ORIGINAL ARTICLE

The Influence of Different Levels of Cardiorespiratory Fitness on Exercise Performance 24 Hour Post Blood Donation in Male Donors

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ABSTRACT

Introduction: Haematological parameters decrease following one unit of whole blood donation which results in a detrimental effect on cardiorespiratory fitness and maximal exercise capacity. However, it remains unclear to what extent blood donation will impact exercise performance across varying levels of cardiorespiratory fitness. The aim of this study is to compare the effects of a standard blood donation on maximal exercise performance performed 24 h post-blood donation in individuals with varying levels of cardiorespiratory fitness. **Methods:** Forty-two males (mean age 22 ± 2.1 years) were recruited and segregated into *Low, Moderate* and *Superior* fitness groups. Subjects performed a multi-stage shuttle run test on the day prior to a standard blood donation procedure, and subsequently 24 h post-blood donation. Blood samples were taken on both test days and analysed for haematological parameters. **Results:** Exercise performance, represented by predicted VO_2max were 0.6%, 1.0% and 4.1% lower in the *Low, Moderate* and *Superior* fitness groups respectively. However, the magnitude of reduction was only statistically significant in the Superior fitness group ($p = 0.017$). Compared to baseline, all fitness groups demonstrated significant reductions in haematocrit (*Low*: -8.4%, *Moderate*: -9.1%, *Superior*: -7.2%) and haemoglobin (*Low*: -7.6%, *Moderate*: -7.8%, *Superior*: -5.5%) levels at 24 h post-blood donation. Spearman correlation analysis revealed that changes in haemoglobin concentrations were not associated with changes in exercise performance in all groups. **Conclusion:** Exercise performance was only significantly reduced in the *Superior* fitness group. Well-trained individuals should be refrained from blood donation just prior to competitions.

Keywords: Aerobic fitness, VO_2max, Haemoglobin, Haematocrit, Blood volume

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INTRODUCTION

Haematological parameters such as plasma volume, hematocrit and haemoglobin decrease immediately following one unit of whole blood donation (1). These haematological parameters play an essential role in the regulation of oxygen-carrying capacity and therefore has a direct effect on maximal oxygen uptake (VO_2max) and aerobic exercise performance. In a recent review by Van Remoortel and colleagues (2), it was concluded that between 24 to 48 hours and until 14 days post blood donation, a reduction by ~ 4 to 7% in haemoglobin is expected. Consequently, a decrease in VO_2max by ~7% was observed within 24 to 48 hours post blood donation, which, resulted in a reduction in exercise time to exhaustion by ~10% (2).

The influence of blood donation on exercise performance was first reported in 1942 by Karpovich and Millman when cycling exercise performance was reduced following blood donation and was only reinstituted to normal levels after three weeks (3). Since then, numerous studies have investigated the effects of blood donation on haematological parameters and demonstrated the detrimental side effects on exercise performance (4-10). Series of studies have shown that the ability to perform maximal exercise is reduced for at least a week following blood donation (5-8), while submaximal exercise performance was not affected (9). The reduced VO_2max following blood donation has also been shown to take place up to 2 weeks (10).

Nonetheless, the study by Markiewicz et al. (4) that studied the effects of 400 ml blood donation in male blood donors aged 19-23 years, demonstrated that a significant reduction in VO_2max was observed within the hour post-donation, but not at 24 h post-donation. Similarly, Strandenes et al. (2013) demonstrated that VO_2max and combat performance were not affected immediately after whole blood donation in Norwegian special force soldiers (11). In another study by Gordon and colleagues examined the effect of blood donation on an incremental cycle ergometer test in 15 young adult athletes (12). The fitness testing and haematological parameter were assessed before donation and 48 h post-donation. Despite a significant 9.4% and 5.6% reductions in haemoglobin concentration and VO_2max respectively at 48 h, there was no reduction in the exercise time to exhaustion, suggesting that exercise time to fatigue was unaffected by blood donation (12).

The reason for the inconclusive findings relating to VO_2max and exercise performance following blood donation remains unclear. There is a possibility that the level of cardiorespiratory fitness could have an impact on the exercise performance following blood donation. Therefore, this proposition warrants for an investigation to determine the effects of a standard 450 ml whole blood donation on exercise performance performed 24 h post-blood donation in individuals with varying levels of cardiorespiratory fitness. A secondary aim of the study was to determine the association between changes in haemoglobin concentrations with changes in cardiorespiratory fitness across varying levels of cardiorespiratory fitness.

MATERIALS AND METHODS

Participants

Forty-two (n = 42) subjects were recruited via convenience sampling to participate in the study. They were approached from the registered blood donor list in the National Blood Centre, Kuala Lumpur (NBCKL) and Sarawak General Hospital (SGH) Blood Bank. Subjects were included if they were males between 18 to 29 years old, body mass index (BMI) between 18.0 – 23.0 kg/m2, a non-smoker and a repeat blood donor (i.e., has had donated previously at least once before the commencement of the study). All subjects were physically examined by a qualified medical doctor and had undergone resting electrocardiogram (ECG) to exclude any possibility of cardiac abnormality, and were required to complete the Physical Activity Readiness Questionnaire (PAR-Q) before the start of the study to ensure that subjects were able to optimally engage in maximal exercise. None of the participants had a history of cardiovascular disease and musculoskeletal problem. All subjects gave informed consent form prior to enrolling in the study. Ethical approvals were obtained from the Malaysian Ministry of Health and registered with the National Medical Research Register (NMRR) (NMRR-15-1805-28386) and the Human Research Ethics Committee, Universiti Sains Malaysia (USM/JEPeM/15110489).

Procedures

The study was conducted over three consecutive days. On the morning of the first day, subjects completed a baseline measurement which included a fasting blood withdrawal and a 20-m multistage shuttle run test to determine their cardiorespiratory fitness. Based on the maximal oxygen consumption (VO_2max) values predicted from the shuttle run test, subjects were categorised into either the *Low, Moderate* and *Superior* aerobic fitness group (13). On the morning of the second day, subjects underwent a standard whole blood donation at the blood bank facility. Only subjects with haemoglobin more than 13.0 g·dL-1 were allowed for blood donation. A qualified nurse phlebotomized the subjects by using the sterile blood collection technique. Approximately 450 ml of blood was collected using standard blood donation procedures. Subjects were instructed to rest on the bed for 10 min after the donation before proceeding to the refreshment area. The donors were encouraged to increase oral fluid intake throughout the 24 h after the donation. On the morning of the third day, approximately 24 h post-blood donation, subjects completed a fasting blood withdrawal and underwent another 20-m shuttle run test. Subjects were instructed to refrain from strenuous exercise in the 24 h prior to each test session and to consume approximately the same amount of fluids prior to each laboratory test session.

20-m Multi-Stage Shuttle Run Test (20mMSRT)

Cardiorespiratory fitness was assessed using the 20-m multi-stage shuttle run test. The test involved running between two lines 20 m apart at a pace dictated by a beeping tone. For the first minute of the test, the running velocity was 8.5 $km \cdot h^{-1}$, which increased by 0.5 $km \cdot h^{-1}$ every minute thereafter. The test score was based on the number of 20 m shuttles that a subject managed to complete before voluntarily withdraw from the test, or unable to get within 3 m of the end lines on two consecutive beeping tones. Heart rate (HR) was recorded continuously during the test (Polar®, UK). The maximum HR achieved during the tests was used to provide confirmation that individuals have reached their maximum effort during the test, of which maximum HR was estimated from the formula, 220 minus age. For each session, up to a maximum of three subjects were tested at any one time. All tests were performed on hard ground running surface and at approximately the same time of the day to reduce intra-individual variation.

Blood Sampling and Analysis

Blood sampling sessions were conducted at baseline (24 h prior to blood donation), immediately after blood donation and 24 h post-blood donation. For baseline and 24 h post blood donation, blood samples were collected in the morning after 12 hours of fasting. The second blood sampling was collected immediately after the blood donation procedure. Blood samples were collected under the following standardized conditions: 5.0 mL of venous blood was collected in vacuum tubes containing EDTA/K3 for the determination of haemoglobin and haematocrit concentrations. All samples were then transported to the laboratory at 4°C and were analyzed within an hour after the blood collection using Sysmex® XS- 800i™ haematology analyser.

Total blood volume (BV) at baseline, immediately post- and 24 h post-blood donation periods were estimated according to the formula described by Mora-Rodriguez *et al*. (2012) as follows (14):

(i) $BV_{baseline}$ (ml) = [(0.045 x body mass in kg) + 1.672] x 1000

We then combined the calculated initial BV value with the changes in blood volume to determine post-donation BV:

(ii) $BV_{immediately\ post\text{-}donation}$ (ml) = $BV_{baseline}$ x ($Haemoglobin_{baseline}/Haemoglobin_{during\ blood\ donation}$) – 450 ml

(iii) $BV_{24\ h\ post\text{-}blood\ donation}$ (ml) = $BV_{baseline}$ x – 450 ml

Statistical Analysis

The study's sample size was calculated by using the Power and Sample Size software version 3.0 (15). The estimation was based on alpha (α) value of 0.05, power of 90% and effect size of 0.75 L/min, the latter of which was based on differences in VO_2max pre- and post-blood donation according to an earlier study by Birnbaum et al. (2006) (4). The initial calculated sample size was 12 and taking into account for a possibility of dropouts, 14 individuals were chosen per group. All data were analysed with the Statistical Package for the Social Sciences (SPSS) version 22.0 software (IBM Corp., New York). Results are reported as mean ± standard deviation if data were normally distributed, while median and interquartile ranges (IqR) are reported if data were not normally distributed. Cardiorespiratory fitness was represented by predicted VO_2max values and the number of successfully completed shuttles in the 20mMSRT. Percentage of change at 24 h post-blood donation period was calculated as: [(24 h post-blood donation value – baseline value)/baseline value] x 100. Due to data being not normally-distributed, non-parametric tests were used. The Wilcoxon signed rank test was used to compare predicted VO_2max values, maximum heart rate and a number of completed shuttles in 20mMSRT, between baseline and 24 h post-blood donation periods in the three fitness groups. The Friedman test was used to compare haemoglobin, haematocrit and estimated total blood volume between baseline, immediately after and 24 h post-blood donation in the three fitness groups. Differences in the percentage of change for VO_2max, haematocrit, haemoglobin and estimated total blood volume between three fitness groups were tested using the Kruskal-Wallis test. The Spearman rank-order correlation was used to assess the association between percentages of change in haemoglobin concentrations and VO_2max at 24 h post-blood donation across all fitness groups. A p-value of less 0.05 was considered statistically significant.

RESULTS

Subjects' Baseline Characteristics

Table I shows the baseline characteristics of the study subjects according to *Low, Moderate*, and *Superior* cardiorespiratory fitness groups. Overall, the mean age, BMI and cardiorespiratory fitness (VO_2max) of the subjects were 22 ± 2.1 years, 21.9 ± 2.2 kg/m², and 41.2 ± 8.1 ml/kg/min respectively. The median values for baseline VO_2max were 33.3, 38.9, and 50.8 ml/kg/min for *Low, Moderate*, and *Superior* fitness group respectively. Except for VO_2max, none of the parameters were statistically different between the groups.

Table 1: Subjects' Baseline Characteristics

Parameters	Combined (n=42)	Fitness Group		
		Low (n=14)	Moderate (n=14)	Superior (n=14)
Age (years)	22 ± 2.06	20 (19.0 - 21.2)	23 (21.7 - 23.2)	24 (22.0 - 25.0)
Body mass index (BMI, kg/m²)	21.9 ± 2.2	21.5 (20.8 - 22.2)	21.9 (19.5 - 23.1)	22.3 (20.2 - 23.7)
Predicted VO_2max (kg/ml/min)	41.2 ± 8.1	33.3 (30.7 - 35.5)	38.8 (36.8 - 42.6)	50.8 (50.2 - 52.6)
Haematocrit (%)	46.1 ± 2.3	45.7 (43.4 - 48.5)	47.2 (44.8 - 48.7)	46.2 (44.1 - 46.9)
Haemoglobin (g/dL)	15.3 ± 0.7	14.9 (14.4 - 15.3)	15.3 (14.8 - 15.9)	15.5 (14.9 - 16.0)

For combined, values are expressed as mean ± SD
For fitness categories, values are expressed as median with interquartile ranges (IqR)

Cardiorespiratory Fitness

Table II shows the comparison of predicted VO_2max values, number of completed shuttles in the 20mMSRT and maximum heart rate between baseline and 24 h post-blood donation periods in all three fitness groups. Overall, there were no significant differences observed in predicted VO_2max values, the number of completed shuttles in the 20mMSRT and maximum heart rates between baseline and 24 h post-blood donation periods when all subjects were combined. However, predicted VO_2max values and number of completed shuttles in the 20mMSRT were significantly reduced by 4.1% and 7.3% respectively, in the *Superior* fitness group at 24 h post-blood donation period compared to baseline (p=0.017). VO_2max values were lower by 0.6% and 1.0% in the 24 h post-blood donation period for *Low* and *Moderate* fitness groups respectively. However, these changes were non-significant. No changes were seen for other parameters in the *Low* and *Moderate* fitness groups. Further analysis indicated the percentage of change for VO_2max values in the 24 h post-blood donation period were significantly different between *Superior* fitness vs. *Moderate* fitness groups (p=0.009), but not between other fitness groups.

Haematological Parameters and Total Blood Volume

Table III shows the comparison of haematocrit, haemoglobin, and estimated total blood volume between baseline, immediately after and 24 h post-blood donation periods in all three fitness groups. Overall, haematocrit (-8.0%), haemoglobin (-7.0%), and estimated total blood volume (-2.1%) were significantly lower in the 24 h post-blood donation period compared to baseline in all subjects combined. Compared to baseline, all fitness groups demonstrated significant reductions in haematocrit (*Low*: -8.4%, *Moderate*: -9.1%, *Superior*: -7.2%) and haemoglobin (*Low*: -7.6%, *Moderate*: -7.8%, *Superior*: -5.5%) levels at 24 h post-blood donation. Overall, subjects lost approximately 8.8% of the total blood volume during the blood donation period. Total blood volume was significantly reduced immediately post-donation compared to baseline in all fitness groups, but showed significant restoration (*Low*: +96%, *Moderate*: +99%, *Superior*: +98%) towards baseline values in the following 24 h. Further analysis showed no significant differences in the percentage of change in 24 h post-blood donation haematocrit and haemoglobin levels, as well as total blood volume between *Low, Moderate* and *Superior* fitness groups.

Association between Changes in Haemoglobin and VO_2max 24 h Post-Blood Donation

A series of Spearman rank-order correlation analysis was performed to determine the association between the percentage of change in haemoglobin and cardiorespiratory fitness (VO_2max) values 24 h post-blood donation in all subjects combined, as well as according to *Low, Moderate* and *Superior* fitness groups. No significant associations were observed between the parameters as shown in Table IV.

Table II: Comparison of Cardiorespiratory Fitness Parameters Between Baseline and 24 h Post-Blood Donation Periods in *Low, Moderate* and *Superior* Fitness Groups

Parameters	n	Baseline	24 h post-blood donation	p value
Predicted VO_2max (kg/ml/min)				
Combined	42	41.2 ± 8.1	40.5 ± 7.7	0.079
Low fitness group	14	33.3 (30.7 - 35.5)	33.1 (30.7 - 34.5)	0.937
Moderate fitness group	14	38.8 (36.8 - 42.6)	38.4 (36.5 - 41.9)	0.805
Superior fitness group	14	50.8 (50.2 - 52.6)	48.7 (48.5 - 50.9)	**0.017**
Completed shuttle in 20mMSRT				
Combined	42	67.4 ± 23.4	65.6 ± 21.7	0.074
Low fitness group	14	44 (39 – 48)	42 (36 - 53)	0.694
Moderate fitness group	14	58 (52 - 69)	57 (51 - 67)	0.805
Superior fitness group	14	96 (93 - 102)	89 (86 - 96)	**0.014**
Maximum Heart Rate (bpm)				
Combined	42	189.9 ± 5.8	190.7 ± 4.5	0.380
Low fitness group	14	190 (186 - 195)	190 (187 - 193)	0.944
Moderate fitness group	14	188 (183 - 191)	189 (186 - 191)	0.160
Superior fitness group	14	191 (184 -199)	191 (187 - 197)	0.729

For combined, values are expressed as mean ± SD
For fitness categories, values are expressed as median with interquartile ranges (IqR)

Table III: Comparison of Haematological Parameters and Blood Volume Between Baseline, Immediately After and 24 h Post-Blood Donation Periods in Low, Moderate and Superior Fitness Groups

Parameters	Baseline	Immediately post-blood donation	24 h post-blood donation
Haematocrit (%)			
Combined	46.1 ± 2.3	45.5 ± 2.1*	42.4 ± 2.4*‡
Low fitness group	45.7 (43.4 - 48.5)	45.5 (43.2 - 46.6)*	41.2 (39.7 - 43.8)*‡
Moderate fitness group	47.2 (44.8 - 48.7)	46.3 (43.8 - 48.2)	42.6 (40.6 - 45.5)*‡
Superior fitness group	46.2 (44.1 - 46.9)	44.7 (44.0 - 45.8)*	42.5 (40.5 - 43.8)*‡
Haemaglobin (g/dL)			
Combined	15.3 ± 0.7	15.1 ± 0.7*	14.2 ± 0.8*‡
Low fitness group	14.9 (14.4 - 15.3)	14.8 (14.3 - 15.1)	13.8 (13.2, 14.5)*‡
Moderate fitness group	15.3 (14.8 - 15.9)	15.0 (14.5 - 15.8)	13.9 (13.4 - 15.1)*‡
Superior fitness group	15.5 (14.9 - 16.0)	15.1 (14.9 – 15.9)	14.3 (13.7, 15.1)*‡
Blood Volume (L)			
Combined	4.60 ± 3.49	4.19 ± 3.83*	4.5 ± 3.53*‡
Low fitness group	4.44 (4.31 - 4.55)	4.00 (3.84 - 4.19)*	4.29 (4.19 - 4.50)*‡
Moderate fitness group	4.46 (4.31- 4.76)	4.08 (3.92 - 4.37)*	4.42 (4.29 - 4.64)*‡
Superior fitness group	4.71 (4.48 - 5.02)	4.24 (4.01 - 4.68)*	4.61 (4.27 - 4.91)*‡

For combined, values are expressed as mean ± SD
For fitness categories, values are expressed as median with interquartile ranges (IqR)
*significant ($p< 0.05$) in comparison to baseline
‡significant ($p< 0.05$) in comparison to immediately after blood donation

Table IV: Correlation Analysis Between Percentages of Change in Haemoglobin and Cardiorespiratory Fitness (VO_2max) at 24 h Post-Blood Donation Across Fitness Groups

% Change Between Haemoglobin and VO_2max	r_s	p value
Combined	0.409	0.072
Low fitness group	0.001	0.999
Moderate fitness group	0.639	0.140
Superior fitness group	0.532	0.050

DISCUSSION

Given the mixed findings of the effects of whole blood donation on exercise performance, the purpose of the present study was to examine the impact of varying levels of cardiorespiratory fitness on exercise performance performed at 24 h post-blood donation. The salient finding of the present study was that whole blood donation did not reduce exercise performance 24 h post-blood donation in a large group of individuals with a heterogeneous profile of cardiorespiratory fitness. However, when these individuals were separated into the *Low, Moderate* and *Superior* levels of cardiorespiratory fitness, there was a significant decline in exercise performance as result of blood donation observed in the *Superior* fitness group but not in the *Low* and *Moderate* aerobic fitness. Interestingly, the magnitude of decline in exercise performance in the *Superior* fitness group was significantly lower when compared to that observed in the *Moderate* fitness group. The main finding of our study, therefore, supported our hypothesis that exercise capacity was differently affected after blood donation in individuals with varying levels of fitness. This finding also suggests that whole blood donation may have the greatest impact in individuals with higher levels of aerobic fitness relative to the less aerobically fit individuals.

Our main finding concurs with the pooled results of eight studies which reported 4% to 7% reductions in cardiorespiratory fitness within 24 to 48 h post-whole blood donation (3). It, however, remains unclear why the reduction in exercise performance at the 24 h mark after blood donation was only significant in the *Superior* fitness group but not in the *Low* and *Moderate* fitness groups. The 20 m multi-stage shuttle run test is predominantly an aerobic event with minimal contribution from the anaerobic energy resources (16). Thus, given that oxygen delivery is a critical limiting factor in the aerobic energy system pathways (2,4). Therefore we can only speculate that individuals with higher fitness level are more sensitive towards factors that can impair oxygen delivery in the cardiovascular system relative to individuals in the lower fitness groups. It is possible that individuals with somewhat higher fitness would have had exhausted their aerobic capacities to greater extent during the exercise test as compared to the lesser fit individuals. Our view of a greater involvement of aerobic fitness influencing aerobic performance is supported by the study Hill et al. (7), in which trained subjects performed a time to exhaustion cycle before and after a whole blood withdrawal. The investigators calculated the involvement and contribution of aerobic and anaerobic pathways during the time to exhaustion exercise via indirect oxygen uptake and maximal accumulated oxygen deficit and subsequently showed that blood donation led to a lower VO_2max which caused significantly lower exercise time to exhaustion in their trained subjects. The authors reasoned that the lower exercise performance was due to in part, reduction in oxygen carrying capacity resulting from the lower haemoglobin and modest reduction in blood volume (7).

Oxygen delivery has been identified as the main limiting factor in the maximal rate of oxygen utilization (VO_2max) in healthy individuals, thus, haemoglobin and total blood volume are two factors that can potentially affect VO_2max (17-19). Typically, haemoglobin concentration is reduced by 1.2 g/dL or approximately 8% one or two days after the removal of 450 mL of blood (20,21). It has been previously reported that VO_2max and exercise performance decreased for every g/dL loss of haemoglobin mass (22, 23). A study by Schmidt et al. demonstrated a change in total haemoglobin mass by 1 g will result in a change in VO_2max of approximately 4 mL/min (20,23). A study by Burnley et al. (20) recorded a 6.5% reduction in haemoglobin 24 h after blood donation with a significant VO_2max reduction of 4% (5), comparable to the reductions of 4.1% for VO_2max and 5.5% for haemoglobin observed in the *Superior* fitness group in the present study. Our observation conforms to the concept of the relationship between the oxygen carrying capacity of haemoglobin with exercise performance as previously reported by Calbet et al. (24). On this note, it was of interest in the present study to examine the association between changes in haemoglobin concentrations with the changes observed in exercise performance. The present finding, however, showed that changes in exercise performance were not associated with changes in haemoglobin concentrations. Earlier studies, however, had demonstrated a strong relationship between changes in haemoglobin and cardiorespiratory fitness (19, 25-27). The mean of haemoglobin reduction in these studies ranged from 10-30%, and the magnitude of reduction was much more significant in comparison to current study findings, which was between 5-8%. Therefore it can be deduced that the relationship between these two factors is significant only when there is a substantial reduction in haemoglobin concentration and/or mass. Furthermore, some studies have indicated that VO_2max is closely related to total haemoglobin mass (tHb-mass) and blood volume, but not haemoglobin concentrations (20, 28). In addition, early recovery of VO_2 max has been observed even though haemoglobin concentrations had not been recovered following blood donation (10, 29), suggestive of other possible regulators that may mitigate the effects of haemoglobin reduction on cardiorespiratory fitness following blood donation.

The lack of association between haemoglobin reduction with VO_2max in this study could also be partly explained by the restoration of blood volume almost to baseline levels in the following 24 h after blood donation in our subjects. About 24 – 48 h after withdrawal of blood, blood volume return to almost normal (around 4–6% lesser than baseline), while the concentration of hemoglobin is reduced by hemodilution (19). Restoration of blood volume soon after blood donation generally occurs through plasma expansion by reconstitution of fluid from extravascular space to intravascular space as well as increased fluid absorption from the kidneys and gastrointestinal tract (29). Recovery of haemoglobin mass, on the other hand, takes 36 days in average following a typical blood donation (21). Studies have shown that an increase in blood volume through plasma expansion can compensate for moderate reductions in haemoglobin through the increase in cardiac output, allowing VO_2max to increase or remain unchanged (30-32). Based on our observation, however, the restoration of blood volume in our study was apparently not sufficient to maintain the exercise performance in the *Superior* group, indicating that oxygen carrying capacity plays a greater role than blood volume in determining exercise performance.

The present study is not without limitations. It would have been ideal if the individuals' VO_2max have been measured directly using indirect calorimetry method rather than relying on indirect testing procedures to predict their maximum aerobic capacity. It also remains to be determined whether similar effects observed in this study would be observed in other populations, e.g. female donors and elderly subjects. The present study has some practical implications. In view of the small, albeit significant, reduction in exercise performance following blood donation, physical exercise can be safely resumed following 24 h of physical rest for individuals, in particular, those who possess low to moderate levels of aerobic fitness. However, for competitive athletes who clearly possessed much higher aerobic capacity and exercise tolerance, the potential of decrements in aerobic performance should be made aware. This decrement in aerobic performance may persist for at least a week following a standard blood donation, therefore, individuals can better decide regarding blood donation.

CONCLUSION

In conclusion, the present study indicates that exercise performance was negatively affected following 24 h post-blood donation in individuals possessing higher levels of cardiorespiratory fitness as compared to those with lower levels of fitness. Athletes and fitness enthusiasts constitute a healthy potential donor population, therefore measures to counteract the alterations in hematological parameters should be developed to minimise alterations in exercise capacity and thereby encouraging this population in particular to become blood donors.

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REFERENCES

1. Ziegler AK, Grand J, Stangerup I, Nielsen HJ, Dela F, Magnussen K, Helge JW. Time course for the recovery of physical performance, blood haemoglobin, and ferritin content after blood donation. Transfusion. 2014. 55, 898-905.
2. Van Remoortel H, De Buck E, Compernolle V, Deldicque L, Vandekerckhove P. The effect of a standard whole blood donation on oxygen uptake and exercise capacity: a systematic review and meta-analysis. Transfusion. 2017. 57, 451-462.
3. Karpovich PV, Millman N. Athletes as blood donors. Res Quart Exerc Sport. 1942. 13, 166-8.
4. Birnbaum L, Dahl T, Boone T. Effect of blood donation on maximal oxygen consumption. Sports Med Phys Fitness. 2006. 46, 535-539.
5. Burnley M, Roberts C, Thatcher R, Doust JH, Jones AM. Influence of blood donation on O2 uptake on-kinetics, peak O2 uptake and time to exhaustion during severe-intensity cycle exercise in humans. Exp Physiol. 2006. 91, 499-509.
6. Dellweg D, Siemon K, Mahler F, Appelhans P, Klauke M, Kohler D. Cardiopulmonary exercise testing before and after blood donation. Pneumologie. 2008. 62, 372-377.
7. Hill DW, Vingren JL, Burdette SD. Effect of plasma donation and blood donation on aerobic and anaerobic responses in exhaustive, severe-intensity exercise. Appl Physiol Nutr Metab. 2013. 38, 551-7.
8. Judd TB, Cornish SM, Barss TS, Oroz I, Chilibeck PD. Time course for recovery of peak aerobic power after blood donation. J Strength Cond Res. 2011. 25, 3035-3038.
9. Markiewicz K, Cholewa M, Gorski L, Jaszczuk J, Chmura J., Bartniczak Z. Effect of 400 ml blood loss on adaptation of certain functions of the organism to exercise. Acta Physiol. Pol. 1981. 32, 613-621.
10. Panebianco RA, Stachenfeld N, Coplan NL, Gleim GW. Effects of blood donation on exercise performance in competitive cyclists. Am Heart J. 1995. 130, 838-840.
11. Strandenes G, Skogrand H, Spinella PC, Hervig T, Rein EB. Donor performance of combat readiness skills of special forces soldiers are maintained immediately after whole blood donation: a study to support the development of a prehospital fresh whole blood transfusion program. Transfusion. 2013. 53, 526-530.
12. Gordon D, Wood M, Porter A, Vetrivel V, Gernigon M, Caddy O, *et al*. Influence of blood donation on the incidence of plateau at VO_2max. Eur J Appl Physiol. 2014. 114, 21-27.
13. Deborah R., Ehrman J.K., Liguori G., and Magal M. ACSM's Guidelines for Exercise Testing and Prescription. 10th ed. Philadelphia: Wolters Kluwer, 2018.
14. Mora-Rodriguez R, Aguado-Jimenez R, Del Coso, Estevez E. A standard blood bank donation alters the thermal and cardiovascular responses during subsequent exercise. Transfusion. 2012. 52, 2339-2347.
15. Dupont WD, Plummer WD. PS power and sample size program available for free on the internet. Control Clin Trials. 1997. 18, 274.
16. Ahmaidi S, Collomp K, Prйfaut C. The effect of shuttle test protocol and the resulting lactacidaemia on maximal velocity and maximal oxygen uptake during the shuttle exercise test. Eur J Appl Physiol 1992. 6, 475-479.
17. Saltin B, Calbet JAL, Wagner PD. Point: In health and in normoxic environment, VO_2max is limited primariy by cardiac output and locomotor muscle blood flow. J Appl Physiol. 2006. 100, 744–748.
18. Wagner PD. Determinants of maximal oxygen transport and utilization. Ann Rev Physiol. 1996. 58, 21–50.
19. Kanstrup IL, Ekblom B. Blood volume and hemoglobin concentration as determinants of maximal aerobic power. Med Sci Sports Exerc. 1984.16, 256-262.
20. Otto JM, Montgomery HE, Richards T. Haemoglobin concentration and mass as determinants of exercise performance and of surgical outcome. Extrem Physiol Med. 2013. 2, 33-40
21. Pottgiesser T, Specker W, Umhau M, Dickhuth HH, Roecker K, Schumacher YO. Recovery of hemoglobin mass after blood donation. Transfusion. 2008. 48, 1390-1397.
22. Bonne TC, Lundby C, Jшrgensen S, Johansen L, Morgan M, Bech SR, *et al*. "Live High-Train High" increases hemoglobin mass in Olympic swimmers. Eur J Appl Physiol 2014. 114, 1439-1449.
23. Schmidt W, Prommer N. Impact of alterations in total hemoglobin mass on VO_2max. Exerc Sport Sci Rev. 2010. 38, 68-75.
24. Calbet JA, Lundby C, Koskolou M, Boushel R. Importance of hemoglobin concentration to exercise: acute manipulations. Respir Physiol Neurobiol. 2006. 151, 132-140.
25. Ekblom B, Goldbar AN, Gullbring B. Response to exercise after blood loss and reinfusion. J Appl Physiol. 1972. 33, 175-180.
26. Ekblom B, Wilson G, Astrand PO. Central circulation during exercise after venesection and reinfusion of red blood cells. J Appl Physiol. 1976.

40, 379–383.
27. Woodson RD, Wills RE & Lenfant C. Effect of acute and established anemia on O2 transport at rest, submaximal and maximal work. J Appl Physiol Respir Environ Exerc Physiol. 1978. 44, 36-43.
28. Gore CJ, Hahn AG, Burge CM, Telford RD. VO_2max and haemoglobin mass of trained athletes during high intensity training. Int J Sports Med. 1997. 18, 477-482.
29. Ziegler AK, Grand J, Stangerup I, Nielsen HJ, Dela F, Magnussen K, Helge JW. Time course for the recovery of physical performance, blood haemoglobin, and ferritin content after blood donation. Transfusion. 2014. 55, 898-905.
30. Berger NJA, Campbell IT, Wilkerson DP, Jones AM. Influence of plasma volume expansion on VO_2 kinetics, VO_2peak, and performance during high-intensity cycle exercise. J Appl Physiol. 2006. 101, 707–714.
31. Gledhill N, Warburton D, Jamnik V. Haemoglobin, blood volume, cardiac function, and aerobic power. Can J Appl Physiol. 1999. 24, 54-65.
32. Mier CM, Domenick MA, Turner NS, Wilmore JH. Changes in stroke volume and maximal aerobic capacity with increased blood volume in men women. J Appl Physiol. 1996. 80, 1180-1186.