EFFECT OF BLOOD DONATION ON BLOOD DONOR CARDIORESPIRATORY FITNESS LEVEL

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DECLARATION

I hereby declare that this research has been sent to Universiti Sains Malaysia for the degree of Masters of Medicine in Transfusion Medicine. It is also not to be sent to any other universities. With that, this research might be used for consultation and will be photocopied as reference.

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LIST OF ABBREVIATIONS

- **2,3 DPG** 2,3 Diphosphoglycerate
- **20 m MST** 20 meters Multistage Shuttle Run Test
- **ATP** Adenosine Triphosphate
- **BMI** Body Mass Index
- **bpm** Beats per minute
- **BV** Blood Volume
- **FBC** Full Blood Count
- **HR** Heart Rate
- **IqR** Interquartile Range
- **kg** Kilogram
- **ml** Millilitre
- **MOH** Ministry of Health
- **NBCKL** National Blood Center, Kuala Lumpur
- **PAR-Q** Physical Activity Readiness-Questionnaire
- **SGH** Sarawak General Hospital
- **USM** University Science of Malaysia
- **VO_2 max** Maximum oxygen uptake
- **WHO** World Health Organization

ABSTRAK

Tajuk: *Effect of Blood Donation on Blood Donor cardiorespiratory fitness level*

Latar Belakang. Pendermaan darah adalah penting untuk memenuhi keperluan darah dan produk-produk darah untuk pesakit. Tetapi, pengurangan *haemoglobin*, *haematocrit* dan isipadu darah selepas pendermaan darah mungkin memberi kesan terhadap kecergasan kardiorespiratori. Tujuan kajian ini adalah untuk mengkaji kesan pendermaan darah kepada kecergasan fizikal kardiorespiratori penderma iaitu *predicted maximum oxygen uptake* (*VO_2 max)*.

Kaedah. Sebanyak 42 penderma darah lelaki telah terlibat dalam kajian keratan lintang ini dengan 14 peserta untuk setiap kumpulan kecergasan rendah, sederhana dan tinggi. Protokol *20 meter Multistage Shuttle Run fitness test* telah digunakan untuk menguji kecergasan kardiorespiratori. Ujian kecergasan kardiorespiratori dijalankan 24 jam sebelum dan selepas pendermaan 450 ml darah utuh. Pada masa yang sama, *Haemoglobin* dan *haematocrit* diuji pada 24 jam sebelum, sebaik selepas dan 24 jam selepas pendermaan.

Keputusan. Median *VO_2 max* sebelum pendermaan adalah 33.30 (30.73, 35.5), 38.85 (36.80, 42.65) and 50.80 (50.20, 52.60) ml/kg/min untuk kumpulan kecergasan rendah, sederhana dan tinggi, secara masing-masing. Kecergasan kardiorespiratori pada 24 jam selepas pendermaan darah telah berkurangan sedikit sebanyak 0.61%, 1.29% and 3.43% untuk kumpulan kecergasan rendah, sederhana dan tinggi, secara masing-masing. Tetapi, hanya pengurangan di dalam kumpulan kecergasan tinggi adalah signifikan secara statistik ($p = 0.017$). *Haemoglobin* dan *haematocrit* pada 24 jam selepas pendermaan darah telah berkurangan dengan signifikan secara statistik untuk semua kumpulan. *Haemoglobin* telah berkurangan sebanyak 7.63% ($p <$

0.001), 7.82% ($p < 0.001$) dan 5.46% ($p < 0.001$) untuk kumpulan kecergasan rendah, sederhana dan tinggi, secara masing-masing. *Haematocrit* telah berkurangan sebanyak 8.40% ($p < 0.001$), 9.08% ($p < 0.001$), and 7.21% ($p = 0.002$) untuk kumpulan kecergasan rendah, sederhana dan tinggi, secara masing-masing. Analisa korelasi *Spearman* menunjukkan hubungan yang tidak signifikan di antara perubahan *haemoglobin* dan perubahan *VO_2 max* untuk semua kumpulan, kumpulan kecergasan rendah ($r_s = 0.001$), kumpulan kecergasan sederhana ($r_s = 0.639$) dan kumpulan kecergasan tinggi ($r_s = 0.532$).

Kesimpulan. Tahap kecergasan kardiorespiratori sedikit berkurangan pada 24 jam selepas pendermaan 450 ml darah utuh, ini turut disertai dengan pengurangan signifikan *haemoglobin* dan *haematocrit*. Tetapi, hubungan di antara perubahan haemoglobin selepas pendermaan darah dan perubahan kecergasan kardiorespiratori tersebut adalah tidak signifikan.

Kata kunci: Pendermaan darah, Kecergasan kardiorespiratori, *VO_2 max*, *Haemoglobin, Haematocrit*

ABSTRACT

Title: Effects of Blood Donation on Blood Donor Cardiorespiratory Fitness Level

Background. Blood donation is vital to meet patients' needs for blood and blood products. However, reduction of haemoglobin, haematocrit and blood volume following blood donation might affect the cardiorespiratory fitness level. The aim of this study is to determine the effect of blood donation on the level of cardiorespiratory fitness as measured by predicted maximum oxygen uptake (VO_2 max).

Methods. A total of 42 male blood donors were involved in this cross sectional study with 14 participants each for poor, average and excellent fitness groups. The 20 meters Multistage Shuttle Run fitness test protocol was used to measure cardiorespiratory fitness level. The test was performed 24 hours before and after a 450 ml whole blood donation. Simultaneously, haemoglobin and haematocrit were assessed 24 hours before, immediately after and 24 hours after the donation.

Results. The median baseline pre-donation predicted VO_2 max were 33.30 (30.73, 35.50), 38.85 (36.80, 42.65) and 50.80 (50.20, 52.60) ml/kg/min for poor, average and excellent fitness groups, respectively. The cardiorespiratory fitness level were slightly reduced at 24 hours after blood donation by 0.61%, 1.29% and 3.43% in the for poor, average and excellent fitness groups, respectively. However, the reduction was only statistically significant in the excellent fitness group ($p = 0.017$). The haemoglobin and haematocrit significantly reduced for all groups at 24 hours after donation. The haemoglobin was reduced by 7.63% ($p < 0.001$), 7.82% ($p < 0.001$) and 5.46% ($p < 0.001$) for poor, average and excellent fitness groups, respectively.

The haematocrit was reduced by 8.40% ($p < 0.001$), 9.08% ($p < 0.001$) and 7.21% ($p = 0.002$) for for poor, average and excellent fitness groups, respectively. The Spearman correlation analysis revealed no significant relationship between haemoglobin changes and predicted VO_2 max changes in all groups, poor fitness level group ($r_s = 0.001$), average fitness level ($r_s = 0.639$) and excellent fitness level group ($r_s = 0.532$).

Conclusion. The cardiorespiratory fitness level was slightly reduced at 24 hours following a 450 ml whole blood donation, which was concomitant with significant haemoglobin and haematocrit reduction. However, there was no significant relationship between the changes in haemoglobin and cardiorespiratory fitness after blood donation.

Key words: Blood donation, Cardiorespiratory fitness, VO_2 max, Haemoglobin, Haematocrit

CHAPTER 1: INTRODUCTION

1.1 OVERVIEW

1.1.1 The importance of blood donation

The theme for World Health Organization's (WHO) world blood donor day on 14 June 2015 is "Thank You for Saving My Life". Through this theme, blood donors' contributions must be appreciated. The pool of healthy blood donors worldwide is vital to ensure enough blood supply to meet clinical demands. A healthy donor is able to donate blood even until the age of 65 years (Ayob *et al.,* 2008). One of the options to improve donor's well-being is by advocating physical activity, which is the best practice to reduce the risk in the development of chronic diseases namely cardiovascular disease, hypertension, type 2 diabetes, stroke, osteoporosis, colon cancer, and breast cancer (Warburton *et al.,* 2010).

Approximately 92 million blood donations have been recorded all over the world in 2011 (WHO Global Database on Blood Safety Summary report 2011). More than nine million patients were transfused and had benefited from these donations. The global need for blood is expected to increase in the future with the advancement of chemotherapy, heart surgery, stem cell and organ transplantation (Osaro and Njemanze, 2010). Bleeding emergencies in obstetric, trauma as well as transfusion dependent individuals are also contributing factors for increasing demand worldwide. WHO advocates voluntary blood donations as part of its strategies to ensure safe and sustainable blood supply (WHO, 2013).

There are two main types of blood donation methods; whole blood donation and apheresis donation. Whole blood donation is a process of blood collected from donor that is processed into blood components later in the processing laboratory. Donated bloods are processed into packed red cell, platelet, fresh frozen plasma, cryoprecipitate and cryosupernatant or retained without further processing as a whole blood. Blood components have different specific gravity and the components separation is achieved by manipulating centrifugation duration and speed. Apheresis is a process where blood is removed from the donor and passed through the automatic cell separator machine (Harmening, 2012). Only the desired blood components are collected by the apheresis machine and the rest of unwanted components will be returned to the donor's blood circulation during the donation.

1.1.2 Blood donation in Malaysia

According to Malaysian Blood Transfusion Service Annual Report, 675,315 donations were recorded in 2014. This was a 3.5% increment from 653,124 donations in 2013. There were 230,467 new blood donors in 2014. The Malaysian blood transfusion service advocates volunteer blood donors as studies have shown that paid and replacement donors are more likely to harbour transfusion transmittable disease (WHO, 2013). In 2014, only 0.01% replacement blood donors were recorded in Malaysia from a total of 675,315 donations. From this figure, 70.34% of bloods donated in 2014 were by male and remaining of 29.76% was contributed by female. In regards to racial distribution, Malays were the biggest number of donors with 55.4% (304,049), followed by Chinese 33.86% (185,427), and Indians 8.3% (45,683). In 2014, Malaysian blood donors have donated a total of 656,742 (9.7%) whole blood and 18,573 (2.7%) apheresis blood products (Annual Report Blood Transfusion Service Malaysia, 2014).

The Malaysian Blood Transfusion Service has set several criteria to ensure blood donors' safety (Ayob *et al.,* 2008). For instance, all prospective blood donors must have a minimum weight of 45 kg, with age not less than 17 years old and not more than 65 years old. A total of 350 ml of blood donation shall be taken from donors who weigh less than 55 kg and 450 ml for those who weigh more than 55 kg. The blood donation volume restrictions are based on the body weight. This is important to ensure not more than 10% from the total blood volume is removed during the blood donation (Shaz *et al*., 2013).

1.1.3 Physical activity and blood donation

Physical activity has been universally accepted as an important factor for health and well-being (Warburton *et al.,* 2010). Among normal adults in Malaysia, only a small percentage (14.2%) of Malaysians had sufficient exercise (Poh *et al.,* 2010), in comparison with 43.7% who were physically inactive (Nor *et al.,* 2006). To date, little is known about the physical activity patterns among Malaysian blood donors.

Lack of physical activity or physical inactivity is a modifiable health risk against chronic non-communicable diseases. It is reflected in a study by Warburton *et al.* (2010) that physical activity is effective in the primary prevention of type 2 diabetes, hypertension, stroke, cardiovascular disease, osteoporosis, breast cancer, and colon cancer. Therefore, Malaysian Ministry of Health recommends physical activity as one of the key areas in the "National Strategic Plan for Non-Communicable Diseases 2010-2014" (Mustapha *et al.,* 2014). A pool of healthy Malaysian blood donors, which are eligible to donate blood from the age of 17 to 65 years old are important to provide sustainable blood supplies to meet increasing demands for

blood and blood products. Promoting physical activity is a good strategy to encourage healthy lifestyle among Malaysian blood donors population.

In practice, majority of the overseas blood transfusion services recommends 12 to 24 hours following blood donation completion, before any strenuous physical activity can be resumed. Different recommendations are given among countries with blood transfusion services; for example, the Red Cross Society of America recommends blood donors to avoid strenuous exercise and heavy lifting for the rest of the day after donation (American Red Cross, 2013), while Hong Kong Red Cross suggests 24 hours (Hong Kong Red Cross, 2013), Belgian Red Cross-Flanders recommends at least 24 hours after blood donation (Van Remoortel *et al*., 2016), and Australian Red Cross Blood Service advocates only 12 hours following blood donation (Australian Red Cross, 2016). In Malaysia however, there is no proper documented latency period of physical activity post donation as far as National Transfusion Guideline for Clinical and Laboratory Personnel (Ayob et *al.,* 2008) is concern. It does however, covers extensively on the donor selection criteria, adverse donation reaction as well as management of reactive donors.

In order to advocate physical activity for blood donors, it is important to ascertain whether blood donation will influence cardiorespiratory fitness. As the main function of haemoglobin is to carry oxygen via blood from lungs to tissues (Hoffbrand and Moss, 2011), the reduction in haemoglobin (Gordon *et al.,* 2013; Dellweg *et al.,* 2008; Birnbaum *et al.,* 2006) and also blood volume following any successful blood donation may potentially decrease oxygen delivery to the working muscles. A systematic review and meta-analysis report by Van Remoortel *et al*. (2016) revealed that cardiorespiratory fitness as measured by VO_2 max was reduced by 7 to 8% and haemoglobin was reduced by 7% after 24 to 48 hours following blood donation. Van Remoortel *et al*. (2016) concluded that blood donation leads to physiologically

small but potentially significant reduction in cardiorespiratory fitness level. Unfortunately, to the best of the author's knowledge, there is no local data available concerning the effect of blood donation on cardiorespiratory fitness level as a reference to establish physical activity recommendations for Malaysian blood donors. Thus, this preliminary study was carried out to examine the effect of blood donation on cardiorespiratory fitness among Malaysian blood donors population.

1.1.4 Maximum oxygen uptake (VO_2 max) as a gold standard for cardiorespiratory fitness indicator

Maximum oxygen uptake (VO_2 max) is one of the most universally used parameters in the cardiorespiratory fitness testing (Levine, 2008). VO_2 max is defined as the upper limit of cardiorespiratory system's ability to supply oxygen to the working muscles and tissues and during maximal demanding activity (Basset and Howley, 2000). It has been found that VO_2 max demonstrates cardiovascular and respiratory system's ability to supply oxygen during maximum physical activity to support oxidative production of Adenosine Triphosphate (ATP) as an energy source to perform physical work (Wagner, 1996). While Boyadijiev (2014) defined the VO_2 max as the aerobic capability of body to provide energy for muscles and tissues through the aerobic mechanism of oxidation and reflect to a larger extent the oxygen transport and delivery to the working muscles.

VO_2 max is also known as maximum aerobic capacity, maximum oxygen carrying capacity, and maximum oxygen consumption. These names reflect the ability of the cardiorespiratory system to supply oxygen during demanding physical activity.

1.2 RESEARCH JUSTIFICATION AND BENEFITS

Most of the foreign blood transfusion service authorities such as American Red Cross and Hong Kong Red Cross Organization recommend physical activity resumption at one day after the blood donation. However, this has yet to be addressed in the current National Transfusion Guideline (Ayob *et al.*, 2008). To date, there is no local data concerning the effect of blood donation on cardiorespiratory fitness level. By providing local data, this preliminary study is potentially important in the process of developing a post blood donation physical activity recommendation that tailors towards the Malaysian blood donors' population. These recommendations can also be adopted in the Transfusion Practice Guideline for Clinical and Laboratory Personnel at National Blood Centre Kuala Lumpur (NBCKL).

1.3 RESEARCH OBJECTIVES

1.3.1 General Objectives

To examine the influence of blood donation on cardiorespiratory fitness level.

1.3.2 Specific Objectives

i. To determine the effect of a standard 450 ml whole blood donation on blood donor's cardiorespiratory fitness level based on the predicted maximum oxygen uptake (VO_2 max) value in male blood donors with the poor, average, and excellent cardiorespiratory fitness level.

ii. To determine haemoglobin and haematocrit levels 24 hours before, immediately, and 24 hours after a standard 450 ml whole blood donation in male blood donors with the poor, average, and excellent cardiorespiratory fitness level.

iii. To determine the relationship between haemoglobin changes with cardiorespiratory fitness level changes 24 hours after a standard 450 ml whole blood donation in male blood donors with the poor, average, and excellent cardiorespiratory fitness level.

1.4 RESEARCH HYPOTHESIS

Null Hypothesis

i. The cardiorespiratory fitness level is not significantly affected 24 hours following blood donation in male blood donors with the poor, average, and excellent cardiorespiratory fitness level.

ii. There is no significant change in haemoglobin and haematocrit levels 24 hours following blood donation in male blood donors with the poor, average, and excellent cardiorespiratory fitness level.

iii. There is no correlation between the changes of VO_2 max and haemoglobin level in male blood donors with the poor, average, and excellent cardiorespiratory fitness level.

Alternate Hypothesis

i. The cardiorespiratory fitness level is significantly affected 24 hours following blood donation in male blood donors with the poor, average, and excellent cardiorespiratory fitness level.

ii. There is a significant change in haemoglobin and haematocrit levels before and after blood donation in male blood donors with the poor, average, and excellent cardiorespiratory fitness level.

iii. There is a correlation between the changes of VO_2 max and haemoglobin level in male blood donors with the poor, average, and excellent cardiorespiratory fitness level.

1.5 LIST OF DEFINITIONS

1.5.1 Blood Donation

Blood donation is a process of collecting blood from eligible donors by sterile phlebotomy technique. Blood is collected into a pyrogen free plastic blood bag that contains anticoagulant and preservative. There are two types of blood donations, which consist of whole blood donation and apheresis donation.

1.5.2 Physical Fitness

Physical fitness is defined as the ability to carry out daily tasks with vigour and alertness without undue fatigue and with ample energy to enjoy leisure-time pursuits and to meet unforeseen emergency (McArdle *et al.,* 2006).

1.5.3 Cardiorespiratory Fitness

Cardiorespiratory fitness refers to ability of lungs, cardiac, and vascular system to supply oxygen during sustained physical activity and to eliminate fatigue products after supplying oxygen and fuel (McArdle *et al.,* 2006).

CHAPTER 2: LITERATURE REVIEW

2.1 EFFECT OF BLOOD DONATION ON THE CARDIORESPIRATORY FITNESS LEVEL

Karpovich and Millman (1942) accidentally discovered blood donation's effects on cardiorespiratory fitness and performance when one of their subjects donated blood. The unauthorized blood donation during the study period by one of the subjects had complicated their original experiment. However, they had observed that the cycling performance had reduced after 500 ml donation and later returned to normal after three weeks. Based on this finding, they had discouraged athletes from donating blood except in emergency situations (Karpovich and Millman, 1942).

The subsequent study by Balke *et al.* (1954) on 14 subjects aged from 22 to 45 years has concurred the earlier finding by Karpovich and Millman (1942). The subjects' cardiorespiratory fitness was measured before blood donation and at two and eight days after blood donation. A 500 ml blood donation had caused a significant reduction in VO_2 max even until eight days post donation. They found that the VO_2 max at one hour and eight days post donation was significantly reduced by 8% and 5%, respectively (Balke *et al.,* 1954).

Unpublished study by Dennison (1960) showed different results in comparison with the two prior studies. Twenty subjects from the University of British Columbia Athletic teams were involved in this study and further divided equally into the blood donor and non-blood donor groups. A 500 ml of blood was withdrawn from the blood

donors group whereby no blood was withdrawn from the non-blood donors group. All subjects were tested with the cycle ergometer to measure the cardiorespiratory fitness level before donation, at 24 hours, and seven days after donation. It was observed that there was no significant difference between the two groups. The researcher speculated that the work capacity in the blood donors group was not affected by 500 ml blood donation because of motivational factors (Dennison, 1960).

Another study by Howell and Coupe (1964) entitled "Effect of Blood Loss upon Performance in the Balke-Ware Treadmill Test" showed significant reduction in VO_2 max after 500 ml of blood donation. The researchers studied 12 subjects at five to seven days pre-donation, immediately after donation, and at 24 hours and seven days post donation. Pre-donation VO_2 max was 5.43 L/min, which was reduced immediately after donation by 7.1%, 11.4% reduction after 24 hours, and finally 15% reduction after seven days post donation. This was the only report that recorded the VO_2 max had progressively reduced immediately after the blood donation and also as the post donation period was increased. Most of the studies had observed that the VO_2 max had shown an improvement as post donation time was increased. Although there was a significant drop in VO_2 max, the treadmill performance of time to the exhaustion was not affected. The authors concluded that the psychological factors influenced the result of the treadmill performance (Howell and Coupe, 1964).

Subsequently in 1978, Woodson *et al.* studied the effect of isovolumic anaemia in 11 healthy male subjects by withdrawing 500 ml of blood and replacing an equal amount of albumin (50 g/L) in isotonic saline. The subjects' cardiorespiratory fitness was tested with the Bruce treadmill protocol before and immediately after blood withdrawal.

The VO_2 max was significantly dropped by 16% from 43.0 to 36.1 ml/kg/min immediately after blood withdrawal. Meanwhile, haemoglobin level was dropped acutely from 15.30 g/dL to 10.04 g/dL (30% reduction) immediately after blood withdrawal and haemodilution. This was by far the largest haemoglobin reduction after 500 ml venesection compared to previous studies (Balke *et al.,* 1954; Howell and Coupe, 1964). The submaximal effort, however, was not significantly affected (Woodson *et al.,* 1978).

Markiewicz *et al.* (1981) studied the effect of 400 ml blood donation in 18 male blood donors aged 19-23 years. The subjects' cardiofitness level was tested 24 hours before and at one hour and 24 hours after donation by cycle ergometer protocol. There was a significant reduction in VO_2 max within one hour completion of the donation. After 24 hours, the cardiorespiratory fitness changes were observed to be insignificant in comparison to pre-donation value (Markiewicz *et al.*, 1981).

Fritsch *et al.* (1993) conducted a study on 16 young and healthy subjects to examine the effect of 450 ml blood donation on their exercise capacity. Subjects' cardiorespiratory fitness was tested with the maximal incremental cycle ergometer protocol. The VO_2 max was measured at one to seven days before donation and at two days post donation. A significant VO_2 max reduction by 11.2% after the donation was observed. Haemoglobin was significantly dropped to 13.0 g/dL two days after donation from the baseline of 14.5 g/dL (Fritsch *et al.,* 1993i).

Another study on ten male cyclists revealed a significant decrease in VO_2 max (Panebianco *et al.,* 1995) after donation of one unit of blood (450 ml) within seven days post donation period. The VO_2 max was 4.85 L/min before donation and had significantly

been reduced to about 8.2% two hours after, 8% after two days, and 7% at seven days post donation period. The authors also noticed that the oxygen uptake was not affected at the submaximal exercise intensity. Contrary to the study by Woodson *et al.* (1978), the haemoglobin immediately after donation was not affected, but had significantly been lowered at two and seven days post donation from 14.8 to 13.8 g/dL. Panebianco *et al.* (1995) concluded that donations of one unit of blood had caused a decrease in cycling performance and haemoglobin level plays an important role in VO_2 max.

Subsequently, a study on ten young male subjects (mean age of 24 years old) with the Bruce protocol 24 hours before and after donation observed a significant reduction in cardiorespiratory fitness level after the blood donation (Birnbaum *et al.,* 2006). Absolute VO_2 max 24 hours after 500 ml blood donation had significantly been reduced by 9.7% to 2.87 L/min from the pre-donation value of 3.18 L/min. Similarly, the haemoglobin value was observed to be significantly decreased after the donation. The authors postulated that a significant VO_2 max reduction was solely due to a decrease in oxygen carrying capacity as indicated by haemoglobin reduction since the cardiac output had remained unchanged (Birnbaum *et al.,* 2006).

In the same year of 2006, another study described similar findings of Birnbaum *et al.* (2006) in identical demographics of 11 young subjects (Burnley *et al.,* 2006). Ten males and one female underwent bicycle ergometer testing 24 hours before and after a 450 ml blood donation. A significant VO_2 max reduction by 4% was observed after the blood donation. Time to exhaustion similarly had significantly been decreased (Burnley *et al.,* 2006). Likewise, in 2008, another study on 11 subjects demonstrated significant

VO_2 max reduction of 9% after one unit whole blood donation with haemoglobin was significantly reduced by 1.2 g/dL (Dellweg *et al.,* 2008).

In an effort to support the army pre-hospital fresh whole blood transfusion program in Norway, Strandenes *et al.* (2013) studied the effect of blood donation on combative and physical performance of special force soldiers. The authors observed that the VO_2 max and combat performance were not affected immediately after whole blood donation. A total of 25 male soldiers were involved in this study and the cardiorespiratory fitness was tested by the Bruce Treadmill Protocol. The subjects were very fit by the evidence of their mean baseline VO_2 max of 61.1 ml/kg/min. There was an insignificant drop of VO_2 max to 60.1 ml/kg/min (1.6%) immediately after donation. In addition, combat performance such as pistol shoot test and uphill hiking exercise were not affected (Strandenes *et al.,* 2013). However, this study population was extremely fit and motivated hence the finding is not applicable for the general population.

A recent study was carried out by Gordon *et al.* (2013) on 15 young athletes (mean age of 23.3 years old) by the incremental cycle ergometer test to examine the effect of reductions in blood volume to VO_2 max. The fitness testing and haematological parameter were assessed 24 hours before donation and at 24 to 48 hours after donation. The authors reported a significant haemoglobin reduction was observed after a 450 ml blood donation from 15.5 to 14.1 g/dL. The VO_2 max was similarly affected with a significant reduction (5.6% of absolute VO_2 max reduction and 5.2% of relative VO_2 max reduction). Even with the haematological and VO_2 max changes, there was no reduction in the exercise exhaustion time. The authors attributed that time to fatigue was dependent on anaerobic capacity, which was less affected by blood donation (Gordon *et al.,* 2013).

Most of the studies had examined the effect of 450 to 500 ml blood donation on the cardiorespiratory fitness level, but in Malaysia the minimum regular donation volume is 350 ml for potential blood donors with body mass of 45 to 55 kg. A study in the neighbouring country of Indonesia revealed that 350 ml donation in regular blood donors did not have any significant effect on VO_2 max (Tarawan and Purwasasmita, 2009). The researchers tested 21 regular male blood donors with the mean age of 35 ± 7.7 years old (age ranged from 30 to 50 years old) with the Rhyming Astrand bench protocol one day before and after donation. The researchers had observed a non-significant VO_2 max reduction by 3% from the pre-donation value of 36.66 ml/kg/min. The researchers had concluded that a smaller 350 ml whole blood donation did not lead to significant cardiorespiratory fitness level reduction in their study population. However, the heterogeneous nature of participant's age may possibly played a big role in the study outcome. The VO_2 max is essentially influenced by age (McArdle *et al.,* 2006) and the researchers did not include age as a confounding factor in their study.

A couple of studies followed the subjects up to one month post donation in order to investigate the full time frame required for full VO_2 max recovery after donation as shown in Table 2.1. Judd *et al.* (2011) conducted a prospective study on 12 moderately fit individuals who donated one unit (450 ml) blood before the donation and weekly until four weeks after donation. The participants completed a total of six maximal bicycle ergometer exercise tests; the day before donation, 24 hours after donation, and every one week for four weeks after donation. There was a significant reduction of VO_2 max from baseline after one day and up to two weeks following donation. The VO_2 max recovery occurred after three weeks post donation (Judd *et al.,* 2011). Ziegler *et al.* (2014) conducted a similar study recently on 19 healthy men. The subjects underwent VO_2 max

testing, haemoglobin measurement, and three km time trial before blood donation. These fitness tests were repeated at three days, seven days, 14 days, and 28 days after donation. Ziegler *et al.* (2014) noted an earlier recovery of VO_2 max (two weeks) compared to the findings by Judd *et al.* (2011). Haemoglobin was significantly reduced, but VO_2 max recovery occurred even before haemoglobin level had returned to the baseline level.

Table 2.1: Comparison of VO_2 max (ml/kg/min) recovery after blood donation by Judd *et al.* (2011) and Ziegler *al.* (2014)

Authors	VO2 max						
	Pre-donation	**Post donation Day 1**	**Post donation Day 3**	**Post donation Week 1**	**Post donation Week 2**	**Post donation Week 3**	**Post donation Week 4**
Judd *et al.* (2011)	46.6	44.0*	Not done	45.4	44.5*	45.9	45.5
Ziegler *et al.* (2014)	49.7	Not done	46.5*	47.2*	48.9	Not done	49.0

* Statistically significant ($p < 0.05$)

Meurrens *et al*. (2016) subsequently studied effect of a 470 ml whole blood donation in 24 moderately trained young subjects with a mean baseline VO_2 max of 56.9 ± 4.63 ml/kg/min. The participants were further randomly divided into donation group (n = 16) and non-donation group as a placebo (n = 8). Both group were followed up prospectively for one month. The cardiorespiratory fitness in the donation group as measured by peak oxygen consumption was observed to be significantly reduced by 5%, 7% and 10% after two, seven and 14 days of blood donation, respectively. Meanwhile, for haematological parameters, the maximal decrease after blood donation were 11% for haematocrit and 10% for haemoglobin during the study period (Meurrens *et al.*, 2016).

The researchers however observed a longer cardiorespiratory fitness recovery of 28 days in comparison to previous studies (Judd *et al.*, 2011; Ziegler *et al.*, 2014).

There was a paucity of information concerning the effect of the blood donation on cardiorespiratory fitness level for the elderly blood donors. To address this issue, Janetzko *et al.* (1998) studied pre and post donation maximal working capacity in the 54 elderly blood donors (age range 55 to 69 years). There was no significant maximal working capacity reduction observed after the blood donation in this study. Based on this observation, the authors concluded that there was no negative effect of blood donation on cardiorespiratory fitness level in the healthy elderly blood donor. However, VO_2 max was not measured in this study (Janetzko *et al.,* 1998).

From exhaustive literature review, it was observed that most studies concluded that the standard whole blood donation of 450 to 500 ml led to a significant reduction in VO_2 max as shown in Table 2.2. The full recovery of VO_2 max was expected to take place in two to three weeks' time after blood donation, except for single study by Markiewecz *et al.* (1981) that reported VO_2 max recovery at 24 hours following blood donation. However cardiorespiratory fitness at submaximal intense exercise was less likely to be affected and other parameters such as time to fatigue and VO_2 max kinetics generally were not much affected due to the human physiological reserve and the role of anaerobic metabolism. To date, only three studies recorded insignificant VO_2 max reduction after blood donation; a study in 350 ml whole blood donors (Tarawan and Purwasasmita, 2009), in a very fit athletes (Dennison, 1960), and soldiers (Strandenes *et al.,* 2012).

Table 2.2: Summary of the effects of blood donation and blood loss on cardiorespiratory fitness level, haemoglobin, and haematocrit

First author, year, country	Study design	Population	Comparison	Exercise Protocol	VO_2 max changes (%)	Haemoglobin changes (g/dL)	Haematocrit changes (%)
Birnbaum, 2006, USA	Pre and post No control	10 males healthy subjects Age 23 ± 4 years old	24 hours before and after 500ml blood donation	Bruce protocol Incremental cycle ergometer test until exhaustion	42.8 to 38.6 significant reduction (9.81%)	15.3 to 13.5significant reduction (11.76%)	Not measured
Tarawan, 2009, Indonesia	Pre and post No control	21 males Age 35 ± 7 year old	A day before and after 350 ml blood donation	Rhyming Astrand bench test	36.66 to 35.44 insignificant reduction (1.22%)	Not measured	Not measured
Burnley, 2006, UK	Pre and post No control	10 males, 1 female Age 23 ± 6 years	Before and 24 hours after 450ml blood donation	Incremental cycle ergometer test until exhaustion	3.79 to 3.64 significant reduction (4%)	15.4 to 14.7 significant reduction (6.55%)	44 to 41 significant reduction (6.81%)
Howell, 1963, Canada	Pre and post With Control (no donation)	12 males (6 each in each group) Age 19 ± 2 year	Before, immediately, 24 hours after and 7 days after 500 ml blood donation	Incremental treadmill test until 180/min heart rate	5.43 to 4.61 significant reduction in study group (5.3%)	Not measured	Not measured
Ziegler, 2014, Denmark	Pre and post No control	20 males Age 33 ± 2 years old	Before, 3 days after, 7 days after, 14 days after and 28 days after 450 ml donation	Incremental cycle ergometer test until exhaustion	Baseline 49.7 to 46.4 at day 3 post donation significant reduction (6.5%)	7.9% significant reduction at day 3 post donation	7.0% significant reduction at day 3 post donation

First author, year, country	Study design	Population	Comparison	Exercise Protocol	VO_2 max changes (%)	Haemoglobin changes (g/dL)	Haematocrit changes (%)
Judd, 2011, Canada	Pre and post No control	12 (2 women, 10 men) Age 24 ± 5 years old Moderately active	1 day before, 24 hours after, weekly until 1 month after 450 ml donation	Incremental cycle ergometer test until exhaustion	46.6 to 44.0 Significant reduction 5% at 24 hours post donation, 46.6 to 44.5 4.3% reduction at 2 weeks after blood donation	Only baseline measured, no post donation value	Only baseline taken, no post donation value
Gordon, 2013, UK	Pre and post No control	15 well trained Age 23.3 ± 4.5 years old	Before and 48-72 hours after 450 ml donation	Incremental cycle ergometer test until exhaustion	51.3 to 48.4 significant reduction (5.7%)	15.6 to 14.1 significant reduction (9.6%)	48.76 to 43.99 significant reduction (9.8%)
Strandenes, 2012, Norway	Pre and post No control	25 fit male soldier Age 29 years old	Before and immediately after 450 ml donation	Incremental treadmill test until exhaustion	61.1 to 60.1 Insignificant reduction (1.6%)	Not measured	Not measured
Panebianco, 1995, USA	Pre and post No control	10 male cyclists Age not mentioned	Before, 2 hours after, 2 days after and 7 days after donation	Incremental cycle ergometer test until exhaustion	Baseline: 4.85 Significant reduction 2 hours 4.45 (8.2%) 2 days 4.46 (8.0%) 7 days 4.50 (7.2%)	Only baseline measured	Only baseline measured

First author, year, country	Study design	Population	Comparison	Exercise Protocol	VO_2 max changes (%)	Haemoglobin changes (g/dL)	Haematocrit changes (%)
Hill, 2013, USA	Pre and post No control	9 males Age 23 years old	Before, 2 hours after, 2 days and 7 days after 450 ml donation	Incremental cycle ergometer test until exhaustion	Baseline 40.4 Significant reduction 2 hours after 34.0 (15%) 2 days after 36.0 (10%) 7 days after 37.0 (7.5%)	Baseline 14.9 2 hours after 14.9 2 days after 14 (-6.4%) 7 days after 14 (-6.4%)	Not measured
Fritsch, 1993, Germany	Pre and post No control	10 males 6 females Age 25.3 ± 2 years old	1 to 7 days before and 2 days after 450 ml blood donation	Incremental cycle ergometer test until exhaustion	11% reduction	14.5 to 13.0 Significant reduction (10.3%)	Not measured
Markiewicz, 1981, Poland	Pre and post No control	18 males volunteer blood donor Age 21 years old	Before and 24 hours after 400 ml blood donation	Incremental cycle ergometer test until 170/min heart rate	Reduced immediately after donation, after 24 hours no significant difference	Not measured	Not measured

First author, year, country	Study design	Population	Comparison	Exercise Protocol	VO_2 max changes (%)	Haemoglobin changes (g/dL)	Haematocrit changes (%)
Dellweg, 2008, Germany	Pre and post No control	7 males 4 females age 36 years old	Before and 24 to 48 hour after 500 ml blood donation	Incremental cycle ergometer test until exhaustion	9% significant reduction	9% reduction	Not measured
Gordon, 2010, UK	Pre and post No control	6 males 4 females Age 21 ± 2 years old	Before and 24 hours after 450 ml blood donation	Constant workload testing on a cycle ergometer until 6 minutes duration	Only VO_2 kinetic studied (no significant difference for VO_2 kinetic)	14.2 to 13.1 significant reduction (7.7%)	43.55 to 40.20 significant reduction (7.6%)
Meurrens, 2016, Belgium	Pre and post With control (no donation)	24 males Age 27 ± 4 years old 16 in donation group 8 in placebo group	Before, 2, 7, 14 and 28 days after 470 ml donation	Incremental cycle ergometer test until exhaustion	Baseline 56.7 Significant reduction in the donation group 2 days after 5% reduction 7 days after 7% reduction 14 days after 10% reduction	Significant reduction in the donation group 2 days after 15.6 to 13.7 (12.1%) 7 days after 14.2 (9.0%) 14 days after 14.2 (9.0%)	Significant reduction 2 days after 45.8 to 40.9 (10.7%) 7 days after 41.9 (8.5%) 14 days after 42.4 (7.4%)

2.2 COMPARISON OF THE EFFECTS OF WHOLE BLOOD AND APHERESIS DONATION ON CARDIORESPIRATORY FITNESS LEVEL

With the advent of component therapy in transfusion medicine and the development of the automated cell separator, apheresis donation of plasma and platelet had become more prevalent as source of blood products. The apheresis donation method is capable of collecting red cells, plasma, platelets, and granulocytes as well as haematopoietic stem cells. According to the Malaysian Annual Blood Transfusion Report, Malaysian blood donors have contributed a number of 8,820 platelet apheresis donations and 10,353 plasma donations in 2014 (Annual Report Blood Transfusion Service Malaysia, 2014).

Hill *et al.* (2013) compared the effects of plasma apheresis donation and regular whole blood donation on the cardiorespiratory fitness level in a 19 healthy university students and observed that the plasma donations had not led to significant VO_2 max changes compared to the whole blood donation as shown in Table 2.3. The whole blood donors group donated 350 ml to 450 ml blood and the apheresis donors group donated about 700 ml plasma. The authors utilized the cycle ergometer to test the cardiorespiratory fitness before donation, and at two hours, two days, and seven days after donation. Authors observed a significant reduction in VO_2 max after whole blood donation with no significant VO_2 max changes in plasma donation. The haemoglobin level at two hours after a whole blood donation was not significantly affected. However, the haemoglobin level was significantly reduced at two and seven days post whole blood donation. The blood volume and total haemoglobin mass loss in whole blood donation had led to a

significant drop in aerobic capacity (Hill *et al.,* 2013). In contrast to whole blood donation, haemoglobin level showed a significant increase after plasma donation and had normalized after two days. Haemoconcentration after plasma removal led to a higher haemoglobin level reading post donation. Reconstitution of fluid into the intravascular circulation usually begins 2 to 48 hours after blood donation and the haemoglobin level will be reduced to baseline level within the same duration.

Table 2.3: Effect of plasma and whole blood donations on VO_2 max and Haemoglobin (Hill *et al.*, 2013)

Type of donation	parameter	Before	2 hours after	2 days after	7 days after
Whole blood	VO_2 max (ml/kg/min)	40	34*	36*	37*
	Hb (g/dL)	14.9	14.9	14.0*	14.0*
Plasma Apheresis	VO_2 max (ml/kg/min)	38	37	38	38
	Hb (g/dL)	14.2	16.1*	14.7	14.4

*Statistically significant ($p < 0.05$)

For red cell apheresis, a maximum of two units of red cell can be collected from a single donor. This can help to reduce risk of multiple donors' exposure to a patient who requires more than one unit red cell transfusion. Additionally, a larger red cell volume can be collected from a single donor with very rare blood type with red cell apheresis donation. Nevertheless, red cell apheresis is costly and the interval between donations is similar when compared to whole blood donation, these disadvantages make red cells apheresis a less favourable option for red cell donation. In 2014, all red cells components were prepared from whole blood donation in Malaysia and no red cell apheresis donation was recorded in the same reporting year (Annual Report Blood Transfusion Service Malaysia, 2014). Sherman *et al.* (1994) reported that there was a significant reduction of VO_2 max after 24 hours of two units' red cell apheresis donation. The cardiorespiratory