donation group between

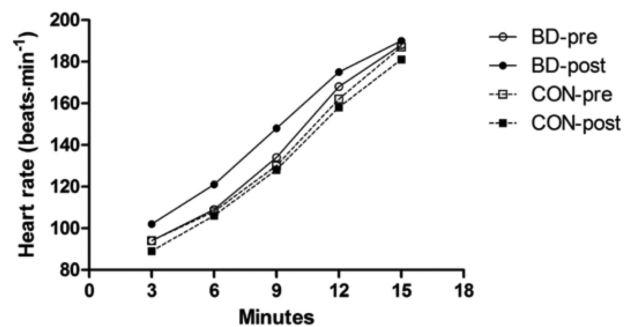


Fig. 2. Figure shows mean submaximal HR (y-axis) level at the end of each level of the Bruce protocol (x-axis). The four groups are: BD pre – Donation group pre-exercise, BD post – Donation group post-exercise, CON pre – Donation group pre-exercise, CON post – Donation group post-exercise.

Test 1 and Test 2 (Fig. 1), compared to no change in the control group. Absolute VO<sub>2max</sub> decreased by 11.2 and 3.6% between pre and posttest in the donation and control groups, respectively (Fig. 1). Differences were statistically significant in both cases.

Mean ETT in the donation group was on average 92 seconds shorter compared to baseline, which represents a decrease of 9.5% (Fig. 1). Yet, this change did not reach significant level ( $p = 0.06$ ).

Figure 2 shows mean submaximal HR level at the end of each level of the Bruce protocol. Donor HR increased significantly compared to baseline at all submaximal levels. Control group HR did not differ at submaximal performance levels post-exercise.

## Hemoglobin

Mean Hemoglobin (Hb) decreased significantly in both the donation and control groups, but differences within groups were not significant at any time point ( $p \geq 0.05$ ). Test subject mean Hb values were 14.6 and

14.0 g/dL on baseline day and donation day, respectively ( $p < 0.05$ ), and those for the control group were 15.5 and 14.7 g/dL, respectively ( $p < 0.05$ ).

## DISCUSSION

In this study, we found that donation of 450 mL of blood after a strenuous military field exercise reduced  $VO_{2max}$  by 3.6%-11.2% compared to baseline values. The subjects in the control group did not display a similar decline in performance or  $VO_{2max}$  after the military exercise. Both results are in line with our expectations that losing 450 mL of blood will result in some degree of physical performance loss.

Donating blood increased submaximal HR during physical exercise in the donation group. Thus, both maximal and submaximal exercise performance were reduced after blood donation; however, ETT was not significantly different in either of the two groups either pre- or post-exercise, and this might be explained by the low numbers of participants.

The significant drop in  $VO_{2max}$  we found in the donation group correlates with the findings of Balke et al.,<sup>5</sup> who concluded in their 1954 paper that "Loss of blood in amounts customary in blood donation imposes significant limitations on physiological adjustments to severe exercise." This study has a time frame from blood donation to testing of 1 hour. Sixty years later, Gordon et al. examined 15 athletes on a bicycle test with incremental increase in resistance to volitional exhaustion after donation of 450 mL blood (6), reporting in 2014 that they found significant decreases in  $VO_{2max}$  compared to pre-donation exercise. Burnley et al. examined 11 young healthy males on a bicycle trial with incremental increase in resistance pre- and post-donation of 450 mL of blood (4), and recorded changes in  $VO_2$  kinetics, ETT, and  $VO_{2max}$ . As in the above-mentioned studies, significant decreases in  $VO_{2max}$  were noted between donation and control groups, and lower  $VO_{2max}$  after blood donation alone has been reported in multiple studies.<sup>6-9</sup> In every case, performance testing was delayed for 24 hours after donation, which allows Hb levels to decrease as a result of Starling forces, and thus normal circulating blood volume was reestablished before  $VO_{2max}$  was assessed. In a test of 10 male cyclists 2 hours post-donation of one unit of blood Panebianco et al. found that  $VO_{2max}$  performance decreased, but submaximal performance remained unchanged.<sup>6</sup> Because time between blood donation and testing was shorter (2 hr), this work was more similar to our study design; however, the method for measuring  $VO_{2max}$  differed, and thus results are difficult to compare.

The commonly accepted explanation for the decrease in  $VO_{2max}$  after blood donation is that Hb concentrations decrease, thus, as described by Fick's principle, delivery of oxygen ( $DO_2$ ) during maximal performance is affected.

Decreases in Hb concentrations were seen in both groups at the time of the posttest, most likely due to the effect of rehydrate fluids available to study participants during blood donation and the posttest blood sampling period. This study did not record the amount of fluid intake among study subjects before blood sampling, thus a full analysis is not possible. It is unlikely that the decrease in Hb concentrations can be explained by hemodilution after water intake in the timeframe between completion of exercise and blood sampling and we can neither rule out test variability, thus differences could not be fully explained at this time. The change in Hb may partly explain the decrease in  $VO_{2max}$  but it does not explain the differences between groups. Hb decreased in both groups, but the difference in  $VO_{2max}$  was limited to the donation group. A more likely explanation for the decrement in the donation group's  $VO_{2max}$  is that blood donation resulted in a reduction in circulation volume, with a concomitant reduction in venous return to the heart, and thus in reduced cardiac output and  $DO_2$ .

ETT was not significantly different in either the donation group or non-donation group between pre- and post-exercise testing. This finding was not consistent with that reported by Burnley et al. in 2006, which found a significant reduction in the time to exhaustion (of 54 sec or approx. 14%) after blood donation.<sup>10</sup> Possible reasons for the differences between that study and ours include that Burnley did not use a double-blinded study design, and our subjects were highly motivated Special Forces soldiers at peak physical condition. The low numbers of participants and the borderline p-value of 0.06 (9.5%) can also be of relevance to this difference from Burnley's work.

Factors that limit  $VO_{2max}$  were described by Bassett and Howley<sup>18</sup> in their seminal paper based on findings of A.V Hills published in the early 1920s. This work is often referred to as the "catastrophic model" because according to the authors, maximal exercise is eventually terminated due to a lack of oxygen delivery capacity to exercising muscle. The resulting anaerobic conditions that develop limit the production of ATP, causing muscle fatigue and eventual collapse. This model has been broadly criticized by Noakes et al.<sup>19</sup> who propose an alternative model termed the "Central Governor Model," with the main difference being the role of neural recruitment of skeletal muscle during exercise. Our test candidates were all Special Forces soldiers and they have been through extensive selection on skills like mental strength and stamina. This might affect their ability to perform under stress and thus affect their oxygen uptake by neural recruitment as explained by the "Central Governor Model."

Another difference in our population compared the normal population is both the weight and level of physical fitness. It has been shown that physical performance and bodyweight affects the blood volume, and thus the affect

of blood donation.<sup>20</sup> Due to these facts, our findings can probably not translate to the general population.

Conversely, HR at submaximal effort differed significantly in the donation group. This early change has not been previously reported and was not significant in the control group, although HR was significantly elevated in both groups at maximal effort. Potential reasons for the early changes in the donor population may be due to the reduction in blood volume resulting in reduced  $\text{DO}_2$ . During submaximal performance increased HR will compensate for the reduction in stroke volume to maintain  $\text{DO}_2$ . However, during maximal performance, and at maximal HR the reduction in stroke volume explains the change in peak performance between the two groups. Other studies may not have detected this early change due to longer intervals between donation and testing which allowed circulatory volumes to equilibrate. The closest comparator was the study design reported by Balke et al., who performed testing within 1 hour of blood donation. This study also detected a significant decrease in  $\text{VO}_{2\text{max}}$  after blood donation, but a change in HR was not reported. Gordon et al.<sup>12</sup> and Burnley et al.<sup>10</sup> performed post-donation testing at the 24 hours mark, after normovolemia was presumably established due to rehydration, and interstitial fluid shifts into the systemic circulation.

Our research group has previously found that  $\text{VO}_{2\text{max}}$ , rapid shooting skills and hiking performance are not compromised by blood donation. This study was conducted in a similar group of Special Forces soldiers, but not under duress but rather well fed and rested.<sup>13</sup> This study demonstrates that compensatory mechanisms are impaired under stress conditions, and loss of performance begins to occur.

These findings raise an important question, regarding whether the combined effects of reduced physical capacity and blood donation result in an unacceptable level of risk when donating blood under battlefield conditions. Ultimately, soldiers and care providers will have to make these determinations based on both medical and operational considerations; however, these study findings impart valuable information to assist operational decisions. While it is clear that implementation of a buddy transfusion protocol to provide WFWB for the resuscitation of severely bleeding casualties is not without risk, it is equally clear that even under stressful conditions Special Forces soldiers perform well and demonstrates that they can tolerate the loss of a unit of blood without compromising battle readiness. The shortest ETT time was 813 seconds, which is still a remarkably good result.

The limitations of this study include a small sample size, the lack of a method of estimating pulmonary artery pressure, and the ad libitum administration of rehydration fluids. In addition, the unique characteristics of these fit and motivated Special Forces soldiers limit generalizability of the study findings. Conversely, this study was not

limited by open study group allocation, as was previous research evaluating the effect of blood donation on donor performance. Whereas the subjects in previous studies were not blinded and may have been influenced by awareness of their blood loss, donors and investigators in this study were not aware of study assignments. The lack of cognitive testing is another weakness of this study, and therefore we cannot make conclusions regarding cognitive performance after blood donation.

To our knowledge, this is the first study to implement blinding of both donors and investigators, there by strengthening the validity of the results.

Understanding effects of blood donation on submaximal performance in a combat setting is a valuable addition to information regarding maximal performance results. Special Forces personnel are more likely to perform at a submaximal level when on assignment, whereas maximal performance is infrequently required. Additionally, study conditions cannot model the actual maximal capacity in life-threatening conditions when these highly trained elite forces will likely recruit additional reserves due to a massive sympathetic response.

For non Special Forces and civilian health care workers a similar decrease in performance might even be more tolerable, thus allowing them to safely serve as emergency donors under extreme situations.

The authors would like to call for additional studies on this field with more general populations, including cognitive testing after blood donation. Walking blood banks and emergency donors can be of great benefit and donor safety is important in such a setting.

## CONCLUSION

Donating blood after a week of strenuous physical activity is feasible for Special Forces soldiers. While the donation results in some diminishment of  $\text{VO}_{2\text{max}}$ , a 3.6%-11.2% decrease in relative  $\text{VO}_{2\text{max}}$ , and in elevation of submaximal HR levels, highly trained personnel continue to perform well both at both submaximal and maximal effort levels.

## CONFLICT OF INTEREST

Heather Pidcoke is employed by Terumo BCT. The remaining authors declare that they have no conflicts of interest relevant to the manuscript submitted to TRANSFUSION.

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