ANGLIA RUSKIN UNIVERSITY

FACULTY OF SCIENCE AND ENGINEERING, SCHOOL OF PSYCHOLOGY AND SPORTS SCIENCE

THE PHYSIOLOGICAL RESPONSES TO A STANDARD UK BLOOD DONATION DURING SUB-MAXIMAL AND SUPRA-MAXIMAL EXERCISE ACROSS A FIVE-DAY INTERVENTION PERIOD

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A thesis in fulfilment of the requirements of Anglia Ruskin University for the degree of Doctor of Philosophy

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ANGLIA RUSKIN UNIVERSITY

ABSTRACT

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Introduction: Previous studies investigating the effects of blood donation (BD) on exercise are limited primarily to $\dot{V}O_{2max}$ and adopt short intervention periods; thus, do not fully consider sub-maximal or supra-maximal exercise or fully appreciate the impact that BD can have on exercise in excess of 24 hours (h) post BD. Thus, results may be relevant to BD services, donors and (recreational) athletes.

Methods: A systematic literature review and meta-analysis (1st experimental chapter) provided the grounding theory to aid in the formulation of further experimental chapters. Following ethical approval volunteers participated in four experimental studies. The first of which determined the effects of body composition and markers of cardiorespiratory fitness on haematological responses as a consequence of donating a unit (~470 ml) of blood. The next three experimental chapters determined the effects of BD over a period of up to 96 hours on O_2 uptake kinetics (moderate and heavy domains), cardiac and ventilatory outputs, and power outputs during four repeated 15 s sprints.

Results: The systematic review highlighted that sub-maximal and supra-maximal exercise data was limited. Experimentally, 3 h post BD Haemoglobin (Hb) decreased by 6.51 %, 24 h post BD a decrement of 7.09 % occurred. Further decreases of 2.68 and 2.55 % continued between 24-48 h and 48-72 h, with an increase of 1.98 % between 72-96 h ($P < 0.0001$). The $\dot{V}O_2$ amplitude was unaffected by BD, tHb data via Near infrared spectroscopy (NIRS) had significant increases for moderate ($P = 0.018$) and heavy domains ($P = 0.038$). At 72 h, ventilatory and circulatory parameters were most affected ($P < 0.05$). Power outputs increased following BD; a main effect for time in Peak Power Output (PPO) occurred across 24-96 h BD period ($P = 0.001$, $\eta_p^2 = 0.35$), at 24 h ($P = 0.001$, $\eta_p^2 = 0.39$), 48 h ($P = 0.013$, $\eta_p^2 = 0.35$), 72 h ($P = 0.006$, $\eta_p^2 = 0.31$) and 96 h ($P = 0.002$, $\eta_p^2 = 0.37$).

Conclusion: The UK BD guidelines advise caution with or avoiding strenuous exercise 12-24 h post BD. However, Hb continues to decrease in excess of 24 h highlighting implications for individuals wishing to undertake exercise for several days post BD. Ramifications of these findings are of benefit to BD athletes and non-athletes alike as well as blood transfusion services.

Key words: *Blood donation (BD), Haemoglobin (Hb), O_2 kinetics, High Intensity Intermittent Exercise, Ventilation, Circulation*

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List of Abbreviations and Chemical Definitions

ATT	Adipose tissue thickness
BD	Blood donation
BF	Body fat
BLa	Blood lactate
BP	Blood pressure
BSA	Body surface area
BV	Blood volume
Ca O_2	Arterial oxygen content
CO_2	Carbon dioxide
CI	Cardiac index ($\dot{Q}$ from the left ventricle/BSA, during one minute)
CI	Confidence interval (such as 95% CI: a parameter is within it)
CV	Coefficient of variation
EDFR	Early diastolic filling ratio (blood flow from the left atrium to left ventricle)
EFD	Food and drug administrator
EVF	Erythrocyte volume fraction
FDA	Food and drug administrator
FI	Fatigue index
GET	Gas exchange threshold
Hb	Haemoglobin
Hct	Haematocrit
HHb	Deoxygenated haemoglobin
HIIT	High intensity interval training
HR	Heart rate
Hz	Hertz
I-HSA	Iodine-Human serum albumin (radioactive plasma protein)
ISAK	International society for the advancement of kinanthropometry
LBM	Lean body mass
MPO	Mean power output
m$\dot{V}O_2$	Muscle O_2 consumption
NHS	National Health Service
NIR	Near infrared
NIRS	Near infrared spectroscopy
O_2	Oxygen
O_2Hb	Oxygenated haemoglobin
PaO_2	Partial pressure of O_2 in arterial blood

pBLa	Peak blood lactate
PCV	Packed cell volume
PCO_2	Partial pressure of CO_2
PO_2	Partial pressure of O_2
PPO	Peak power output
PV	Plasma volume
$\dot{Q}$	Cardiac output
RBC	Red blood cell
rBLa	Resting blood lactate
RCV	Red cell volume
RER	Respiratory exchange ratio
rhEPO	Recombinant human erythropoietin
RPM	Revolutions per minute
SaO_2	Arterial oxygen saturation
SD	Standard deviation
SpO_2	Peripheral oxygen saturation (percentage of oxygenated Hb in the blood)
SRS	Spatially resolved spectroscopy
SV	Stroke volume
SVR	Systemic vascular resistance
SVRi	Systemic vascular resistance index
RS	Spatially resolved spectroscopy
TBV	Total blood volume
TEM	Technical error of measurement
tHb	Total haemoglobin
TSI(%)	Tissue saturation index
VAV	Variable average value
$\dot{V}CO_2$	Carbon dioxide output
$\dot{V}_E$	Pulmonary ventilation (expired)
$\dot{V}_E/\dot{V}CO_2$	Ventilatory equivalent for carbon dioxide
$\dot{V}_E/\dot{V}O_2$	Ventilatory equivalent for oxygen
$\dot{V}O_2$	Pulmonary oxygen uptake
$\dot{V}O_{2max}$	Maximum oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake
W	Watt
WHO	World Health Organisation

Azide-methemoglobin: Formed when sodium nitrite converts the Hb iron from the ferrous to the ferric state to form methemoglobin, which then combines with azide (Von Schenck, falkensson and Lundberg, 1986).

Cyanmethemoglobin: a reasonably nontoxic compound produced by combining hydrocyanic acid (cyanide ion) with methemoglobin, a pigment used in hemoglobinometry (Farlex, 2012).

Drabkins reagent: a solution consisting of sodium bicarbonate, potassium ferricyanide and potassium cyanide

Haemiglobincyanide: another term for cyanmethemoglobin method

Methhaemoglobin: formed to protect Hb (to bind to cyanide) cannot carry oxygen due to the addition of the methyl group via nitrite.

Potassium cyanide (KCN): a very poisonous crystalline salt

Potassium ferricyanide ($K_3Fe(CN)_6$): a red soluble crystalline salt (compound) used in laboratory tests (Merriam-Webster, 2018)

Sodium bicarbonate ($NaHCO_3$): a salt of sodium that neutralizes acid (Oxford, 2010).

Sodium desoxycholate: a specific bile acid, resulting from de-conjugated bile salts (Lab M, 2017). A standardised selective inhibitor for enteric pathogens (Mast Group, n.d)

Sodium nitrite ($NaNO_2$): an antidote for cyanide poisoning and also used to reduce systemic BP and relieve local vasomotor spasms such as Raynaud disease (Farlex, 2012).

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Chapter 1: Introduction

It is often taken for granted that in times of need following an accident/trauma or on-going medical requirements that should a blood transfusion be vital then it can be given. In order for the National Health Service Blood and Transplant (NHS Blood and Transplant) to meet the current life-saving demands 400 new blood donors are needed every day (NHS Blood and Transplant, 2019). Maintaining and recruiting new donors depends on the altruism of the general public whose knowledge as to the effects of blood donation (BD) is either accepting or ignorant. A BD comprises firstly a haemoglobin (Hb) level check via capillary blood from a finger to ascertain the quality of the oxygen (O_2) carrying protein and then a ~470 ml venesection is taken from a vein in the arm. With the population comprising many combinations of male and female statures the effect of having a reduced haemoglobin (Hb) level following BD are likely varied, due to each person donating the same volume regardless of size. Nevertheless, for all donors having a reduced circulating O_2 in the blood will have consequences such as increasing heart rate and increased mean arterial blood pressure (Zöllei, et al., 2003). Although general advice at BD is to avoid strenuous exercise the day of BD, beyond this time frame little is known with regards to the effects of donation on exercise. Indeed, experimental work completed in this field to date has mainly concerned maximal oxygen uptake ($\dot{V}O_{2max}$) as opposed to sub-maximal exercise intensities (Gordon, et al., 2013). It is possible that this lack of research is contributing to the difficult task of keeping or recruiting donors. It may foster a lack of knowledge within the general population, in particular physically active and/or trained individuals who perceive that there are detrimental effects of BD on athletic performance (Van Remoortel et al., 2016). It is a moot point though that athletes (particularly endurance trained) would actually make good donors if they were not worried about performance. This is because they have an augmented amount of circulating red blood cells (RBCs) and Hb compared to inactive individuals (Mairbäuri, 2013). Nevertheless, there are anecdotal stories of negative exercise performance post BD and additionally an informal short survey (of 68 people, unpublished data) revealed that almost 23 % of people surveyed did not donate due to the worry of their athletic performance being affected.

It is recognised that the eligible BD population are not all athletic, but it is nevertheless reasonable that everyone should have access to information based on data regarding the consequences of having a reduce O_2 carrying capacity on and beyond the day of BD.

1.1 Aim and Objective

The aim of this research was to provide an awareness of BD on the delivery and utilisation of O_2 during exercise across a five-day intervention period. It is hoped that this research will provide insight into the effects that BD has both sub-maximally and supra maximally and that the results may assist individuals to make more informed decisions to donate or not. The impact of this work has the potential to change or provide additional information within guidance that is given at BD and additionally affect BD numbers.

It was consequently the objective of this thesis to extract and review the available literature on BD and exercise, and also to run experimental chapters on BD to encompass the exercising general public. The first of which, was an experimental chapter that considered the body composition of individuals, that enabled the collection and synthesis of data which discovered who was most affected by BD. Further experimental chapters involved exercising up to 96 hours post BD. Sub-maximal exercise was examined as workouts are mainly performed at this intensity as opposed to maximally. Additionally, supra-maximal exercise in the form of repeated multiple sprints to replicate High Intensity Interval Training (HIIT) was examined due to its prevalence in community exercise classes and the strenuous nature of the exercise.

A conceptual framework (Figure 1.1) was developed to provide an overview of the topic in order to provide a perspective on the contribution to knowledge.

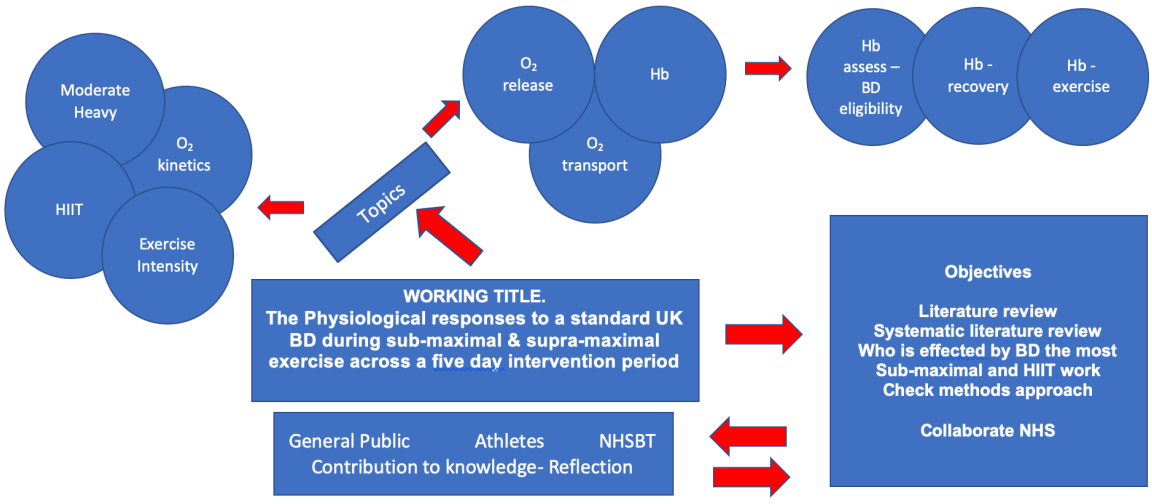

Figure 1.1: Conceptual framework of thesis.

Chapter 2: Review of Literature

The following section relates to the relevant background literature to provide insight and theory for the thesis.

2.1 Blood Volume Overview

Blood is essentially composed of two constituents: plasma (~55 %) and red blood cells (RBCs) (~45 %), which equate to total blood volume (TBV). Additionally, there are platelets and white blood cells, but equate to less than 0.1 % of TBV (Gómez and Jesús, 2015). The haematocrit (Hct) also known as packed cell volume (PCV) (Ertl, Diedrich and Raj., 2007) or erythrocyte volume fraction (EVF) denotes the fraction of RBCs that is within TBV (Ertl, Diedrich and Raj., 2007; Manzone, et al., 2007) and thus represents the volumetric connection between RBCs and plasma. Haematocrit values range between 37-43 % and 42-47 % in women and men respectively (Manzone, et al., 2007 and Gómez and Jesús, 2015).

In clinic/hospitals Hct is frequently used as a proxy measure for red cell volume (RCV) (Gómez and Jesús, 2015); in this instance an assumption could be made that PV is normal. However, in cases of reduced or amplified PV the Hct reading would provide high RCV or low RCV respectively, resulting in false values. This is illustrated in Figure 2.1.

Postural pseudoanaemia should also be considered with Hct readings, as values will be decreased when lying down compared to being upright (Jacob, et al., 2005; Ertl, Diedrich and Raj, 2007), thus Hct is sensitive to body positioning. Additionally, Hct values will differ in varying diameter vessels, for example, capillaries offer a decreased Hct compared to a peripheral vein and also whole-body Hct and venous Hct will vary, this is deemed to be the f-cell ratio (Gómez and Jesús, 2015).

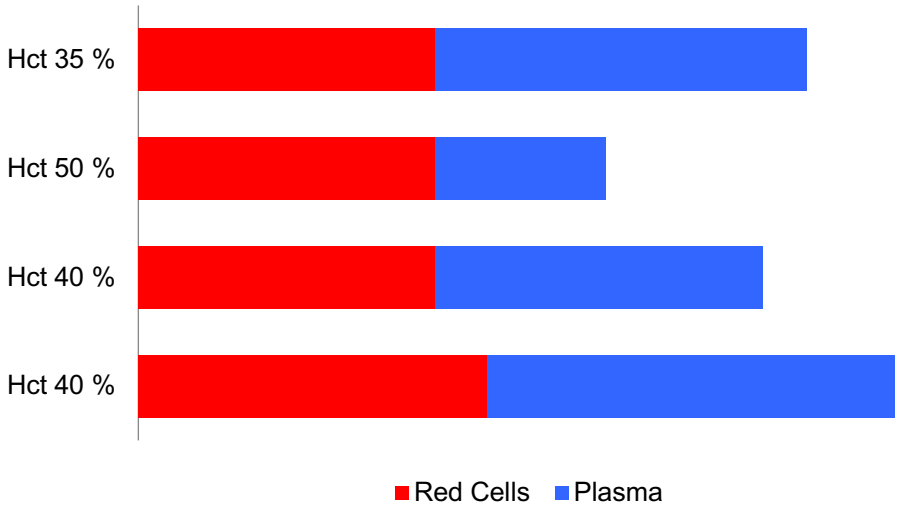

Figure 2.1: An illustrative guide of four blood samples. What is depicted is that the lower two bars /blood samples have the same Hct, however the RCV is higher in the lower bar/sample. Additionally, RCV is the same for the top three samples, but Hct varies, illustrating the variance of PV. Information adapted from Gómez and Jesús, (2015).

Plasma volume (PV) contains solutes (particularly plasma proteins such as albumin) and is ~92 % water. Although plasma proteins transport materials like hormones they also have the important oncotic role of keeping pressure of the blood, which via osmosis draws water into blood vessels (Manzone, et al., 2007). PV can be affected by circumstances such as hot or cold environments, dehydration, exercise and posture, for example when recumbent the volume can increase by 10 % within an hour (Gómez and Jesús, 2015). PV is also regulated by physiological means such as cardiac, renal and hormonal status. Thus, PV is not static and division of fluid from interstitial tissues and the circulatory system results in variations. RCV is controlled by growth factors and erythropoietin (Gómez and Jesús, 2015). Post BD a prompt expansion of PV counterbalances the loss of BV; this decreases Hb values (Schmidt and Prommer, 2010). However only after interstitial fluid is moved will a drop in Hb and Hct be seen (Belfort, et al., 2010). The fast compensatory shift of the interstitial fluid moves into the circulation from skin and skeletal muscle (Saito, et al., 2013). When interstitial fluid is moved to intravascular compartments it is called transcapillary refill (the movement of protein and fluid) and it is responsible for restoring BV following blood loss. This process is due to changes in the Starling Forces, which concern passive water exchange between capillary microcirculation and the interstitial fluid. Initially, after a decrease in capillary hydrostatic pressure a rapid movement of protein-free fluid goes to capillaries from the interstitium, following this protein then returns to aid plasma oncotic pressure (Drucker, et al., 1981). Thus, water moves from interstitial to intravascular compartments. Thus, Hct will decrease due to the increase in BV/PV.

Fluid is a component of the blood and balancing fluid is important, essentially fluid output such as vomit, faeces or sweat needs to equal fluid input. Daily fluid intake is generally 2-3 L included in food and drink, plus a minor contribution from cell metabolism (Manzone, et al., 2007). It is suggested that to maintain hydration daily fluid intake should be 35 $ml \cdot kg^{-1}$ for 18-60 years and 30 $ml \cdot kg^{-1}$ for over 60 years (Todorovic and Micklewright, 2011).

2.2 Measuring Blood Volume

Normal BV in adults is proposed to be 7 % of ideal body weight and in children it is 8-9 % (Stainsby, MacLennan and Hamilton, 2000). As an estimated guide an adult has about 4.7-5.7 L (Manzone, et al., 2007).

According to Rowntree, Brown and Roth (1929) gruesome direct methods were employed in the 19th century using humans and animals. They report that Bischoff in1857 used two condemned beheaded felons to establish BV, which was calculated at 73 $ml \cdot kg^{-1}$, via exsanguination, washing out vessels with water and extracting Hb via mincing the organs. This process was carried out with dogs a few years earlier by Welcker in 1854 who established that blood volume was 7.7 % of body mass, a conclusion that Bischoff (1857) concurred with (Rowntree, Brown and Roth, 1929). Fortunately for today's society more civilised methods are used, but the methods are indirect.

Early attempts at establishing BV by using dyes was made by Keith, Rowntree and Geraghty (1915) who used Evans 'brilliant vital red dye', however this was problematic and was replaced with Evans blue dye (T-1824) for slower diffusion purposes. Processes further evolved with the presentation of radioactive elements, which gave the means of tagging erythrocytes. Combining this method with the dye dilution or I-HSA (Human serum albumin) method for plasma volume provided a more accurate BV overview (Yurkon, 1965).

BV can be assessed with radioisotope; assessing PV or RCV measurements (or both with a double isotope technique, the gold standard method) using indicator dilution system. Generally, with a patient recumbent, a tracer/dye is administered and after mixing within the body a blood sample is taken, however, this can be a long and arduous task (Manzone, et al., 2007), plus it is invasive for the individual. Manzone, et al. (2007) and Gómez and Jesús. (2015) describe the Indicator dilution technique; a known volume of indicator (A), within a known volume V_1 with a known strength C_1 (Figure 2.2), is infused into a system whose volume V_2 is unspecified. After mixing, a sample from V_2 is taken, which can then

provide concentration of the indicator C_2, resulting in the equation $C_1V_1 = C_2V_2$. So, an unspecified volume of a closed system can be calculated thus $V_2=C_1V_1/C_2=A/C_2$. For RCV and PV alike specific markers are needed, for example I-HAS (Human serum albumin) for PV and Cr-sodium chromate for RCV, therefore TBV is often calculated using haematocrit, as the specific markers only yield RBC or PV. It is possibly worth pointing out at this stage that some documents/individuals will refer to red cell mass (RCM), when they actually mean RCV, as it is actually volume that is measured.

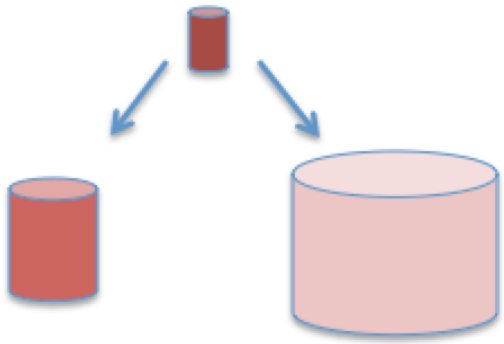

Figure 2.2: A schematic of the indicator dilution technique. If a known amount of red dye such as 10 ml (represented by the top glass) is placed into a large volume of water such as 1000 ml the concentration would be low and the water would look light pink, as shown by the container on the right. However, the converse would be true if the known amount of red dye went into a small volume of water such as 500 ml, the colour would be dark pink, as shown by the left-hand container. Thus, if amounts of indicator and final concentrations are known then blood volume can be worked out. Information adapted from Ertl, Diedrich and Raj, 2007.

BV will adjust depending on stature and composition, for example individuals of identical mass may present conflicting body compositions due to factors such as body fat and musculature. Additionally, the larger and more athletic individual will have a greater BV in comparison to a sedentary, small individual (Maughan, 2013). In earlier work from Wilmore and Costill (1994) BV in a sedentary man increased by 8.51 % from training/exercise and becoming more athletic. Body type has been considered in the past for working out normal reference values for BV (Gregersen and Nickerson, 1950), but somatotyping was too intuitive. Ectomorphs, endomorphs and mesomorphs tended to produce conflicting BV to body mass ratios (Gregersen and Nickerson, 1950).

BV values are often expressed in terms of unit of mass, for example $ml \cdot kg^{-1}$. Researchers such as Inkley, Brooks and Krieger (1955) found similar values for BV with I-HAS and T-1824 providing 73.3 $ml \cdot kg^{-1}$ and 71.4 $ml \cdot kg^{-1}$ respectively, with Feldschuh and Enson (1977) assessing BV to be 60-70 $ml \cdot kg^{-1}$. But this type of expression that is units per mass is possibly controversial, when considering ectomorphic individuals who are below ideal

mass will convey more BV per unit of mass in comparison to an obese person (Retzlaff, et al., 1969), as BV is reduced in adiposity. Pearson, et al. (1995) believes that results for RCM/PV based on body mass alone should be discarded, due to inappropriate result interpretation from adiposity particularly in obese individuals.

Feldschuh and Enson (1977) discuss the shortcomings of BV to mass or BV to body surface area (BSA) (based on Du Bois and Du Bois (1916) BSA = mass(kg) $^{0.425}$ x Height(cm)$^{0.725}$ x 0.007184) methods to predict normal BV, and they report that BV is not a constant fraction of body weight or height, or BSA (a combination of height and mass), which hence reflects body compositional variation. By using the Metropolitan Life height and weight tables Feldschuh and Enson (1977) avoided systematic errors seen in body weight and BSA norms in 160 well people of varying body compositions. The Metropolitan Life tables determined ideal weights by assuming that the proportion of lean tissue to fat was approximately the same in differing height individuals. The blood volume was forecast on the basis of the percentage that the body weight would vary from ideal weight and therefore gave an estimate of body composition. For example, a higher proportion of body fat is expected in overweight individuals giving a lower predicted normal blood volume per unit of mass. Conversely for an underweight person who is imagined to have a greater amount of lean tissue there is a higher anticipated blood volume per unit of mass.

There are formulas for PV and RCM such as Retzlaff, et al. (1969) and Hurley (1975), but the UK blood donor services use the Nadler formula (Nadler et al, 1962) to work out BV (Table 2.1). These formulas are based on body surface and as such take into account height and mass. The Nadler formula was established using the I-HSA (Human serum albumin) method (where radioactive plasma protein labelling of red cells is merged with determination of plasma volume via dye dilution) (Yurkon, 1965). Other researchers have noteworthy formulas (based on I-HSA) too also using body surface according to Yurkon (1965), these are Baker, Kozoll and Meyer (1957) whose formula is BSA (sq.M) x 2.68 = BV (L) and Allen, et al, (1956) who established for women TBV (L) = 0.414 x Height (M)^3 + 0.0328 x Mass (kg) – 0.30 and for men/boys TBV (L) = 0.417 x Height (M)^3 + 0.045 x Mass (kg) – 0.30.

Yurkon (1965) computed another formula with I-HSA, as it was felt that Evans blue dye gave too high norms and radioactive isotopes too low norms based on clinical findings. The formula was:

Males

$$TBV\ (ml) = \frac{100\ (Height(") \times Mass(lb))}{Height + Mass} + \frac{(Height \times Mass)}{10}$$

RCV (ml) = 47 % of TBV

PV (ml) = 53 % of TBV or TBV - RCV

Females

$$TBV\ (ml) = \frac{100\ (Height(") \times Mass\ (lb))}{Height + Mass} + \frac{(Height \times Mass)}{100}$$

RCV (ml) = 42 % of TBV

PV (ml) = 58 % of TBV or TBV - RCV

Pearson, et al. (1995) believed that there were notable formulae from varying researchers (Table 2.1). However, Pearson and colleagues (1995) also noted they all have varying shortcomings, for example Retzlaff and colleagues (1969) were lacking in recruitment and after injection of indicator solution for PV did not extrapolate time to zero despite measuring it 10-15 minutes later, as was the case for Nadler, et al. (1962) and Hurley (1975). Wennesland and partners (1959) only examined RCM and Nadler (1962) only researched PV. Pearson and collaborators (1995) recommended their own formulae show in Table 2.2.

Table 2.1: Formulae for Red Cell Mass and Plasma Volume for varying researchers. Where S = surface area (m^2); DuBois & DuBois, (1916) Mass.(kg) $^{0.425}$ x Ht.(cm)$^{0.725}$ x 0.007184. M = males, F = females, RCM = Red Cell Mass, PV = Plasma Volume

	Hurley 1975 n= 481 M, 303 F	**Retzlaff et al, 1969 n= 40 M (22-66 yrs), 38 F (20-64 yrs)**	**Wennesland et al, 1959 n= 201 M (19-44 yrs)**	**Nadler et al, 1962 n= 92M, 63 F (17-90 yrs)**
Mean RCM M (ml)	$1486S^2$-4106S+4514	1100S	1550S - 890	Resulting from BV
Mean PV M (ml)	$995e^{0.6085S}$	1630S	1580S – 520*	$(366.9H^3)$ + (32.19M) + 604
Mean RCM F (ml)	1167S - 479	840S	N/A	Resulting from BV
Mean PV F (ml)	$1278S^{1.289}$	1410S	N/A	$(356.1H^3)$ + (33.08M) + 183.3

* Indirectly from haematocrit

Table 2.2: Pearson and collaborators (1995) formulae. Where S = surface area (m^2); DuBois & DuBois, (1916) Wt.(kg) $^{0.425}$ x Ht.(cm)$^{0.725}$ x 0.007184. M = males, F = females, RCM = Red Cell Mass, PV = Plasma Volume

Type of measured volume	Equation	
Mean RCM M (ml)	(1486 x S) – 825	98 % limits = ± 25 %
Mean PV M (ml)	1578 x S	99 % limits = ± 25 %
Mean RCM F (ml)	(1.06 x age) + (822 x S)	99 % limits = ± 25 %
Mean PV F (ml)	1395 x S	99 % limits = ± 25 %

In 1998, a semi-automated BV analysis system called the BVA-100 was approved for clinical use by the Food and Drug administration (USA). By injecting ^{131}I-HAS (in saline) via a patented volumetric flow chamber PV is measured. The Hct is used to ascertain RCV and TBV, a method comparable to the gold standard double labelling technique. Other benefits consist of short time frames to get results, a less complicated procedure and the machine compares results to normal values based on deviation from ideal weight (Manzone, et al., 2007).

Identifying what is normal/typical blood volume is difficult, due to the fact that it continually oscillates with metabolism, physical activity, body positioning, sleep and temperature (Gómez and Jesús, 2015). Additionally, it varies according to age, sex, musculature, body proportionality and weight (Yurkon, 1965; Gómez and Jesús, 2015). Gómez and Jesús, (2015) believe that there is no perfect system for calculating BV and more accurate methods for normal reference values (eliminating the obesity issue) would be advantageous. Within this thesis minimising any possible fluctuations in BV were managed by the participants. Thus, any repeated laboratory visits were attended at exactly the same time, normal daily activity was maintained as was dietary intake (food and drink). Participants were weighed at each visit and no weight changes were observed, additionally hydration status was recorded daily, alongside Hct concentration, thus aiding to indicate stability in BV.

2.3 Body Surface Area

When considering BV, BSA although better than using mass alone still lacks adequate reflection of body mass and tissue vascularity (Manzone, et al., 2007). It appears that many people turn to the Dubois and Dubois (1916) formula when it comes to calculating BSA, although it does appear to be lacking in participants (one female child and eight adults), questioning scientific integrity. They compared the casting of the bodies to geometric calculations of individual body segments relating to length and diameter, this showed close

approximation. The Dubois brother's formula is based on relating body mass and height to BSA. Other formulas can be seen in Table 2.3. Haycock and colleagues (1978) used the geometric notion that spheres and cylinders represented body parts to determine BSA, but with the values for participants' BSA, height and weight missing within the paper, suppositions against other authors can only be made, this is also the case for Gehan and George (1970). Various authors claim the Dubois equation was not appropriate for obese individuals (Gehan and George, 1970; Livingston and Lee, 2001) or small children (Haycock, et al., 1978; Gehan and George 1970) due to the underestimation of values.

Table 2.3: Varying formulae for calculating Body Surface Area.

Researcher	**No. Participants**	**Formula**
Dubois and Dubois (1916)	9	$BSA = 0.007184 \times Mass(kg)^{0.425} \times Ht(cm)^{0.725}$
Gehan and George (1970)	401	$BSA = 0.0235 \times Ht^{0.42246} \times Mass^{0.51456}$
Haycock, Schwarta and Wisotsky (1978)	81	$BSA = 0.02465 \times Ht^{0.39646} \times Mass^{0.5378}$
Mosteller (1987)	N/A*	$BSA = \sqrt{Ht(cm) \times Mass(kg)}/3600$

* No. participants as the formula is a modified version of Gehan and George (1970).

As a final note, although the Nadler (1962) formula has faults, it is the formula adopted by the NHS when regarding blood volume calculation for blood donation. Furthermore, this approach avoids the confounding issues highlighted by Pearson, et al. (1995) where mass alone is used as the metric of body stature. It is therefore the formula that will be adopted where necessary for this study to keep consistency, as all participants will be NHS blood donors.

2.4 The Structure of Haemoglobin and Oxygen Transport

In terms of arterial O_2 content, > 99 % of O_2 is transported by Hb (Maniatis, 2010). Hb, which gives the distinctive red colour to the blood cells, contains a haem prosthetic group with an iron atom in the middle, with four haem groups per Hb molecule. Thus, Hb is a tetramer of polypeptide chains carrying four oxygen molecules when fully saturated (Figure 2.3). Full saturation occurs at high partial pressures of oxygen in the blood. If iron is bound to O_2 the haem group is red, however it is blue-red if O_2 is lacking, thus striking the difference between oxyhaemoglobin and de-oxyhaemoglobin. Muscles are supplied with oxyhaemoglobin from oxygen diffusing into capillaries during day-to-day activity exercise.

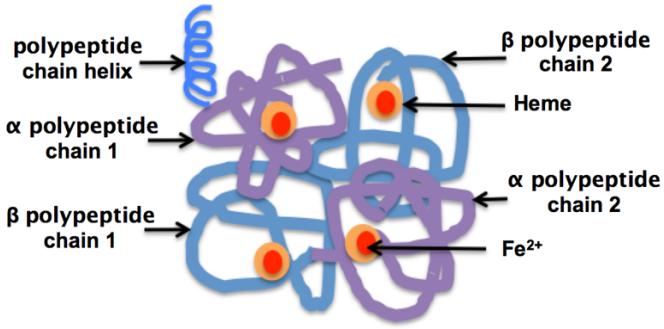

Figure 2.3: A schematic of the haemoglobin molecule. It is comprised of 2 alpha and 2 beta helix shaped polypeptide (globin) chains linked together to form a spherical molecule. A heme group is attached to each chain, which has an iron atom, the site of oxygen binding. The iron is strongly (covalently) bound to the globular protein (this allows for unloading of oxygen).

Hb is accountable for the conveyance of O_2 in blood from the lungs at high pressure (100 mmHg) to varying body tissues at low pressures (40 mmHg) and at full saturation carries ~20 ml of oxygen per dL of whole blood (McArdle, Katch and Katch, 2010). Thus, it is calculated accordingly, O_2 = 1.34 (ml $O_2 \cdot g^{-1}$ Hb) x 15 ($Hb \cdot 100ml^{-1}$) = 20.1 ml $O_2 \cdot 100ml^{-1}$ (Harrison, 2011a). Therefore, 70.5 g Hb is lost in a 470 ml blood donation. Also, Hb is responsible for more than one-half of the total iron in the body (Klein and Anstee, 2007) and therefore it is the largest circulating component of iron with 450 ml of whole blood containing 200 mg (Hoffbrand, Catovsky and Tuddenham, 2005). A standard UK donation removes ~236 mg of iron in men and ~213 mg in women (Abdullah, 2011).

The entire mass of circulating Hb (oxygenated and deoxygenated Hb, carboxyhaemoglobin and methaemoglobin) is known as total haemoglobin mass (tHb-mass) (Otto, Montgomery and Richards, 2013) and Hb values are dependent on PV and tHb-mass. It has been suggested that tHb-mass is more stable than Hb and as such is a better parameter to use when regarding oxygen-transporting molecules. For example, Hb could fluctuate when intravascular fluid moves as a consequence of disease or medical treatment, resulting in a poor indicator of oxygen carrying capacity. Also, Hb is determined by tHb and TBV, thus a contracted PV may mask a reduced oxygen carrying capacity related to tHb and additionally escalations in intravascular fluid may reduce Hb, even if there is normal tHb (Otto, Montgomery and Richards, 2013). Eastwood, et al. (2008) showed low fluctuations of ~ 2 % in the tHb-mass of six active men studied every 1–6 days for 100–114 days (42 ± 3 measurements) with the carbon monoxide rebreathing method. However, it should be recognised that oxygen delivery to a tissue is equal to Hb concentration multiplied by blood flow, thereby having potential performance implications.

2.5 Oxyhaemoglobin Dissociation Curve

Oxyhaemoglobin dissociation (Figure 2.4) or unloading is associated with tension dropping, as the partial pressure falls from 100 mmHg at the lungs to low pressure (40 mmHg) at tissues resulting in oxygen release to the tissues. It is a change in the P_{50} value (which is when Hb is 50 % saturated) which shows an alteration in O_2 affinity for Hb and therefore will display a right or left shift in the dissociation curve (Thomas and Lumb, 2012).

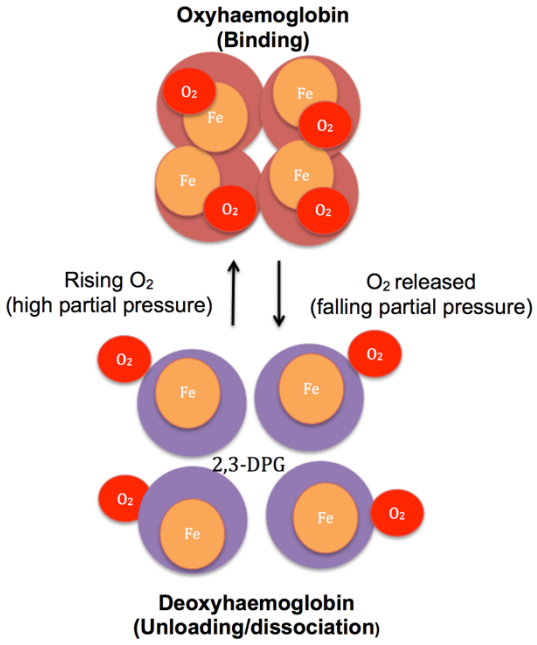

Figure 2.4: A schematic illustrating the release of oxygen from a haemoglobin molecule. High partial pressure (100 mmHg) in the alveoli will move to a low partial pressure (40 mmHg) in the peripheral tissues for Hb unloading. The β chains are drawn apart when O_2 is unloaded, which allows for 2,3-DPG to entre, this results in reducing Hb affinity for O_2.

The oxyhaemoglobin dissociation curve is a graphical illustration to aid understanding of how Hb saturation is determined by the partial pressure of O_2 and therefore how Hb conveys and releases oxygen. The sigmoidal shape expresses the association between Hb saturation (SO_2) and varying oxygen tensions (PO_2) (SpO_2) (Figure 2.5). A rightward shift occurs in the curve because of the Bohr effect, which is generally associated with exercise due to the oxygen requirement in muscles. Increases in temperature and partial pressure of carbon dioxide and decreases in pH levels (as hydrogen ions increase) result in an acidic environment and oxygen dissociating from Hb (McArdle, Katch and Katch, 2010). The right shift is also from 2,3-biphosphoglycerate (2,3-DPG) levels increasing (Mairbäurl, 2013). 2,3-biphosphoglycerate is a phosphate produced in RBCs (from metabolising glucose), it causes a right shift by binding laxly to the subunits of Hb, thus reducing the Hb affinity for O_2 and releasing O_2 to tissues (a benefit in anaemia, chronic hypoxia and intense exercise when releasing O_2 to tissues is needed (Mairbäurl, 2013)) and decreasing pH levels (McArdle, Katch and Katch, 2010). The decreased affinity requires more partial pressure of oxygen to bind to Hb.

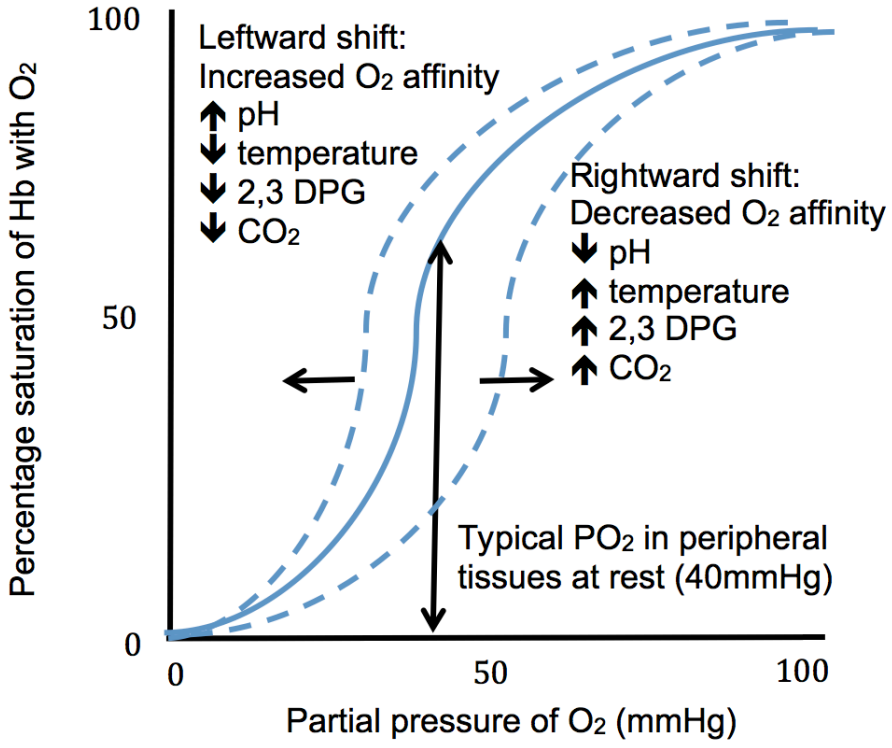

Figure 2.5: An illustrative schematic expressing the oxygen dissociation curve (in solid blue) and the effects of changing pH, temperature, 2,3 DPG and PCO_2 has on the curve. With the rightward shift in the curve known as the Bohr shift. With normal pH (7.4) and 40 mmHg PO_2 in tissues there is about 70 % saturation of Hb (thus 30 % is unloaded). When exercising the pH decreases, a more acidic pH of 7.2 results in saturation of about 60 %, making Hb unload to exercising tissues. In the lungs the pressure is 100 mmHg and saturation is ~98 %, the same for all curves, thus the Hb in the lungs persistently receives oxygen molecules (binding the same percentage of oxygen).

Augmented levels of 2,3-DPG in erythrocytes are accountable for training associated decreases on Hb affinity for O_2. It is likely that the increased levels of 2,3-DPG are a result of aroused erythropoiesis, which reduces RBC mean age (Mairbäurl, et al., 1983) as juvenile RBCs have increased 2,3-DPG compared to older RBCs (Mairbäurl, et al., 1990). The impact of the decreased O_2 affinity is a shift to the right in the dissociation curve thus increasing acidosis (pH ≤ 7.35 (Bohr effect)). Furthermore, with an increased erythrocyte production (via erythropoietin stimulating erythropoiesis) oxygen carrying capacity in the blood is increased.

The CO_2 produced is surplus from cellular metabolism, but it is useful in aiding pH balance. By RBCs transporting CO_2 and by binding hydrogen ions (H^+) to Hb a pH buffer is created (Mairbäurl, 2013). Increased CO_2 results in increased PCO_2 thus moving the dissociation curve rightwards, particularly evident during exercise. Increased H^+ as a result of lactate and carbonic acid formation from exercise results in a more acidic environment thus lowering pH levels and creating a rightward curve shift. Similarly, to CO_2, this effect occurs while RBCs move through the muscle capillaries (Plowman and Smith, 2014). The final variable in the rightward shift is temperature and during exercise it increases to ~41℃ from ~37℃ at rest (Mairbäurl, 2013). The extent of the deviation of the dissociation curve compared to resting will be dependent on exercise intensity and thus active muscle (Mairbäurl, 2013).

When blood loss (haemorrhage or maybe BD) occurs, RBCs are lost and there is no immediate reserve to replace them, this results in reduced Hb levels and lowers arterial O_2 content (Meßmer, et al., 1973) thus the oxygen dissociation curve shifts right to maintain the oxygen supply to the tissues. This means that Hb will decrease its affinity for the oxygen molecule and release oxygen to the tissues at a higher partial pressure. The relevance of the disassociation curve shift is that an understanding can be gained between Hb, oxygen and partial pressure. Thus, an individual who is generally short of breath could indicate a decreased Hb content.

2.6 Haemoglobin and the Blood Donor

Commonly ~ 470 ml is collected from donating a unit of blood (NHS choices, 2014) and this quantity should not exceed >15 % of an individual's blood volume (JPAC, 2013). The National Health Service (NHS) Blood and Transplant (2017) currently allow males and females to donate blood every 12 weeks and 16 weeks respectively, equating to four or three donations per year. However, it is females that dominate as blood donors who

represent 56 % of all donors and furthermore it is white British (92.9 %) and 45 – 54 years of age that are more prevalent (Public Health England, 2016).

There are various guidelines to deem the suitability of an individual to donate blood, however it is not meeting the Hb standards that is the most common cause for donor exclusion (Klein and Anstee, 2007, p. 7). In order to safeguard potential blood donors from becoming (acutely) anaemic post BD (which would impair oxygen transport and symptoms such as shortness of breath, increased HR and faintness may be experienced), sensitive screening of Hb prior to BD is essential. If Hb drops below normal range, which is 13 – 18 $g{\cdot}dL^{-1}$ and 11.5 – 16.5 $g{\cdot}dL^{-1}$ for adult males and non-pregnant females correspondingly (Gledhill, 1985) an individual could be deemed anaemic. Hence, tolerance levels for Hb are set higher than the lowest value of the normal range at donation centres in order to avoid any patient becoming acutely anaemic. There are many types of anaemia, which is classified as an abnormally low amount of RBCs (Home Health UK, 2020) and causes can range from having low iron levels or vitamin B12, heavy menstruation or diseases such as leukaemia. With regard to athletes or exercise enthusiasts, they may have sports anaemia. This concerns the Hb dropping just under average i.e. 15.8 $g{\cdot}dL^{-1}$ for males and 13.9 $g{\cdot}dL^{-1}$ for females (Gledhill, 1985). According to Eichner (1992), athletes have lower Hb values due to expanded plasma volume, which dilutes RBCs, however this is an adaptation to aerobic exercise and as such sports anaemia is a false anaemia.

At a blood donor centre the Hb assessment usually involves capillary blood and is traditionally performed using the finger prick method, due to competitive costing, quickness and user-friendly operation (Daves, et al., 2016). Following the finger prick, blood is released into blue for females or green for males aqueous copper sulphate, which has a specific gravity of 1.053 (comparable to 12.5 $g{\cdot}dL^{-1}$ Hb) and 1.055 (comparable to 13.5 $g{\cdot}dL^{-1}$ Hb) respectively. This simple test ensures that the quality of Hb in a full blood donation is of standard (JPAC, 2013). Hb levels in the UK should not be lower than 12.5 $g{\cdot}dL^{-1}$ and 13.5 $g{\cdot}dL^{-1}$ for women and men respectively preceding donation (NHS, Blood and Transplant 2018; European Directorate for the Quality of Medicines and HealthCare (EDQM), 2015). The European normal cut off values also replicate the UK (EDQM, 2015), in America and Canada the acceptable donation values are 12.5 $g{\cdot}dL^{-1}$ and 13.0 $g{\cdot}dL^{-1}$ for women and men respectively (American Red Cross, 2020; Canadian Blood Services, 2020), in Australia the acceptable donation values are 12.0 $g{\cdot}dL^{-1}$ and 13.0 $g{\cdot}dL^{-1}$ for women and men respectively (Australian Red Cross Blood Service, 2020).

However, the finger prick method according to Pagliaro (2014) is not especially acceptable (particularly in women), as Hb values have been overestimated according to haemocytometry (where RBCs are counted) and indeed capillary blood provides a higher Hb concentration than venous blood. Daves, et al. (2016) studied 1014 blood donors and agreed with Pagliaro (2014) and showed a significant trend to overestimate Hb from capillary blood compared to venous blood, with significance after a paired *t*-test shown as $P < 0.0001$. Patel, et al. (2013), also found this in a study of 150 donors, as did Cable and colleagues (2012) in 2425 donors. Conversely to these conclusions, a 42 month study by Tong and associates (2010) that happened in the Irish Blood Transfusion Service, explored 36,258 paired samples and found that venous Hb was higher than the finger prick (capillary) value by 0.67 $g{\cdot}dL^{-1}$ (females) and 1.07 $g{\cdot}dL^{-1}$ (males) producing significance of $P < 0.001$, which resulted in the researchers endorsing a decision to alter acceptable donor Hb levels to 12 $g{\cdot}dL^{-1}$ and 13 $g{\cdot}dL^{-1}$ for females and males respectively for finger prick Hb. This decision enabled a supplementary 32,900 blood units to be collected over the study period and BD in clinics increased by 9.4 % per year. A factor in the varying outcomes could be due to disparity in participant numbers but, Patel, et al. (2013), suggest that the discrepancies between their study and Tong, et al. (2010) may be due to seasons, as they only collected data for one month in the summer, whereas Tong and colleagues (2010) was over several seasons as it was over 42 months. In the summer capillary Hb is lower than winter, this was reported by Bäckman and associates (2016) comparing July (a.m. mean Hb of 15.41 $g{\cdot}dL^{-1}$ in males and 13.96 $g{\cdot}dL^{-1}$ in females, p.m. mean of 15.38 $g{\cdot}dL^{-1}$ in males and 13.89 $g{\cdot}dL^{-1}$ in females) to January (a.m. mean Hb of 15.72 $g{\cdot}dL^{-1}$ in males and 14.28 $g{\cdot}dL^{-1}$ in females, p.m. mean of 15.69 $g{\cdot}dL^{-1}$ in males and 14.18 $g{\cdot}dL^{-1}$ in females). This resulted in 7.8 % of donor deferrals in July at 7pm compared to only 1.6 % in January at 11am ($P < 0.0001$). The seasonal difference is considered to be due to PV increases that occur (by 9 %) in the summer (Haus, 1992, p.506). Cable and colleagues (2012) suggest that discrepancies between their study and Tong, et al. (2010) may be due to the fact that they had iron depleted donors and Tong and associates did not determine iron status. Once again though participant numbers (25,762 females and 10, 496 males (Tong, et al., 2010) and 65 females and 85 males (Patel, et al., 2013)) are vastly different between these studies, which could be an influencing factor on results.

It should also be noted that fingertip pressure or “milking” sometimes used to extract more blood might give a false reading, as this process may increase plasma content and consequently the extracellular fluid inclusion would decrease red cell components (Meites, 1998). Additional consideration should be given to skin temperature, skin thickness and penetration depth of the puncture (Patel, et al., 2013). According to Patel and

contemporaries (2013), Hb is more reliably assessed with venous blood, as it reflects the blood surging through the heart, arteries and veins, whereas the finger prick sample is from smaller capillaries, arterioles and venules. Although it should be noted that finger prick blood closer approximates venous Hb values when compared to the ear prick, which tends to produce higher results (Wood, Kim and Miller, 2001). Blood obtained from a finger prick/skin puncture, has a larger percentage of arterial blood compared to venous blood, which is because of the amplified pressure in the arterioles leading to the capillaries as oppose to the pressure in the exiting venules from the capillaries (Niwinski, 2009).

The simplicity of the finger prick test should be appreciated in comparison to venipuncture, which requires phlebotomists. However, consideration should be given to the finger from which the blood is drawn; as a study revealed that there was up to 7 % intra-variability from different fingers despite samples being obtained at the same time (Boulton, Nightingale and Reynolds, 1994). Additionally, there may also be intra-subject differences between left and right hands, as with Morris and cohorts study (1999), with a deviation of 0.8 $g \cdot dL^{-1}$. Therefore, for simplicity and to avoid variability, as depicted in section 4.4.1 of this thesis the finger prick test was adopted to measure Hb and Hct, using the 3rd digit on the right hand.

Body position is an extra consideration that has the potential to affect Hb, as during standing there is a pooling of blood to the lower limbs from gravity, blood pressure and heart rate will be elevated and as such will cause plasma (intravascular fluid) to move to interstitial compartments, which would result in Hb (and Hct) to increase and PV to decrease. Therefore, maybe a period of relaxation in a semi reclined position should be adopted prior to taking values. Jacob, et al. (2005) considered postural pseudoanaemia and noted that a PV change occurred rapidly, within 10-15 minutes and was complete at 20 minutes when standing and restoration when supine was of a similar time frame. In terms of this thesis, capillary Hb is used in the experimental chapters and as such the previous discourse on reflecting how blood is drawn and thus adopting good practice is considered to be imperative to providing accurate and consistent results.

2.7 Haemoglobin Recovery Post Blood Donation

Post BD, tHb is in deficit and unlike blood (plasma) volume that regenerates rapidly, a much longer recovery period is required. A study by Falz, Leue and Busse (2014) revealed that a 500 ml BD in 10 subjects (9 males and 1 female) resulted in an average tHb-mass loss of 8.7 ± 2.4 % ($P < 0.0001$) after donation with significance ($P < 0.001$) still seen 2

weeks later, although Hb concentration appeared relatively stable with the lowest concentration seen 1 week after donation. These results were similar to an earlier study by Pottgiesser, et al. (2008) where 550 ml of blood was withdrawn in 29 male volunteers, which resulted in 8.8 ± 1.9 % for tHb loss (measured by carbon monoxide rebreathing). The tHb was recovered after a mean of 36 ± 11 days in the research with Pottgiesser, et al. (2008) and in the more recent work of Falz and colleagues (2014) it took 4 weeks to gain an average 69 % recovery of tHb-mass and by assuming a linear increase they predicted that 100 % would be achieved after 6.55 ± 2.17 weeks. Although this team of scientists wanted to provide a prediction, the accuracy of it is uncertain, as from their reported tHb values they are not increasing linearly (a straight line cannot be detected in the corresponding figure). The data increases in the following format over 4 weeks 876 ± 183 g to 904 ± 179 g to 919 ± 196 g to 922 ± 167 g. It can be observed that the differences between the weeks are not equal and therefore does not produce linearity. Also, with confounding variables such as diurnal variation and fluid intake it is not appropriate to assume a linear increase. As seen in Falz, Leue and Busse (2014) the impact of Hb appearing to recover but not tHb-mass could result in a donor being able to donate again, as donation centres only check Hb. Although the minimum repeat donation time of 12 weeks for UK males does appear to be adequate if the 100 % recovery prediction by Falz and colleauges (2014) is correct. The NHS (2018) report Hb recovery to be approximately 6-12 weeks.

Hb values post BD have been reported in various exercise-based research. The following Table (2.4) highlights how the Hb values reduce after BD; unfortunately, sometimes significance values are not always reported in all the studies even if the authors report that there was significance. If the pre-post BD values (15.1 ± 0.6 and 14.0 ± 0.5 $g{\cdot}dL^{-1}$) for the research that studied Hb reduction over 24-48 h are observed there is a percentage decrease of 7.28 %. The values pre to post (14.5 ± 0.8 and 13.2 ± 0.8 $g{\cdot}dL^{-1}$) for 3-4 days have an 8.97 % reduction and for 7 days pre to post (14.8 ± 0.9 and 13.8 ± 0.9 $g{\cdot}dL^{-1}$) the percentage decrease is 6.76 %. What can be ascertained from this data is that Hb values appear to continue to reduce for a number of days after BD and by the 7^{th} day the Hb may be beginning to restore again. In brief, this restoration is due to the kidneys sensing a decreased oxygen delivery following blood loss. Consequentially, erythropoietin is released to the bone marrow and spleen, which signal to stem cells to develop into RBCs. This process will continue until normal values are regained.

Table 2.4: Hb values pre and post BD for various studies.

Study	Participants (and sex)	BD vol (ml)	Hb ($g \cdot dL^{-1}$)				
			Pre BD	Post BD (2 h)	Post BD (24-48 h)	Post BD (3/4 days)	Post BD (7 days)
Balke, et al., 1954	14 (sex unknown)	500	15.3		14.1		
Bennet & McKay, 2012	8 (male)	450	15.6 ± 6.7		14.4		
Brinbaum, Dahl & Boone., 2006	10 (male)	500	15.3 ± 1.2		13.5 ± 1.6 **		
Burnley, et al., 2006	11 (10 male & 1 female)	450	15.4 ± 0.9		14.7 ± 1.3		
Ekblom, et al., 1972	4 (male)	400	14.9			13.3	
Gordon, et al., 2010	10 (6 male & 4 female)	450	14.2 ± 1.5		13.1 ± 1.5*		
Gordon, et al., 2013	15 (male)	450	15.6 ± 1.2		14.1 ± 1.6**		
Hill, Vingren & Burdette., 2013	9 (7 male & 2 female)	450	14.9 ± 0.8	14.9 ± 1.1	14.0 ± 1.7		14.0 ± 1.2
Mora-Rodriguez, et al., 2012	8 (male)	450	15.3 ± 2.8	14.8 ± 2.5*	14.2 ± 3.6		14.5 ± 2.8
Meurrens, et al., 2016	26 (male)	470	15.6 ± 0.2		13.7 ± 0.3		14.2 ± 0.2
Stangerup, et al., 2017	12 (female)	450	13.5 ± 0.2$^{\$}$			12.4 ± 0.2 $^{\$}$	12.5 ± 0.2 $^{\$}$
Ziegler, et al., 2014	20 (male)	450	15.0 ± 0.2$^{@}$		13.9 ± 0.2$^{@}$	13.9$^{@}$	
Mean ± SD	N/A	455.8 ± 26.1	15.1 ± 0.6	14.9 ± 0.1	14.0 ± 0.5	13.2 ± 0.8	13.8 ± 0.9

* P = < 0.05 ** P = < 0.001 $^{\$}$ denotes values worked out from Hct scores $^{@}$ denotes values converted from $mmol \cdot L^{-1}$

Table 2.4 also reveals that the majority of the studies are male biased, with only one study (Stangerup, et al., 2017) being exclusively female participants. What is also clear from the Hb results is that the females have lower pre and post donation values. Although with only one study representing this outcome and not being able to specifically identify the results of the nominal female participants of the other studies it is difficult to ascertain if this would always be the case. However, it is likely as, females are known to have ~12 % lower Hb (Murphy, 2014) and from calculating the male only studies pre BD Hb mean values (15.3 ± 0.3 $g \cdot dL^{-1}$) against the female only study value in Table 2.4, an 11.8 % reduction in pre BD Hb levels are observed, which would appear to concur with the 12 % value suggested by Murphy (2014). According to Jelkmann (2011) it is the stimulus of erythropoiesis by androgens (testosterone) and the inhibition from oestrogens (on bone marrow) that results in greater Hb concentrations in males compared to females. Another difference between male and females Hb levels is possibly the loss of blood in women that occurs from menstruation, which has the potential to decrease Hb (Kotwaney and Shetty, 2014), but

may vary according to the amount of menstrual blood lost. Furthermore, consideration should be given to males having more muscle mass than females, which promotes Hb content and the higher proportion of body fat percentage that females have compared to males, which doesn't generate BV (and thus Hb).

In terms of comparing the female and males relative change from pre to post BD the results are similar. The relative change pre to 3-4 days post BD for the studies of male (Ekblom, et al., 1972; Ziegler, et al., 2014) and female (Stangerup, et al., 2017) only were -9.03 % and -8.15 % respectively, thus the males Hb loss was marginally greater. The relative change pre to 7 days post BD for the studies of males (Mora-Rodriguez, et al., 2012; Meurrens, et al., 2016) and females (Stangerup, et al., 2017) only were -7.12 % and -7.41 % respectively, thus similar relative Hb loss. It could therefore be postulated that the post BD effects experienced by both sexes in terms of exercise/performance may actually be similar.

2.8 Haemoglobin and Exercise

Exercise tolerance can alter from haematological changes. For example, losing Hb can impair training and performance. Initially, simply by reducing circulating BV (a consequence of BD), it is possible that ventricular preload (diastole functionality) will influence maximal aerobic power ($\dot{V}O_{2max}$), as there will be a reduced force of contraction if Frank-Starling Laws are considered, thus altering $\dot{Q}$ and SV (Krip, et al., 1997). The Frank-Starling relationship considers that ventricular output increases as preload (end diastolic volume (EDV)) increases (Delicce and Makaryus, 2020). If preload (the stretching of the ventricles from EDV) is increased, SV (EDV minus end systolic volume) is increased. However, it is the converse with a reduced SV, which could be a consequence of blood loss. It is likely that the more pronounced the blood loss the more the effects would be heightened. Therefore, the effects may be more pronounced in petite individuals from BD, for example, a 50 kg (the minimum mass requirement for BD) 150 cm female would lose 15.5 % BV from a 470 ml BD, more than the maximum loss of BV recommended by the NHS, which is 15 % (JPAC, 2013). Hb reduction will reduce pulmonary diffusion (Otto, Montgomery and Richards, 2013) and also will theoretically reduce O_2 delivery proportionally for the same muscle blood flow, as CaO_2 will be reduced (Burnley, et al., 2006). There is also the consideration that as a result of diminished Hb O_2 diffusing capacity is lessened, which is possibly associated to slower dissociation of O_2 from Hb or maybe linked to modifications in the intra-capillary spacing of erythrocytes (Schaffartzik, et al., 1993).

Exercise capacity is related to the oxygen carrying capacity of blood and as such alterations in Hb and tHb will likely alter exercising ability, the possibility of this is illustrated in Figure 2.6. It is mainly the lowered oxygen carrying capacity from reduced Hb that adversely impacts issues such as aerobic endurance and $\dot{V}O_{2max}$ (Calbert, et al., 2006), with tHb exhibiting a stronger affiliation with $\dot{V}O_{2max}$ than with Hb and BV (Kanstrup and Ekblom, 1984; Otto, Montgomery and Richards, 2013), this is because tHb governs oxygen transport and it increases BV from erythrocyte volume growth. This has been shown over the years in blood reduction studies from numerous researchers, such as from Balke, et al., 1954; Burnley, et al., 2006; Gordon, et al., 2013; Hill, Vingren and Burdette, 2013, who all found that $\dot{V}O_{2max}$ decreases after blood donation. The decrement is due to a reduced O_2 transport capacity (Gledhill, 1985 p.76), also declined is $\dot{Q}$ (Krip, et al., 1997; Lundby, et al., 2007). Furthermore, Wasserman, et al. (2005) reports that with a poor oxygen delivery anaerobic glycolytic metabolism aids aerobic ATP production and increases blood lactate concentration. Conversely, studies that have augmented tHb from methods such as rhEPO or infusion have been shown to increase $\dot{V}O_{2max}$. For example, Parisotto, et al. (2000) reported a rise of 7 – 12 % in tHb, which amplified $\dot{V}O_{2max}$ by 6 – 7 % in 27 recreational athletes with the use of rhEPO. If $\dot{V}O_{2max}$ is of prime concern then it is worth knowing that if tHb alters by 1 g there is a change in $\dot{V}O_{2max}$ by ~ 4 $ml \cdot min^{-1}$ (Schmidt and Prommer, 2010). Therefore, a high BV and tHb are fundamental if an elevated $\dot{V}O_{2max}$ is required and that is a crucial consideration for successful endurance performance.

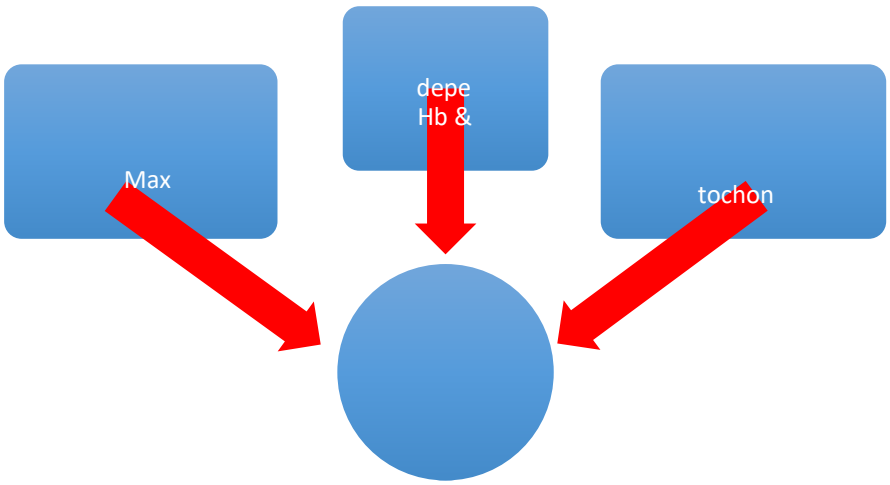

Figure 2.6: Aerobic power and its defining parameters. Information adapted from Jelkmann and Lundby (2011).

Sawka, et al. (1992) reported in a study of 51 male soldiers that erythrocyte (and thus tHb) correlates well (r = 0.83) with lean body mass (LBM). Concurring with this notion Schmidt et al. (2009) studied 114 male subjects of varying athleticism and determined that there was 16.8 g Hb per 1 kg LBM and higher performance athletes displayed ~ 25 % higher

tHb than the lower performance athletes. It is thought that elite endurance athletes have 40-50 % increased tHb, implicit from the erythropoietic effects of training (Schmidt and Prommer 2010).

When regarding BD and exercise research it seems that it is widely accepted that BD decreases values like $\dot{V}O_{2max}$ from having reduced O_2 carrying abilities and increasing O_2 carrying abilities (from infusion type methods) enhances these capabilities. However, the quality of the research is not always thorough. For example power calculations are important as they provide suggested sample size requirements to avoid type I or II errors, yet the following blood reduction and exercise studies do not have power calculations included, Balke, et al., 1954, Bennett and McKay., 2013, Brinbaum, Dahl and Boone., 2006, Burlney, et al., 2006, Duda, et al., 2003, Ekblom, Goldbarg and Gullbring., 1972, Foster, et al., 2008, Gordon, et al., 2010 and 2013, Hill, Vingren and Burdette., 2013, Janetzko, et al., 1998, Krip, et al., 1997, Markiewicz, et al., 1981, Mora-Rodriguez, et al., 2012, Panebianco, et al., 1995, Sliwowski, et al., 2001, Strandenes, et al., 2012, Williams, Lindhjem and Schuster., 1978 and Ziegler, et al., 2014. Thus, the validity of the results within these studies are questionable as there may not have been an appropriate sample size. Additionally, only two of these aforementioned studies (Gordon, et al., 2013 and Mora-Rodriguez, et al., 2012) measured urine to check for hydration. The other studies do not appear to account for or measure hydration levels. This is possibly ill-advised, as Hb and Hct will alter according to hydration levels, which should be an important factor to consider when regarding a BD study and furthermore aerobic performance can be degraded from dehydration (Sawka, et al., 2007). Moreover, only Gordon, et al., 2010 is sub-maximal analysing the moderate and heavy domains. Gordon, et al. (2010) indicate that Hb loss does not reduce sub-maximal performance, they did not control adequately for hydration and a power calculation was absent. This indicates that there is scope for additional work regarding sub-maximal environments.

2.9 O_2 Uptake Kinetics

At the onset of exercise ATP production increases, so that metabolic demand can be met. In a square-wave transition, O_2 travels through the circulation to meet the O_2 demand at the muscle. However, there is not an immediate matching of supply meeting demand, a time delay occurs between exercise onset and the desired rate of oxygen uptake (Gordon, et al., 2010). For example, O_2 uptake will not attain a steady state until 2-3 minutes at a moderate intensity (Jones and Poole, 2005), thus, there is an O_2 deficit. Furthermore, at a heavy intensity/wattage it would take longer for a steady state to be achieved (~8 minutes

(Gordon, et al., 2010)) resulting in increased metabolic stress as a consequence of more power production. The consequences of working in a heavier domain are that anaerobic metabolism must be used and thus $\dot{V}O_2$ responses are affected, which is related to the slow component (discussed further on). Thus, to prevent ATP decline PCr (and as such the enzyme creatine kinase) is utilised, which will increase the glycolytic rate, resulting in increased lactate until aerobic supply meets aerobic demand and ultimately a steady state.

The O_2 deficit is the difference between the demand for energy at the muscle and the energy supply from oxidative phosphorylation prior to steady state being achieved (figure 2.7). If the deficit were reduced, as a result of faster $\dot{V}O_2$ kinetics, PCr, glycogen and metabolic stress would also reduce, as would anaerobic energy provision at exercise commencement (Burnley and Jones, 2007). For example, faster $\dot{V}O_2$ kinetics are present in elite rowers compared to club rowers in the moderate and heavy domains, (Ingham, et al., 2007). Ingham and colleagues (2007) report elite rowers to have a faster time constant on the primary component, that they also consumed less oxygen in both domains and that they have a superior absolute slow component, thus the larger economy suggests beneficial modification of the oxidative process.

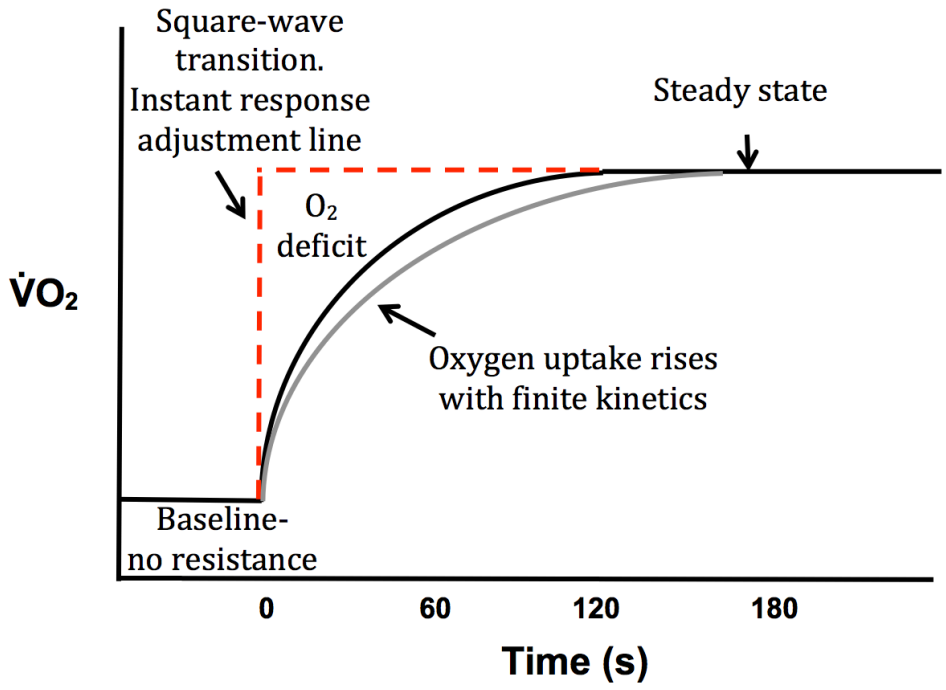

Figure 2.7: A schematic representing the immediate response of a square-wave transition. At the point of instant demand, the muscle requires more O_2 to meet demand, but ATP used and ATP supplied via oxidative metabolism takes several minutes after exercise onset to achieve steady state, and anaerobic energy assists. The grey curved line indicates a slower response, which results in a bigger O_2 deficit compared to the curved black line. It is approximately 2 minutes before steady state is achieved (with the smaller deficit curve), therefore, the intensity is moderate and below gas exchange threshold (GET). When steady state becomes closer to being achieved, less anaerobic energy is used and at steady state almost no anaerobic metabolism contributes to ATP supply. Information adapted from Jones and Poole (2005).

2.9.1 Response of O_2 Uptake Kinetics at the Onset of Exercise

There are three phases to the response of pulmonary O_2 uptake regarding exercise onset (Figure 2.8). Phase 1 is rapid and concerns the initial $\dot{V}O_2$ increase. At the onset of the square-wave transition, as soon as the exercising load is applied a brisk surge in blood flow through the lungs occurs (with $\dot{V}CO_2$ appearing at the lung), as cardiac output has increased, although amplified muscle O_2 intake is not reflected (Jones and Poole, 2005) thus this phase would appear autonomous to the exercising limb. However, conversely to Jones and Poole, (2005), Bangsbo and colleagues (2000) observed O_2 extraction in the thigh to be elevated after < 6 s of exercise. This phase concludes at the point when deoxygenated blood reaches the lung (gas exchange occurs) as muscle O_2 consumption has increased. Phase 2, which is greater in magnitude than phase 1 denotes the exponential rise of $\dot{V}O_2$ prior to steady state (in moderate and heavy domains). It describes the amount of O_2 being taken up at the exercising limb (Gaesser and Poole., 1996), thus representing increased muscle metabolism. This phase also reflects the amount of anaerobic energy needed to get to steady state. Phase 3 is reaching steady state, which if the intensity is above GET (but below critical power (CP)) includes the slow component. The slow component results when oxygen uptake increases, as normal aerobic metabolism is inadequate for the workload needed (Gordon, et al., 2010).

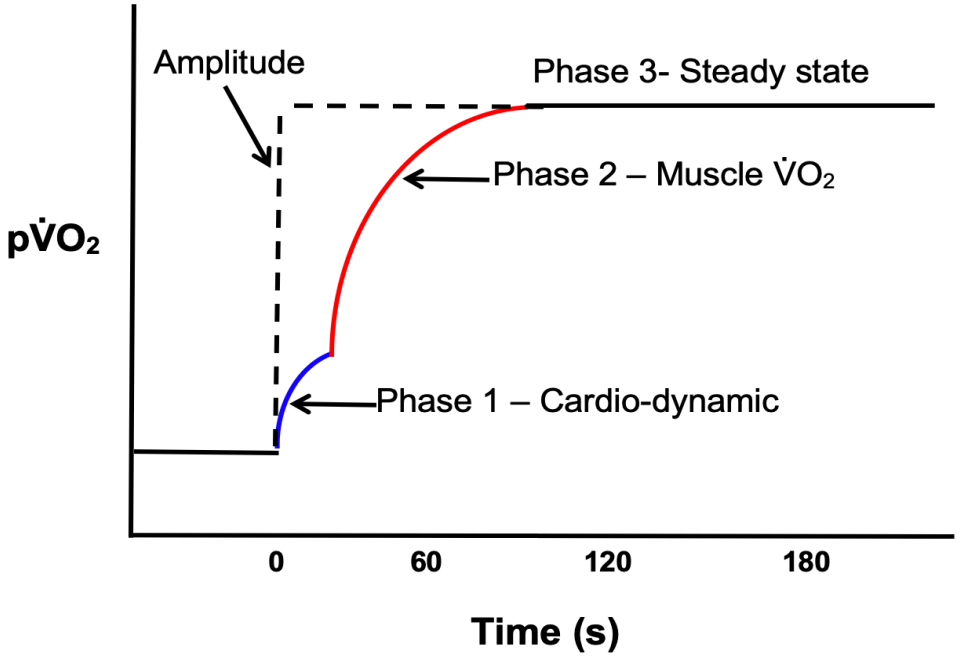

Figure 2.8: The three pulmonary $\dot{V}O_2$ phases following the sudden onset of exercise. Also showing the amplitude of the $\dot{V}O_2$ (that is $\dot{V}O_2$ at steady state minus it prior to exercise commencement) and the time/rate taken to acquire steady state, which represents the scale of the O_2 deficit. The smaller the amplitude the smaller the deficit, thus faster kinetics reduces decreasing PCr and lessens the burden on glycogen reserves. Information adapted from Jones and Poole (2005).

The association between $\dot{V}O_2$ response and the decay of PCr can be appreciated from the work of Rossiter and associates (1999). By using an MRI scanner PCr was scanned (in the calf muscle while flexion and extension of the ankle was performed) and $\dot{V}O_2$ was measured simultaneously at the mouth. The work showed that as the load was applied the PCr decreased (as O_2 was not meeting demand), but eventually as demand was met PCr supply was constant (Figure 2.9).

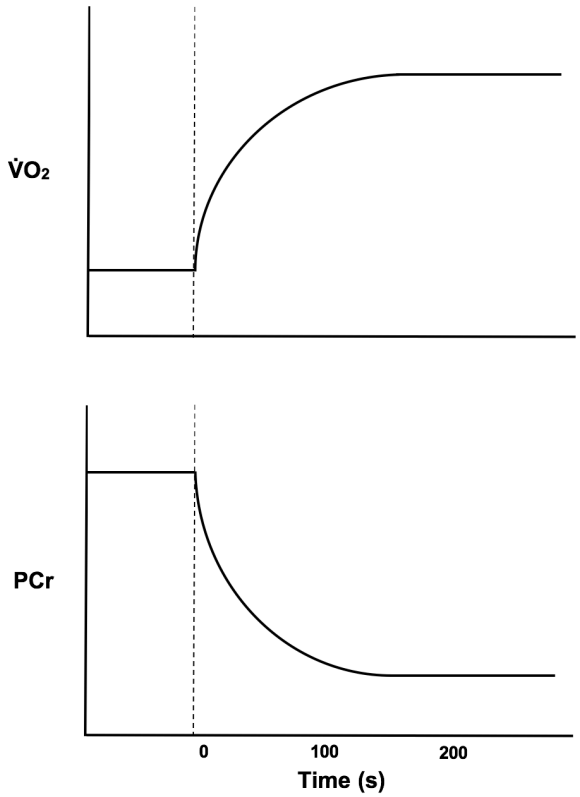

Figure 2.9: A schematic illustrating PCr decay is inverse to $\dot{V}O_2$, adapted from the work of Rossiter and associates (1999). The dashed line represents the onset of exercise and it can be observed that the PCr diminishes mono-exponentially with no pause in reaction to the onset of exercise. Pulmonary O_2 responses can be directly associated to the homogeneous PCr fall in the exercising muscle (Rossiter and associates, 1999). The PCr data is the inverse of $\dot{V}O_2$ and as such the O_2 deficit represent the PCr change in the muscle, hence the smaller the deficit the less PCr would be used and anaerobic energy would also reduce. A lesser deficit could be due to an easy workload or a smaller time constant and would result in achieving steady state quicker.

2.9.2 Exercise Intensity Domains

Exercise in the moderate domain takes place below GET and steady state is achieved in 2-3 minutes (Gordon, et al., 2010). From a constant load square-wave transition the response is exponential (Whipp, et al., 1982). Exercise in this domain can be executed without substantial increase in lactate (thus below GET). Additionally, the O_2 deficit is

encompassed mainly from declines in PCr and O_2 store reduction and a minor anaerobic glycolysis contribution (Gaesser and Poole., 1996).

Exercise above the moderate domain and therefore above GET concerns heavy and severe exercise domains. In the heavy domain (> GET, but < CP), a $\dot{V}O_2$ slow component is produced, which can be explained as the difference between the projected $\dot{V}O_2$ response and the $\dot{V}O_2$ response actually produced. Krustrup, et al. (2009) report that the slow component is mainly due to a gradual loss of muscle proficiency, that is metabolic changes in the contracting skeletal muscle from the requirement of more energy, decrement in PCr and the increase in $\dot{V}O_2$. Rossiter and co-workers (2002) claim that ~90 % of the magnitude of the slow component is reflected in the exercising muscle (according to its PCr response) during high-intensity exercise. More fast twitch muscle fibres are needed, which according to Cannon, et al. (2011) are used to maintain power production but are inadequately efficient. Hence, lactate is produced, which affects muscle and metabolic efficiency, which are indicators of the slow component. To a lesser extent compared to skeletal muscle O_2 consumption, there are other contributions to consider such as, temperature rise (Koga, et al., 1997) and increased pulmonary ventilation (Gaesser, 1994). Consequently, reducing the $\dot{V}O_2$ slow component and quicker $\dot{V}O_2$ kinetics would result in sparing the finite anaerobic reserves.

The heavy domain is placed between GET and CP and steady state can take ~8 minutes (Gordon, et al., 2010) to be achieved. At severe intensity $\dot{V}O_2$ does not reach steady state, O_2 will not meet demand and $\dot{V}O_2$ will rise up to $\dot{V}O_{2max}$. The severe domain is in excess of CP, but not above $\dot{V}O_{2max}$. $\dot{V}O_2$ and lactate cannot be stabilised and therefore rise until fatigue and maximum $\dot{V}O_2$ occur (Gaesser and Poole., 1996).

2.9.3 Blood Donation and Exercise Intensity Domains

Unfortunately, there appear to be very few studies that address the issue of BD and $\dot{V}O_2$ kinetics. In the moderate and heavy domains only one study comparing pre to post BD $\dot{V}O_2$ kinetics appears to exist. This study from Gordon and contemporaries (2010) was undertaken to establish if alterations in the O_2 carrying capacity of blood (following a 450 ml BD) would adjust whole body oxygen on-kinetics in stepwise changes in cycling. The study found that a 7.9 % reduction (with a significance of $P < 0.05$) in Hb following BD did not affect on-transient kinetics. Burnley and associates (2006) address $\dot{V}O_2$ kinetics pre to post BD (450 ml) in the severe domain. They report significant reductions in $\dot{V}O_{2peak}$ (pre BD 3.79 ± 0.64: post BD 3.64 ± 0.61 $L \cdot min^{-1}$; 95 % CL: -0.04, -0.27) and also time to

exhaustion (pre BD 375 ± 129: post BD 321 ± 99 s; 95 % CL -24, -85) from significant Hb reductions (pre BD 15.4 ± 0.9, post BD 14.7 ± 1.3 $g.dL^{-1}$: 95 % CL: -0.04, -1.38).

Gordon, et al. (2010) imply that sub-maximally on-transient kinetics are not affected by BD, but conversely Burnley and associates (2006) at a severe intensity report significant reduction. However, both these studies have flaws. Firstly, hydration status was not measured with any equipment and therefore it is not known if participants were hydrated or not. The issues and the impact of this shortcoming are discussed at length in subsequent chapters. But briefly this has implications with a BD study, particularly when it comes to gathering the results of Hb and Hct levels, as dehydration will provide higher levels of these variables. Secondly the sample size for both studies was low (6 males and 4 females in the study from Gordon, et al., 2010 and 10 males and 1 female for the study from Burnley, et al., 2006). Although justification of the sample was intimated, without a power calculation there cannot be as much guarantee of accuracy if the participant numbers are too low from under power.

With only two studies in this field, more research is required. Within this thesis chapters 6 and 7, similarly to Gordon, et al. (2010), address moderate and heavy domain exercise, but additionally add the use of other testing modalities such as NIRS and address the issues of the shortcomings previously discussed.

2.10 High Intensity Interval Training (HIIT)

Coaches have used the concept of interval training for many years. For example, Emil Zatopek (long distance Olympic champion) used it in the 1950's (Billat, 2001). However, recently HIIT has become more commonplace as an exercise mode for the general public and additionally it has the reputation of being one of the most successful ways of enhancing the physical performance of athletes due to it improving cardiovascular and metabolic function (Buchheit and Laursen, 2013a).

The typical principle for HIIT is that for a short time span such as 20 s (or longer) the exercise of choice is undertaken at a high intensity, maybe sprinting on a bike, this is followed by a rest period such as 10-20 s (or longer) for partial recovery. This pattern of exercise and recovery is repeated several times over the course of the training period/exercise class. It is the period of work and rest intervals plus the intensity that are the major manipulating features of a HIIT session (Astrand, et al., 1960).

Varying protocols exist that involve an assortment of intensities of $\dot{V}O_{2max}$ and different time manipulations over a training session of ~5-40 minutes (Buchheit and Laursen., 2013b). Possibly one of the most familiar forms of HIIT is that of Tabata training. This involves 6 – 8 bouts of 20 s sprints at 170 % of $\dot{V}O_{2max}$ (supramaximal), with 10 s rest between the sprints (Tabata, al., 1997). Indeed, in exercise classes and gyms HIIT classes are sometimes referred to as Tabata training and the work/rest concept of it adopted.

However, work from Buchheit and Laursen, (2013a & b) explain that such exercise is not always HIIT and it could actually be something else such as sprint interval training (SIT) depending on the factors of intensity and duration. Buchheit and Laursen, (2013b) report HIIT to be short (< 45 s) to long (2-4 minutes) sessions of fairly high, but not maximal intensity exercise, or short (≤ 10 s) repeated-sprint sequences (RSS), or long (> 20-30 s) sprint interval session (SIT), combined with recovery. Figure 2.10 depicts an appreciation of the metabolic demands and intensity range depending on the session.

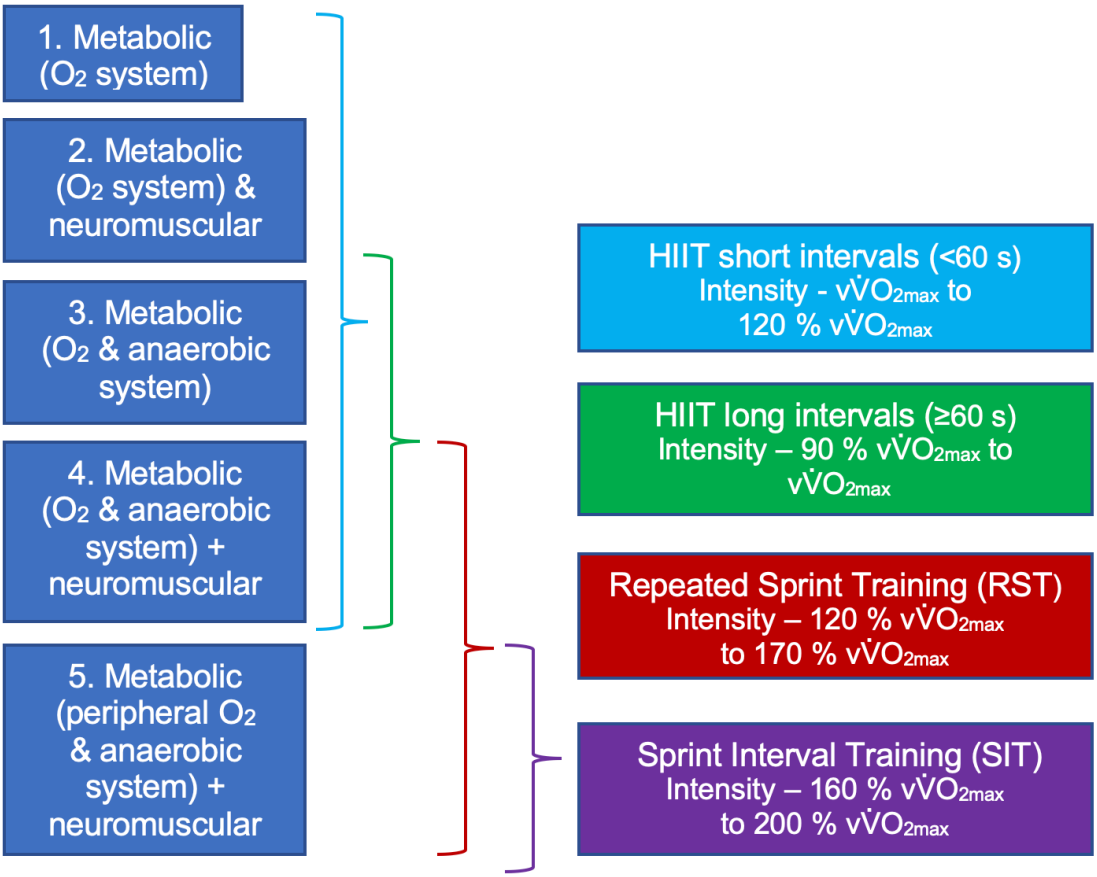

Figure 2.10: The acute responses of HIIT, RST and SIT (categorized from 1 through to 5). The response begins with large requirements from the O_2 transport, exploiting oxidative fibres and cardiopulmonary systems. As the intensity increases demands alter, placing more emphasis on neuromuscular strain and the anaerobic glycolytic energy contribution. The intensity is represented by Velocity at $\dot{V}O_{2max}$ (v$\dot{V}O_{2max}$); it is the uppermost physiological point where aerobic performance can be sustained, and thus delivers a theoretic link between $\dot{V}O_{2max}$ and performance economy (Daniels and Daniels, 1992). The information in this schematic is adapted from Buchheit and Laursen (2013a).

2.10.1 Energy Pathways

All exercise including HIIT will use a combination of aerobic and anaerobic substrate to produce adenosine triphosphate (ATP); with that initial first muscle contraction delivering instant energy via ATP (with replenishment via PCr), however the relative contributions of the substrate according to the event will change. For example, in a 20 s sprint 82 % of energy comes from anaerobic source, whereas the anaerobic energy contribution will reduce to 55 % over a 60 s period (Gastin, 2001).

Astrand and colleagues (1960) suggest that at sub-maximal load (although the specifics of which are unclear) with brief periods of intense bouts (such as 30 s – 180 s), as long as they are supplemented with passive intermissions of low intensity for equal time the work/rest could continue for up to 60 minutes. It is suggested that oxygen bound to myoglobin supplies most of the oxygen requirements with the start of short efforts prior to circulatory and respiratory system stimulation for oxygen demand (Astrand, et al., 1960; Essén, Hagenfeldt, and Kaijser., 1977).

In the instance of the Tabata (1997) method, the work rest ratio is not 1:1, therefore it is likely that the PCr and anaerobic glycolytic energy contribution is going to be high due to the amount of high intensity work done in the total time (Buchheit and Laursen., 2013a). Tabata (1997) suggests that at a 1:1 ratio restoring oxygen and PCr is likely, but with a work rest ratio of 2:1 then full PCr restoration between bouts is unlikely. Also, as intermittent sprint work involves fast twitch fibres lactate production results (a reflection of anaerobic glycolsis). Buchheit and Laursen (2013b) report that with sprints longer than 4 s and recovery less than 20 s the initial level of lactate growth is constantly high.

2.10.2 Responses to HIIT and SIT-Based Exercise

The Tabata (1997) method, after 3 weeks of training, significantly ($P < 0.01$) increased $\dot{V}O_{2max}$ in seven male varsity team members. A study by MacDougall et al. (1998) examined SIT effects in 12 fitness enthusiasts (thus untrained athletes) over a 7 week period using 30 s all out efforts and rest periods between bouts of 4 minutes reducing to 2 minutes and 30 s in the final 3 weeks. Significant ($P < 0.05$) increases were observed in $\dot{V}O_{2max}$, peak power output and the glycolytic enzyme activity of hexokinase, phosphofructokinase (PFK) and oxidative enzymes of citrate synthase (CS), succinate dehydrogenase and malate dehydrogenase. Similarly, Rodas and colleagues (2000) also saw significance increases ($P < 0.05$) from SIT in $\dot{V}O_{2max}$, peak power output PFK and CS.

More recently, Astorino and contemporaries (2012) also used the supramaximal approach and similarly to the earlier studies saw a significant increase in $\dot{V}O_{2max}$ after only six training bouts. Energy expenditure of 16 trained volunteers was studied by Emberts, et al. (2013) using the Tabata (1997) method. By using circuit training type exercises such as lunges, press-ups, burpees and jumps, calorie expenditure was measured, resulting in a range of 240 to 360 Kcals being expended over 20 minutes. Thus, it can be seen that using supramaximal intensity positive adaptations can be gained from HIIT.

Fast twitch fibres will dominate in this Tabata (1997) protocol and they are less dependent on oxygen and have lower oxidative facility, thus it is possible that reduced oxygen availability following BD will have little effect on the exercise. However, due to the potentially intense features of this type of training programme being available for use to the general public and the absence of studies into the effects of repeated sprints post BD it seems prudent to research the field.

Chapter 3: 1st Experimental Chapter

Systematic Literature Review and Meta-Analysis: The Acute Effects of Whole Blood Donation (~470 ml) on Cardio-Metabolic Factors in Exercise

3.1 Introduction

Commonly ~ 470 ml of blood is collected from donating a unit (NHS choices, 2014) and this quantity should not exceed >15 % of an individual's blood volume (JPAC, 2013). In the UK in 2014, blood transfusion in hospitals was distributed in the following ways: 67 % treated medical conditions including cancer, anaemia and blood disorders, 27 % were used in surgery, including emergency and cardiac and 6 % were for blood loss following childbirth (NHS Blood and Transplant, 2016a). Thus, 6,000 donations are needed every day in the UK alone and 200,000 new donors are needed each year in order to replace donors who can no longer donate (NHS Blood and Transplant, 2016a).

"The Missing Type" campaign, which ran from 6-14 June 2015, to encourage people to donate blood and know their blood type, helped to expose a shortage of new donors (McArdle, 2016). The year 2015 revealed NHS Blood and Transplant concern at the 40 % fewer new volunteers for BD in comparison to a decade earlier and reasons cited were having more tattoos, exotic holidays and less time (Slawson, 2015 and McArdle, 2016). The trend for exotic holidays is reinforced by ABTA (Association of British Travel Agents) (2015), who reported a 350 % increase in long haul flights over the last ten years to exotic places. Indeed, to avoid tropical viruses, donation guidelines when returning from exotic places vary, but a 4-week minimum wait post-holiday prior to donation appears common (NHS Blood and Transplant, 2016a). However, other reasons for donor shortage according to research from Lattimore, et al. (2015) are the increase in population from non-white ethnic groups, with the black and ethnic minorities BD contribution being appreciably lower (5.1 and 4.3 per 1000 amongst Asian and black origin respectively) than white British (22.1 per 1000). Furthermore, Lattimore and colleagues (2015) also highlight age and sex influencing BD, as they suggest that repeat new donors are more likely to be male and older (>40). An additional population group who are potentially contributing to this decline are the sport enthusiasts and recreational/professional athletes, who may be worried about the effect of BD on their performance (Van Remoortel et al., 2016). Thus, if there are more people exercising in this population group then this could become a greater factor working against blood donation. Indeed, the Active People Survey reported an increase of 229,400 sporting participants in 16 year olds and older over 12 months (Sport England, 2016).

There was early concern regarding BD affecting performance in the mid 1900's when Springfield College, Massachusetts ruled that no man could be a blood donor while participating in varsity sport, due to unfavourable effects following BD such as wrestlers collapsing after competition and endurance lost in other athletes (Karpovich and Millman, 1942). Additionally, reduced health as a result of BD is a fear of the non-donor (Bednall and Bove, 2011). Guidance for high-performance competitive athletes from the American Red Cross (2017) suggest that a marginal decrease in exercise tolerance may be noticed up to a week after BD. Additionally, where exercise is concerned, prudence should be executed immediately after BD (NHS Blood and Transplant, 2017b and American Red Cross, 2017).

BD is altruistic and according to Nilsson, Sojka and Sojka (2007) altruism is the most common motive for continuing to be a blood donor. However, there are consequences to BD such as decrements in blood/plasma volume (B/PV) and haemoglobin (Hb). While the BV returns to normal within 24-48 h (48 - 72 h according to Klein and Anstee, 2007), due to the progressive PV expansion (Calbert, et al., 2006), red cell mass and thus Hb restoration takes much longer, which according to Klein and Anstee (2007) and the NHS blood and Transplant guidelines (2018) can be 3 - 6 weeks or 6 - 12 weeks respectively. Pottgiesser, et al. (2008), observed (although using a 550 ml donation) reinstatement to be a mean of 36 +/- 11 days (range, 20-59 days).

BD creates a unique biological environment that results in altered systems physiology. An awareness of this was observed in early work from Karpovich and Millman (1942) where a total Hb decrement affected athletic performance. Later work from Balke and colleagues (1954) discovered that from a BD of 500 ml there was a 9 % decline in $\dot{V}O_{2max}$ and more recently Gordon and contemporaries (2013) showed a 4.65 % fall in $\dot{V}O_{2max}$ from a 450 ml donation. Additionally, other variables may be affected or reduced from BD such as maximum aerobic power output (W_{max}) (Duda, et al., 2003) and time to exhaustion (T_{Ex}) (Burnley, et al., 2006; Hill, Vingren and Burdette, 2013 and Meurrens, et al., 2016). There is little doubt that decreasing blood volume via BD will diminish oxygen carrying capacity, as haemoglobin (Hb) concentration will have been reduced and it is the primary source for the conveyance of oxygen.

The aforementioned facts highlight the importance of studies concerning blood donation, which will educate the general public and allow individuals to make informed decisions on the consequences of reducing their blood volume on their health and well-being and additionally performance if they are exercise enthusiasts.

Currently the Cochrane Database of Systematic Reviews (Cochrane Library, 2017) shows no publications concerning the effects that BD has on exercise. There has however recently been a systematic review (Van Remoortel et al., 2016) concerning the effects of a standard whole blood donation on oxygen uptake and exercise capacity. However, there appear to be various methodological issues within this study. For example, a forest plot concerning $\dot{V}O_{2max}$ 24-48 h after BD has data missing, such as a study by Gordon, et al. (2013), which they do however report. Additionally, there is data in the plot (Ekblom, et al., 1972) that should not be there due to the time period being 3 days after donation in the study, as opposed to 24-48 h. The blood volume range that the authors researched is 400 – 500 ml, with a mean of 450 ± 27.56 ml, however, with the data in the forest plots reporting on a wide variety of volumes (400 – 500 ml) and non-standard blood volumes for BD being used too, (such as Ekblom, et al., 1972, who use 400 ml) then the data will possibly be skewed, compared to if only one specific volume was studied.

Therefore, the aim of this chapter is to avoid issues such as skewed data and to collect the relevant historical and current literature to produce an informed analysis of the acute effects on cardio-metabolic factors following whole blood donation (~ 470 ml) during exercise, as straight after blood donation it is suggested that exercise should be approached with caution (NHS, 2017b and American Red Cross, 2017). This will test the hypothesis that blood donation produces either no changes (Null) or produces significant changes (alternate) in haematology, $\dot{V}O_2$, heart rate (HR), exercising power and time.

3.2 Methods

The systematic review was carried out from guidance of Systematic Reviews (Centre for Reviews and Dissemination, 2009) and by using bibliographic management software (RefWorks, Proquest, USA). Subsequent to a thorough literature search for research within the remit of the hypothesis, folders within RefWorks were created for studies extracted from each database explored, followed by the removal of exact duplicates and close duplicates (a term that allows duplicates to be discovered despite minor differences being present, for example varying combinations of author names or differing titles and publication years). The remaining references were subsequently screened according to their title and abstract and moved to a new folder to potentially be included in the review. The full text was then obtained to decide whether to exclude or include the text.

3.2.1 Literature Search of Data Base Sources and Search Terms

From the beginning of December 2016 to the end of January 2018, Pubmed, Web of Science, SPORTDiscus and Scopus were used to obtain literature from the early 1900's to January 2018, due to the databases being medical and science orientated. Search terms were created in order to be sensitive to all possible connections regarding exercise, blood donation/haematology, $\dot{V}O_2$, HR, exercising power and time (Table 3.1). Many of the search terms were the same as the review from Van Remoortel et al. (2016), due to the connectivity with the previously mentioned variables. Also, this was to attempt to repeat their methods, thus extracting similar literature, which will address mistakes that they appear to have made, but to additionally obtain the number of hits from each search engine, as the individual hit numbers were absent from their review.

Table 3.1: Databases and search terms used.

Data Base	Hits	Search Terms
Scopus	5713	"blood donation" OR "blood collection" OR "blood withdrawal" OR "blood centre" OR "Blood center" OR "blood service" OR "blood bank" OR "blood transfusion" OR "phlebotom*" OR "plasma don*" OR "blood volume" OR "blood donor*" "sport" OR "exercise" OR "physical endurance" OR "hiking" OR "cycling" OR "athletic" OR "training" OR "working out" OR "work out" OR "strenuous activity" OR "VO2 max" OR "maximal aerobic power" OR "Oxygen carrying capacity" "men" OR "man" OR "male" OR "sportsman" OR "sportsmen" OR "woman" OR "women" OR "female" OR "sportswomen" OR "Sportswoman" OR "athlete" OR "adult" OR "healthy" OR "humans" OR "people" OR "fit"
Web of Science	426	(TS=("blood don*") OR TS=("blood collection") OR TS=("blood withdrawal") OR TS=("blood centre") OR TS=("Blood center") OR TS=("blood service") OR TS=("blood bank") OR TS=("blood transfusion") OR TS=("phlebotom*") OR TS=("plasma don*")) AND (TS=("sport*") OR TS=("exercis*") OR TS=("physical endurance") OR TS=("hiking") OR TS=("cycling") OR TS=("athletic") OR TS=("training") OR TS=("working out") OR TS=("work out") OR TS=("strenuous activity") OR TS=("VO2max") OR TS=("maximal aerobic power") OR TS=("VO2 max") OR TS=("Oxygen carrying capacity")) AND (TS=("men") OR TS=("man") OR TS=("male") OR TS=("sportsman") OR TS=("sportsmen") OR TS=("woman") OR TS=("women") OR TS=("female") OR TS=("sportswomen") OR TS=("Sportswoman") OR TS=("athlete") OR TS=("adult") OR TS=("healthy") OR TS=("humans") OR TS=("people") OR TS=("fit"))
Pub-med	3243	"blood donation" OR "blood collection" OR "blood withdrawal" OR "blood centre" OR "Blood center" OR "blood service" OR "blood bank" OR "blood transfusion" OR "phlebotom*" OR "plasma don*" OR "blood volume" OR "blood donor*" "sport" OR "exercise" OR "physical endurance" OR "hiking" OR "cycling" OR "athletic" OR "training" OR "working out" OR "work out" OR "strenuous activity" OR "VO2 max" OR "maximal aerobic power" OR "Oxygen carrying capacity" "men" OR "man" OR "male" OR "sportsman" OR "sportsmen" OR "woman" OR "women" OR "female" OR "sportswomen" OR "Sportswoman" OR "athlete" OR "adult" OR "healthy" OR "humans" OR "people" OR "fit"
SPORT Discus	249	TI "blood don*" OR TI "blood collection" OR TI "blood withdrawal" OR TI "blood centre" OR TI "Blood center" OR TI "blood service" OR TI "blood bank" OR TI "blood transfusion" OR TI "phlebotom*" OR TI "plasma don*" OR AB "blood don*" OR AB "blood collection" OR AB "blood withdrawal" OR AB "blood centre" OR AB "Blood center" OR AB "blood service" OR AB "blood bank" OR AB "blood transfusion" OR AB "phlebotom*" OR AB "plasma don*" OR TI "blood volume" OR TI "blood donor*" OR AB "blood volume" OR AB "blood donor*" TI "sport*" OR TI "exercis*" OR TI "physical endurance" OR TI "hiking" OR TI "cycling" OR TI "athletic" OR TI "training" OR TI "working out" OR TI "work out" OR TI "strenuous activity" OR AB "sport*" OR AB "exercis*" OR AB "performance" OR AB "physical endurance" OR AB "hiking" OR AB "cycling" OR AB "athletic" OR AB "training" OR AB "working out" OR AB "work out" OR AB "strenuous activity" OR TI "VO2max" OR TI "maximal aerobic power" OR TI "Oxygen carrying capacity" OR TI "VO2 max" OR AB "VO2max" OR AB "maximal aerobic power" OR AB "Oxygen carrying capacity" OR AB "VO2 max" TI "men" OR TI "man" OR TI "male" OR TI "sportsman" OR TI "sportsmen" OR TI "woman" OR TI "women" OR TI "female" OR TI "sportswomen" OR TI "Sportswoman" OR TI "athlete" OR TI "adult" OR TI "healthy" OR TI "humans" OR TI "people" OR TI "fit" OR AB "men" OR AB "man" OR AB "male" OR AB "sportsman" OR AB "sportsmen" OR AB "woman" OR AB "women" OR AB "female" OR AB "sportswomen" OR AB "Sportswoman" OR AB "athlete" OR AB "adult" OR AB "healthy" OR AB "humans" OR AB "people" OR AB "fit"

3.2.2 Inclusion and Exclusion Criteria

Restricting the publications to English and using the Population, Interventions, Comparators, Outcomes and Study designs (PICOS) strategy enabled studies to be selected accordingly (Table 3.2). Studies that were accessible as conference abstracts were excluded, as were blood expansion studies, studies requiring muscular strength and studies involving participants > 66 years and < 17 years. The age range selected was according to blood transfusion services in the UK (NHS Blood and Transplant, 2017c).

Table 3.2: Inclusion criteria for study selection

PICO Component	Inclusion criteria
Population	Healthy adults (pathology free) Male and female, 17 - 66 years Cardio-vascular exercise
Interventions	Whole blood donation (post/Bled) of 470 ml (UK guidelines) followed by aerobic exercise 24 – 48 hr post donation. Also, donations from 400 ml up to 500 ml.
Comparators	Aerobic exercise performed with no blood donation (pre/Unbled).
Outcomes	Haematological variables such as RBC's, Hb and Hct Aerobic and cardiovascular/respiratory indices including HR, $\dot{V}O_2$, $\dot{V}O_{2max}$, $\dot{V}O_{2peak}$, time to exhaustion, watts and exercise capacity.

3.2.3 Study Retrieval Process and Quality Assessment

Based on the PRISMA statement (Moher et al., 2009) the flow diagram in Figure 3.1 illustrates a summary of the selection process. The final studies (n = 20) selected for review were rated with an adapted scoring method (Mokkink et al., 2019) according to the COnsensus based Standards for the selection of health Measurements Instruments (COSMIN) and the threshold for eligibility set at an average of ≥3 out of 5 for inclusion (Table 3.4).

To ensure that the studies selected were of a good quality two reviewers individually assessed the texts for eligibility according to the exclusion and inclusion criteria and additionally according to the previously mentioned COSMIN threshold.

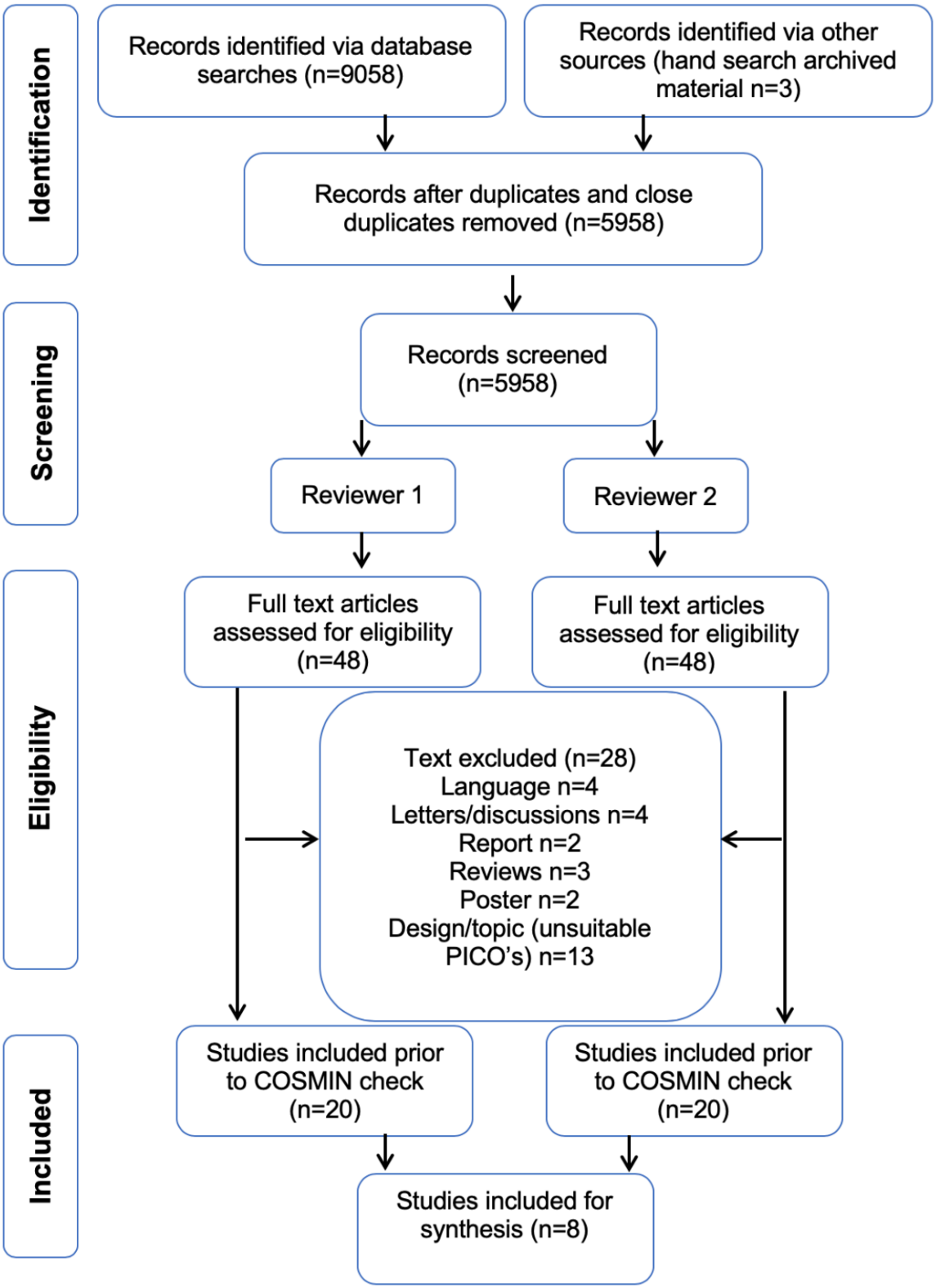

Figure 3.1: Flow diagram based on the PRISMA statement (Moher et al., 2009) illustrating the identification and selection process for the study retrieval.

3.2.4 Data Extraction and Analysis

Using a spreadsheet software package (Microsoft Excel, Microsoft Corp., Washington, USA, 2011) a database was formed with the haematological and cardiovascular/respiratory variables (Table 3.3) extracted from the reviewed studies for analysis.

Table 3.3: Variables extracted from research to form data set for analysis.

Variable	Unit	Acronym
Haemoglobin	$g \cdot dL^{-1}$	Hb
Haematocrit	%	Hct
Erythrocytes	Mio μL^{-1}	RBC's
Maximal oxygen uptake	$ml \cdot kg^{-1} \cdot min^{-1}$ & $l \cdot min^{-1}$	$\dot{V}O_{2max}$
Oxygen uptake	$ml \cdot kg^{-1} \cdot min^{-1}$ & $l \cdot min^{-1}$	$\dot{V}O_2$
Maximum heart rate	$b \cdot min^{-1}$	HR_{max}
Maximum aerobic power output	W & $W \cdot kg^{-1}$	W_{max}
Time/duration to exhaustion	s	TEx

The variables shown in Table 3.3 were all considered for inclusion in the meta-analysis, since they were the most widely reported variables relating to haematological and cardio-vascular responses and regarded according to their mean and standard deviation pre and post donation results. The post donation values were classified according to time frames, which included immediately, 2 h, 24-48 h, 48 -72 h, 7 days, 14 days, 21 days and 28 days. All time period data is included in the systematic review element, however, the meta-analysis only focused on 24 - 48 h for two reasons. Firstly, the time period had sufficient data to analyse and secondly the time period is closest to that suggested by the NHS (2017b) and American Red Cross (2017) to be approached with caution regarding exercise. For an outcome to be included for meta-analysis there needed to be at least two studies that reported a variable of interest. Valentine, Pigott and Rothstein (2010), suggest that although two studies are not ideal it will provide a conclusion and provides an analysis strategy.

Cohens *d* and 95% confidence intervals were calculated for each study. Cohens *d* (standardized difference in means) is expressed as the difference between two (raw) means divided by the pooled standard deviation (SD) of the groups ($M_1 - M_2$ / SD) (Cohen, 1988). Effect sizes for Cohens *d* are evaluated as, 0.8 – > 2.0 (large), 0.3 - 0.5 (medium) and < 0.2 (small). The mean Cohens *d* value was calculated by individual studies being weighted by the variance of the data, thus expressed as

$\overline{SE} = \frac{\sum(ES_i w_i)}{\sum w_i}$ (Harrison, 2011b) or

$$Mean\ Cohens\ d = \frac{Sum\ (individual\ Cohen\ d\ from\ each\ study\ x\ inverse\ of\ variance\ from\ each\ study)}{Sum\ of\ Inverse\ of\ variance}$$

3.3 Results

Following scrutiny of 48 research papers for their eligibility and reducing that number to 20 after further inspection (according to PICO components), 8 studies were deemed suitable for inclusion in the review (Table 3.4) following the COSMIN check, with the publication dates of those included ranging from 1997 - 2016. Regarding assessment of publication bias summarised effects (95 % CI = -1.05, 0.16 and I^2 = %) of the main variable $\dot{V}O_{2max}$ was assessed and a Limits of Agreement plot showed that the two reviewers were considered to be in agreement (bias = 0.12, CI = -0.25, 0.55).

Table 3.4: Eligibility table. Based on the COSMIN doctrine, studies should score an average of >3 (ranked as good) to be considered for inclusion, with 8 studies shown to be eligible using the reviewers average scores and the average combined scores of the reviewers.

Study	Mean score from reviewer 1	Mean score from reviewer 2	Mean combined scores of reviewers	Eligible for review (Yes/No)
Balke, et al., 1954	2.53	2.47	2.50	No
Bennet & McKay, 2013	2.17	2.00	2.08	No
Brinbaum, Dahl & Boone., 2006	2.82	2.88	2.85	No
Burnley, et al., 2006	3.41	2.94	3.18	Yes
Duda, et al., 2003	3.00	2.88	2.94	No
Foster, et al., 2008	2.47	2.71	2.59	No
Gordon, et al., 2010	3.65	3.53	3.59	Yes
Gordon, et al., 2013	3.41	3.29	3.35	Yes
Hill, Vingren & Burdette., 2013	3.35	3.18	3.26	Yes
Janetzko, et al., 1998	2.65	2.65	2.65	No
Judd, et al., 2011	3.24	3.06	3.15	Yes
Krip, et al., 1997	3.06	3.29	3.18	Yes
Markiewicz, et al., 1981	2.88	2.71	2.79	No
Meurrens, et al., 2016	3.50	3.44	3.47	Yes
Mora-Rodriguez, et al., 2012	3.06	2.76	2.91	No
Panebianco, et al., 1995	2.29	2.00	2.15	No
Sliwowski, et al., 2001	2.35	2.18	2.26	No
Strandenes, et al., 2013	2.24	2.29	2.26	No
Williams, Lindhjem & Schuster., 1978	2.82	2.88	2.85	No
Ziegler, et al., 2014	3.35	2.94	3.15	Yes

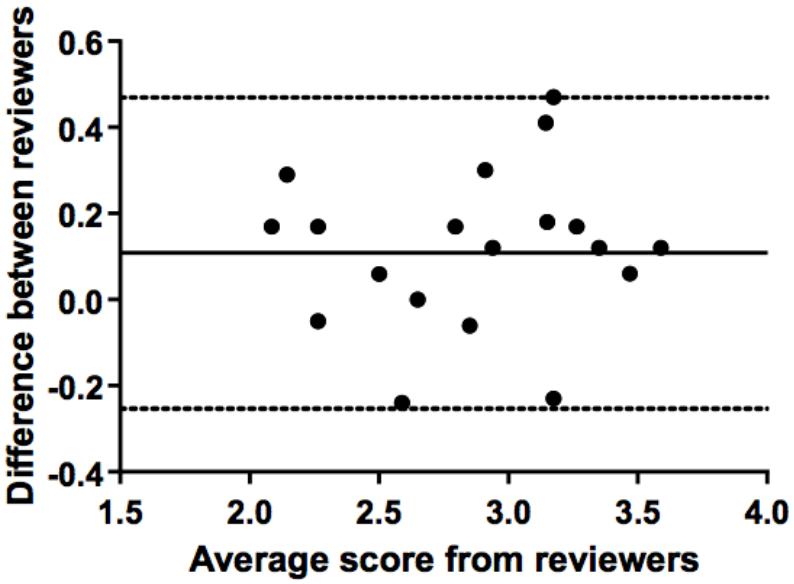

Figure 3.2: Bland Altman (1999) Plot of differences between reviewer 1 and 2 vs the mean of the two measurements (data from table 3.4). The mean score (the bias) of 0.12 is represented by the solid line and lower and upper limits of agreement (dotted lines) from -1.96 to +1.96 are shown as -0.25 and +0.55. All data points are lying within ±2 s of the mean difference, demonstrating the agreement between the two reviewers.

3.3.1 Overview of Studies Included

All 8 studies (Table 3.5) included were experimental in nature, incorporating a before and after blood donation approach, with only the Meurrens, et al. (2016) study using a control (sham bleed) group. Participant numbers ranged from 9 – 24 and hence appeared low, unfortunately only two studies, that of Judd, et al. (2011) and Meurrens, et al. (2016) provided power calculations to reflect sample size, although justification for sample size was presented in all studies except Krip, et al., 1997.

The majority of the participants were male (91 %) and the mean age of all who took part was 23.9 ± 1.5 years with a mean height of 179.2 ± 3.4 cm and a mean mass of 76.4 ± 5.9 kg.

Table 3.5: Overview of the 8 studies included. Sample size varied, as did the combination of male (M) and female (F) participants. Blood donation volumes ranged from 450 – 500 ml. With age, height and mass reported as mean ± SD.

Study	Sample, size (N) & sex (M/F)	Blood drawn (ml)	Age (yrs)	Height (cm)	Mass (kg)	Exercise mode & overview of protocol
Burnley, et al., 2006	10 (M) 1 (F) Physically active & healthy	450	23 ± 6	177 ± 4.0	77.2 ± 11	Cycle: 3 min warm up of 0 W, 25/30 W/min ramp increase at 90 ± 2 rpm to exhaustion. Termination at ≤ 80rpm
Gordon, et al., 2010	6 (M) 4 (F) Regular sport participants	450	21 ± 2	175.2 ± 5.1	66.4 ± 2.8	Cycle: Test 1, 6 min at 80% of respiratory exchange ratio or Test 2, 50% of the difference between $\dot{V}O_{2max}$ and respiratory exchange ratio.
Gordon, et al., 2013	15 (M) Well trained athletes	450	23.3 ± 4.5	180.1 ± 6	77.4 ± 13.1	Cycle: increase ramp from 100 W by 25 W/min at 80 rpm to exhaustion.
Hill, Vingren & Burdette., 2013	2 (F), 7 (M) Healthy university students	450	23 ± 1 (F) 25 ± 3 (M)	170 ± 11 (F) 182 ± 2 (M)	60 ± 8 (F) 92 ± 15 (M)	Cycle: Following a 4 min warm up at 79 ± 15 W and 4 min rest, 80 rpm held at 274 ± 2 W to exhaustion.
Judd, et al., 2011	2 (F) 10 (M) Moderately active	450	24.3 ± 5.2	181 ± 8	87.5 ± 15.3	Cycle: starting at 1.5 /1.0 kp (men and women respectively), ramp increase of 0.5 kp per 2 min at 80 rpm to exhaustion.
Krip, et al., 1997	12 (M) (6 gave blood) Endurance trained	500	24.8 ± 0.5	N.D.	73.9 ± 2.2	Cycle: 6 min at 60 rpm at successive work rates to attain target HR's (maximum of 180).
Meurrens, et al., 2016 #	24 (M) (16 gave blood) Moderately trained	470	25.6 ± 0.76	180.2 ± 1.82	73.8 ± 1.96	Cycle: Starting at 70 W and increasing by 30 W every 2 min at 85-90 rpm to exhaustion.
Ziegler, et al., 2014	20 (M) Healthy men	450	25.9 ± 1.6	185 ± 2	78.7 ± 3.3	Cycle: 6 min warm up at 100 W, 35 W/min to exhaustion.

N.B. N.D. denotes no data, # denotes this study is a RCT design and as such is not included in the meta-analysis.

3.3.2 Cardiorespiratory Variables

3.3.2.1 Oxygen Uptake ($\dot{V}O_2$)

The exercise mode for all studies was cycle ergometer, with a protocol that induced exhaustion and thus $\dot{V}O_{2max/peak}$ with the exception of Gordon, et al. (2010) and Krip, et al. (1997), which were submaximal studies. Table 3.5 shows an overview of the protocols used by all researchers.

Gordon, et al. (2010) has submaximal data in the moderate (below gas Exchange Threshold (GET), specifically 80 % of the difference between rest and GET) and heavy domains (between GET and $\dot{V}O_{2max}$, which was 50 % of the difference between GET and $\dot{V}O_{2max}$), but cannot be compared to any other studies due to no parallels. Similarly, with

little data to compare against and no apparent parallels this is the case also for the Krip, et al. (1997) study.

Athletic descriptions (Table 3.5) included physically active and healthy to well-trained athletes, resulting in a mean $\dot{V}O_{2max}$ value (ml·kg^{-1}·min^{-1}) before BD of 51.3 ± 7.1 (Table 3.6). With values ranging from 40.0 ± 4.0 (Hill, Vingren and Burdette., 2013) – 64.1 ± 1.9 ml·kg^{-1}·min^{-1} (Krip, et al., 1997).

Table 3.6 shows that regardless of the time frame involved in a bled situation there is a trend for BD to decrease $\dot{V}O_{2max}$ values from the pre-BD values. With focus on the time frame of 24 - 48 h post donation, under meta-analysis examination, evidence from the 4 studies inspected showed a mean reduction in $\dot{V}O_{2max}$ (- 2.4 ± 1.6 ml·kg^{-1}·min-1), with a small effect size, -0.26 (Figure 3.3). No statistical significance is present at the mean meta-analysis level due to the 95 % CI passing through 0 (-1.46, 0.94). Hill, Vingren and Burdette (2013) had significance, favouring the intervention (Bled), a large effect size (Cohen's *d*, -1) and confidence intervals of -1.97 to -0.03. The remaining studies all had medium Cohen's *d* and small (Burnley, et al., 2006) effect sizes, but no statistical significance.

Table 3.6: $\dot{V}O_{2max}$ values pre BD (Unbled) in all 8 studies are shown with post BD (Bled) values and time that they were recorded. Gordon, et al. (2010) was a submaximal test post donation in the moderate and heavy domains involving oxygen uptake kinetics and thus the values are not reported in this table.

	$\dot{V}O_2$ & $\dot{V}O_{2max/peak}$ ml·kg^{-1}·min^{-1}							
Study	**Unbled**	**Bled (immediately)**	**Bled (2 h)**	**Bled (24-48 h)**	**Bled (3 days)**	**Bled (7 days)**	**Bled (14 days)**	**Bled (28 days)**
Burnley, et al., 2006	3.79 ± 0.64 *♯			3.64 ± 0.61 *♯				
Gordon, et al., 2010	53.0 ± 4.1							
Gordon, et al., 2013	51.3 ± 7.6			48.4 ± 7.9 ^				
Hill, Vingren & Burdette., 2013	40 ± 4.0		34.0 ± 5.0	36.0 ± 4.0		37.0 ± 4.0		
Judd, et al., 2011	46.6 ± 7.0			44 ± 6.5		44.5 ± 7.2		
Krip, et al., 1997	64.1 ± 1.9 $	55.8 ± 1.8 $				62.1 ± 0.8		
Meurrens, et al., 2016	56.7 ± 1.5			53.9 ± 1.4		52.7 ± 1.4	51.0 ± 1.4	52.7 ± 1.4
Ziegler, et al., 2014	49.7 ± 1.7 ♯				46.5 ± 1.6 ♯	47 .2 ♯	49.2 ♯	49.3 ♯

N.B. * denotes values in l·min^{-1} and ml·min^{-1}, ^ denotes that this time period was 48-72 h. ♯ denotes the Burnley study post donation showing a $\dot{V}O_{2peak}$ value, as this was a test performed in the severe intensity domain to exhaustion and the Ziegler, et al. (2014) study showing $\dot{V}O_{2peak}$ values, with their criteria to exhaustion and a respiratory exchange ratio of more than 1.2. $ denotes the Krip, et al. (1997) study showing submaximal bled end $\dot{V}O_2$ values.

Mean value & 1st Author	Difference in mean	Effect Size Cohens *d*	95% CI for effect size Lower	Upper
Burnley 2006	-0.15	-0.24	-1.08	-0.60
Gordon 2013	-2.90	-0.37	-1.10	-0.35
Hill 2013	-4.00	-1.00	-1.97	-0.03
Judd 2011	-2.60	-0.38	-0.98	-0.21
Mean	-2.41	-0.26	-1.46	0.94

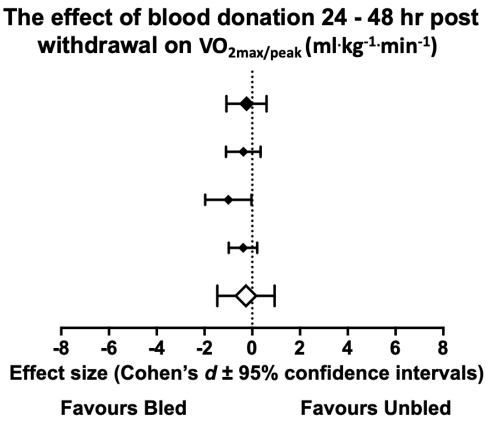

Figure 3.3: Statistical results from four studies of pre and 24 – 48 h post BD in $\dot{V}O_{2max/peak}$. With the diamonds reflecting the effect size of the studies. The white diamond shows the overall mean effect size. All effect sizes are Cohen's *d* and 95 % confidence interval, with heterogeneity of I^2 = 0 %.

3.3.2.2 Heart Rate (HR)

The HR pre and post donation values were only acknowledged in three studies (Table 3.7), with Krip, et al. (1997) only presenting post donation HR results. The meta-analysis showed no statistical significance for any of the results, all the CIs passed through "0", with very small (> 0.2) Cohens *d* effects across all research too (Figure 3.4).

Table 3.7: Maximal HR values pre BD (Unbled) and post BD (Bled) over various time frames.

	HRmax (b·min^{-1})				
Study	**UnBled**	**Bled (24–48 h)**	**Bled (7 days)**	**Bled (14 days)**	**Bled (28 days)**
Gordon, et al., 2013	184.9 ± 10.1	185.0 ± 10.0 ^			
Judd, et al., 2011	186 ± 12	181 ± 19	181 ± 12	185 ± 13	185 ± 13
Krip, et al., 1997		187 ± 3			
Meurrens, et al., 2016	187 ± 4	187 ± 3	183 ± 3	180 ± 3	181 ± 3

N.B. ^ denotes that this time period was 48-72 h. No pre donation value was available for Krip, et al. (1997).

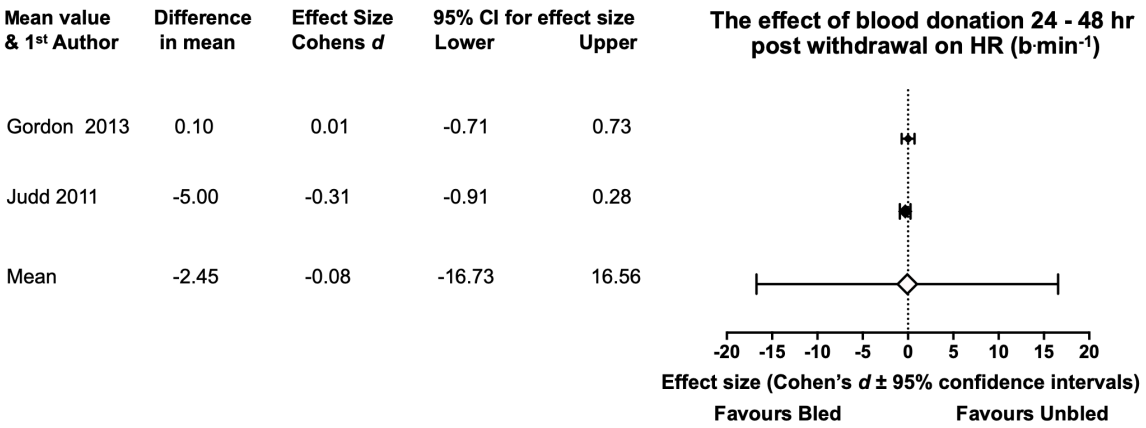

Figure 3.4: Statistical results from two studies of pre and 24 – 48 h post BD in HRmax. With the diamonds reflecting the effect size of the studies. The white diamond shows the overall mean effect size. All effect sizes are Cohen's *d* and 95 % confidence interval with heterogeneity of I^2 = 0 %.

3.3.2.3 Time to Exhaustion (TEx)

TEx pre and post donation evidence was recorded from five studies (Table 3.8). With the exception of the Judd and contemporaries (2011) study TEx shows an inclination to decrease in a Bled condition from Unbled across all time frames. For the time frame of 24 - 48 h the meta-analysis (Figure 3.5) showed a mean reduction in TEx (- 23.52 s), with a large effect size produced (Cohen's *d*, -0.88). No statistical significance is present at the mean meta-analysis level due to the 95 % CI passing through 0 (-35.50, 33.73). Only the Meurrens and colleagues (2016) study demonstrated small significance, as the CI appears just to the left of 0.

Table 3.8: TEx pre and post BD (Unbled and Bled) values over various time periods.

	TEx (s)						
Study	**Unbled**	**Bled (2 h)**	**Bled (24–48 h)**	**Bled (7 days)**	**Bled (14 days)**	**Bled (21 days)**	**Bled (28 days)**
Burnley, et al., 2006	375 ± 129		321 ± 99				
Gordon, et al., 2013	611.4 ± 101.4		610.8 ± 109.8 ^				
Hill, Vingren & Burdette., 2013	268 ± 16	218 ± 32	250 ± 22	258 ± 21			
Judd, et al., 2011	586 ± 168		589 ± 189	610 ± 157	623 ± 173	634 ± 179	649 ± 176
Meurrens, et al., 2016	1014 ± 54		966 ± 48	972 ± 48	978 ± 48		990 ± 54

N.B. ^ denotes that this time period was 48-72 h.

Mean value & 1st Author	Difference in mean	Effect Size Cohens *d*	95% CI for effect size Lower	Upper
Burnley 2006	-54.00	-0.47	-1.32	0.38
Gordon 2013	-0.60	-0.01	-0.72	0.71
Hill 2013	-18.00	-0.94	-1.90	0.03
Judd 2011	3.00	0.02	-0.57	0.61
Mean	-17.40	-0.88	-37.29	35.52

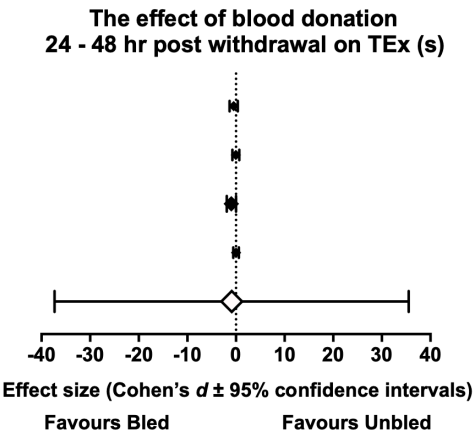

Figure 3.5: Statistical results from four studies of pre and 24 – 48 h post BD in TEx. With the diamonds reflecting the effect size of the studies. The white diamond shows the overall mean effect size. All effect sizes are Cohen's *d* and 95 % confidence interval, with heterogeneity of I^2 = 0 %.

3.3.2.4 Maximal Aerobic Power Output (W_{max})

W_{max} pre (329.7 ± 42.4) and post donation (329.5 ± 45.7) evidence could only be gathered from one study (Gordon, et al., 2013) and the time period was 48-72 h, thus no meta-analysis could be performed.

3.3.3 Haematological Variables

3.3.3.1 Haemoglobin (Hb)

Hb pre and post donation evidence was recorded from six studies (Table 3.9). Across all studies there is a decrement in Hb under Bled conditions across all time periods. The raw mean data (Table 3.9) from Bled to Unbled (24 – 48 h) reveals an 8.06 % decrease in Hb levels. The meta-analysis (Figure 3.6) showed a mean reduction in Hb (- 1.05 $g \cdot dL^{-1}$) across the four relevant studies, with a medium effect size produced (Cohen's *d*, -0.75). No statistical significance is present at the mean meta-analysis level due to the 95 % CI passing through 0 (-2.04, 0.54). Gordon, et al. (2013) showed a large effect size and statistical significance as confidence intervals were to the left of 0 (-1.82, -0.30). Cohen's *d* effects were moderate to high across all the studies. Burnley, et al. (2006), Gordon, et al. (2010) and Hill, et al. (2013) were not shown to have statistical significance under meta-analysis scrutiny for Hb.

Table 3.9: Hb pre and post BD (Unbled and Bled) values over various time frames, with Krip, et al. (1997) not reporting post BD values.

	Hb ($g \cdot dL^{-1}$)						
Study	**Unbled**	**Bled (2 h)**	**Bled (24-48 h)**	**Bled (3 days)**	**Bled (7 days)**	**Bled (14 days)**	**Bled (28 days)**
Burnley, et al., 2006	15.4 ± 0.9		14.7 ± 1.3				
Gordon, et al., 2010	14.2 ± 1.5		13.1 ± 1.5				
Gordon, et al., 2013	15.6 ± 1.2		14.1 ± 1.6 ^				
Hill, Vingren & Burdette., 2013	14.9 ± 0.8	14.9 ± 1.1	14.0 ± 1.7		14.0 ± 1.2		
Krip, et al., 1997	15.8 ± 0.5						
Meurrens, et al., 2016	15.6 ± 0.2		13.7 ± 0.3		14.2 ± 0.2	14.2 ± 0.2	14.5 ± 0.2
Ziegler, et al., 2014	9.3 ± 0.11 *			8.6 ± 0.1 *	8.65 *	8.75 *	9.15 *

N.B. * denotes values in $mmol \cdot L^{-1}$ and ^ denotes that this time period was 48-72 h.

Mean value & 1st Author	Difference in mean	Effect Size Cohens *d*	95% CI for effect size Lower	Upper
Burnley 2006	-0.70	-0.63	-1.48	-0.23
Gordon 2010	-1.10	-0.73	-1.64	-0.17
Gordon 2013	-1.50	-1.06	-1.82	-0.30
Hill 2013	-0.90	-0.68	-1.63	-0.27
Mean	-1.05	-0.75	-2.04	0.54

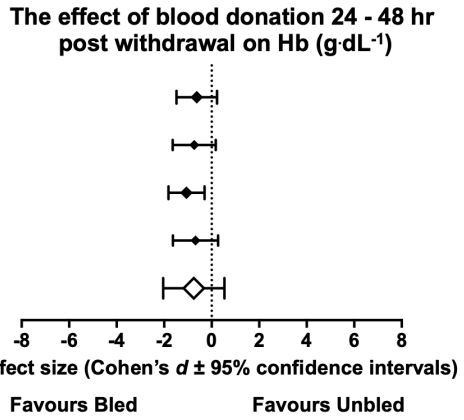

Figure 3.6: Statistical results from four studies of pre and 24 – 48 h post BD in Hb. With the diamonds reflecting the effect size of the studies. The white diamond shows the overall mean effect size. All effect sizes are Cohen's *d* and 95 % confidence interval, with heterogeneity of $I^2 = 0$ %.

3.3.3.2 Haematocrit (Hct)

Hct pre and post donation evidence was recorded from five studies (Table 3.10). Across all studies there is a tendency for Hct to decline in a Bled state across all time periods from the original Unbled value. The raw mean data from Bled to Unbled (24 – 48 h) reveals an 8.81 % decrease in Hct levels. The meta-analysis (Figure 3.7) across the three relevant studies (Gordon, et al., 2010, 2013; Burnley, et al., 2006), showed a mean reduction in Hct (-3.71 %) and a large mean effect size (Cohen's *d*, -1.16). Statistical significance is not present at the mean meta-analysis level due to the 95 % CI passing through 0 (-4.59, 2.28). All studies show large effect sizes and statistical significance.

Table 3.10: Hct pre and post BD (Unbled and Bled) values over varying time frames.

	Hct (%)					
Study	**Unbled**	**Bled (24-48 h)**	**Bled (3 days)**	**Bled (7 days)**	**Bled (14 days)**	**Bled (28 days)**
Burnley, et al., 2006	44 ± 2	41 ± 3				
Gordon, et al., 2010	43.55 ± 3.28	40.20 ± 2.63				
Gordon, et al., 2013	48.76 ± 4.31	43.99 ± 3.91 ^				
Krip, et al., 1997	41.1 ± 1.2					
Meurrens, et al., 2016	45.8 ± 0.6	40.9 ± 0.9		41.9 ± 0.6	42.4 ± 0.5	43.9 ± 0.5
Ziegler, et al., 2014	43.7 ± 0.6		40.6 ± 0.6	40.8 ± 0.6	41.2 ± 0.4	42.8 ± 0.6

N.B. ^ denotes that this time period was 48-72 h.

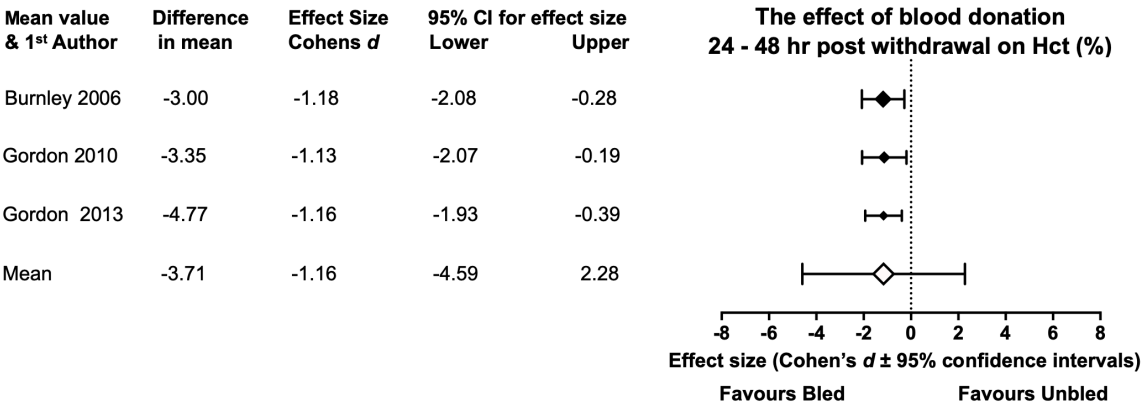

Figure 3.7: Statistical results from three studies of pre and 24 – 48 h post BD in Hct. With the diamonds reflecting the effect size of the studies. The white diamond shows the overall mean effect size. All effect sizes are Cohen's *d* and 95 % confidence interval, with heterogeneity of I^2 = 0 %.

3.3.3.3 Red Blood Cells (RBCs)

RBCs pre and post donation evidence was recorded from three studies (Table 3.11). Across all studies there is a tendency for RBCs to decline in a Bled state across all time periods from the original Unbled value. The raw mean data from Bled to Unbled (24 – 48 h) reveals a 9.49 % decrease in RBCs levels. The meta-analysis (Figure 3.8) across the two relevant studies (Ziegler, et al., 2014; Gordon, et al., 2013), revealed a mean reduction in RBCs (-0.44 Mio μL^{-1}) and a very large mean effect size (Cohen's *d*, -4.23). Statistical significance is present at the mean meta-analysis level due to the 95 % CI being placed notably to the left of 0 (-4.37, -4.10). All studies show large to very large effect sizes and statistical significance.

Table 3.11: RBCs pre and post BD (Unbled and Bled) values over varying time frames.

	RBC's (Mio µL^{-1})					
Study	**Unbled**	**Bled (24-48 h)**	**Bled (3 days)**	**Bled (7 days)**	**Bled (14 days)**	**Bled (28 days)**
Gordon, et al., 2013	5.28 ± 0.50	4.71 ± 0.59 ^				
Meurrens, et al., 2016	5.2 ± 0.1	4.6 ± 0.1		4.8 ± 0.1	4.8 ± 0.1	4.9 ± 0.1
Ziegler, et al., 2014	4.9 ± 0.07	4.6 ± 0.07	4.6 ± 0.07	4.6 ± 0.08	4.7 ± 0.07	4.8 ± 0.07

N.B. ^ denotes that this time period was 48-72 h.

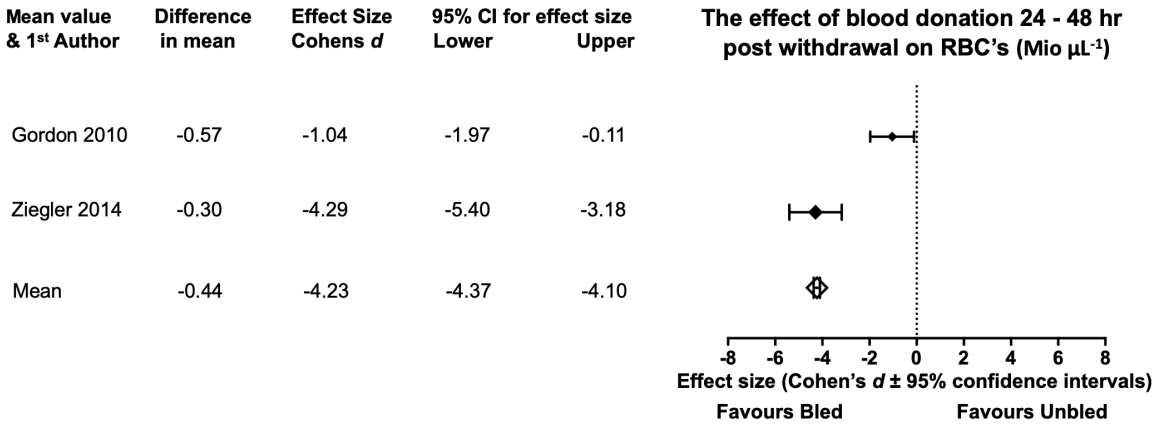

Figure 3.8: Statistical results from two studies of pre and 24 – 48 h post BD in RBCs. With the diamonds reflecting the effect size of the studies. The white diamond shows the overall mean effect size. All effect sizes are Cohen's *d* and 95 % confidence interval, with heterogeneity of I^2 = 0 %.

3.4 Discussion

Based on the hypotheses stated that BD produces either no changes (H_0) or produces significant changes (H_1) in haematology, $\dot{V}O_2$, heart rate (HR), exercising power and time, the following can be concluded. For $\dot{V}O_{2max/peak}$, based on the mean Cohen's *d* ± 95 % confidence intervals, the Null hypothesis cannot be rejected, as the line passes through "0" and thus is not statistically significant; this is also the case for HR, TEx and W_{max}. For the blood variables, the alternate hypothesis is accepted only for RBCs, rejecting the Null hypothesis, as the mean Cohen's *d* ± 95 % confidence intervals favour donation.

Research papers have historically determined that BD decreases $\dot{V}O_{2max}$ (Hill, Vingren and Burdette, 2013; Gordon, et al., 2013 and Meurrens, et al., 2016), however, the confidence intervals have not produced statistical significance. Individual studies have too much variance and therefore the pooled data using the Cohen's *d* allows for comparisons across all papers. One of the reasons for the lack of significance could be the absence of statistical power in the other studies due to low sample sizes (please refer to the power calculation section further on in the discussion).

The issues of too much variance as previously mentioned in $\dot{V}O_{2max}$ across the individual studies could also occur from various issues such as differing training status, sex, age or even barometric pressure. When considering training status, it is known that $\dot{V}O_{2max}$ values between 70 and 85 ml·kg^{-1}·min^{-1} exist for champion male endurance athletes (Joyner and Coyle, 2008) and that female values are ~10 % lower (shown as 67.1 ml·kg^{-1}·min^{-1} in an elite group) due to higher body fat and lower Hb concentration (Pate, et al., 1987). Barnard, Grimditch and Wilmore (1979) highlighted how $\dot{V}O_{2max}$ in Master sprinters (age 41 - 58 years) differed to Master distance runners (age 40 - 78 years) with values being 47.4 ± 2 and 54.4 ± 3 ml·kg^{-1}·min^{-1} respectively and additionally the values decreased by 34.5 % from the age of 40 to 70 years. Normative values from American College of Sports Medicine (ACSM, 2014) based on Balke treadmill test suggest superior values of 54.5 and 60.5 ml·kg^{-1}·min^{-1} for females and males respectively in their twenties, which then decrease to 52 and 58.3 ml·kg^{-1}·min^{-1} during the 30-39 years age range.

Regarding barometric pressure, a change in $\dot{V}O_{2max}$ of 1 % will occur if the pressure alters by 7-8 mmHg (Howley, Bassett and Welch, 1995). For example, a fall in barometric pressure (which is ~760 mmHg at sea level (McArdle, Katch and Katch, 2010)) will result in reduced pressure gradients that drive gas exchange from the alveoli and blood, resulting in less oxygen from the lungs due to reduced partial pressure of oxygen, highlighting the importance of calibration. Examples of changes in $\dot{V}O_{2max}$ with varying barometric pressures can be read in a study by Wagner (1996) where conditions at sea level, 15,000 ft and Everest summit resulted in values of 3.82, 2.81 and 1.46 L·min^{-1} respectively. This assortment of environmental changes caused modifications in the oxygen-haemoglobin dissociation curve.

An overall mean reflection of blood variables for the meta-analysis data collected showed that under Bled conditions (24-48 h) RBCs, Hct and Hb values were significantly reduced. Hb is accountable for the conveyance of O_2 in blood from the lungs (at high pressure (100 mmHg)) to varying body tissues (at low pressures (40 mmHg)) and at full saturation carries ~ 20 ml of O_2 per dL of whole blood (McArdle, Katch and Katch, 2010). There is 1.39 ml O_2·g^{-1} of fully saturated Hb (Otto, Montgomery, Richards, 2013) (or empirically Hb oxygen binding capacity is 1.34 ml O_2·g^{-1} Hb) and 100 ml of blood carries 15 g of Hb, which provides a 20.1 ml oxygen-binding capacity, additionally O_2 binding capacity is proportional to haematocrit (Pittman, 2011). Where data allowed there were three studies (Burnley, et al., 2006; Gordon, et al., 2010 and 2013) that showed proportional reductions in Hct and Hb post BD (Table 3.12). Mathematically for normal Hct (37- 41/43 % and 42 – 47 % in women and men respectively (Manzone, et al., 2007 and Gómez and Jesús, 2015)) of 15g

$Hb \cdot 100ml^{-1}$ (blood), the Hb carrying capacity of O_2 = 1.34 x 15 = 20.1 mL $O_2 \cdot 100ml^{-1}$ (Harrison, 2011a, p. 251). This would equate to loosing 94.47 ml O_2 and 70.5 g Hb in a 470 ml BD, which has been postulated to affect variables such as $\dot{V}O_{2max}$ (Hill, Vingren and Burdette, 2013; Gordon, et al., 2013; Meurrens, et al., 2016). Table 3.13 shows the Hb binding capacity and Hb loss from BD in three studies. Therefore, it seems prudent for all studies that involve BD and exercise together to collect relevant blood variables, such as Hb, Hct and RBCs, which seem to be more commonly reported, but additionally others such as iron and ferritin, as the figures for these could help provide insight to research questions. For example, iron status from ferritin levels could indicate BD frequency. Yet, various authors neglected to report these values fully. For example, Krip, et al. (1997) failed to report Hb and Hct post BD, which would render the pre-BD values that they had collected as lacking in external validity, as there was nothing to compare it with. Judd, et al. (2011) did not report any blood variables, however the study was about the time course for recovery, which would be affected by O_2 in the blood and thus by default Hb.

Table 3.12: Hb and Hct percentage decrease to show if oxygen-binding capacity is proportional to haematocrit.

Study	Hb ($g \cdot dL^{-1}$) Unbled	Hb ($g \cdot dL^{-1}$) Bled	% Decrease	Hct (%) Unbled	Hct (%) Bled	% Decrease
Burnley, et al., 2006	15.4 ± 0.9	14.7 ± 1.3	4.76	44 ± 2	41 ± 2	7.32
Gordon, et al., 2010	14.2 ± 1.5	13.1 ± 1.5	7.75	43.55 ± 3.28	40.20 ± 2.63	7.69
Gordon, et al., 2013	15.6 ± 1.2	14.1 ± 1.6	9.61	48.76 ± 4.31	43.99 ± 3.91	9.78
Meurrens, et al., 2016	15.6 ± 0.2	13.7 ± 0.3	12.18	45.8 ± 0.6	40.9 ± 0.9	10.70

Table 3.13: Hb-binding capacity Unbled and Bled and Hb loss from BD.

Study	ml drawn from BD	Hb ($g \cdot dL^{-1}$) Unbled	g Hb Unbled ml O_2/100ml	g Hb lost from BD	Hb ($g \cdot dL^{-1}$) Bled	g Hb Bled ml O2/100ml	% decrease Hb from Unbled to Bled
Burnley, et al., 2006	450	15.4	1.34 X 15.4 =20.64	69.3	14.7	1.34 X 14.7 =19.70	4.55
Gordon, et al., 2010	450	14.2	1.34 X 14.2 =19.03	63.9	13.1	1.34 X 13.1 =17.55	7.77
Gordon, et al., 2013	450	15.6	1.34 X 15.6 = 20.90	70.2	14.1	1.34 X 14.1 = 18.89	9.62
Meurrens, et al., 2016	470	15.6	1.34 X 15.6 = 20.90	73.32	13.7	1.34 X 13.7 = 18.36	12.15

One issue worth noting is that only one study (Gordon, et al., 2013) controlled for hydration adequately, in the other studies it was not controlled properly or sometimes not mentioned

at all. It is important as dehydration (or even over hydration) affects blood readings. Hct and Hb will alter according to hydration levels. Euhydration (normal body water content) is a combination of fluid intake, stable total body weight and water (intra and extra cellular) and normal blood chemistry (Oppliger and Bartok, 2002). With dehydration, as the plasma part of blood is reliant on water, Hct and Hb values are elevated due to intra cellular and extra cellular space water loss, as the plasma diminishes blood viscosity increases. Hct is usually ~3 times the value of Hb (Quinto, et al., 2006), therefore, if Hct changes so should Hb. It is suggested that for maintaining hydration (in a clinical setting), fluid daily intake should be 35 $ml \cdot kg^{-1}$ for 18 - 60 years and 30 $ml \cdot kg^{-1}$ for over 60 years (Todorovic and Micklewright, 2011), therefore the variance in fluids could be attributed to the change in fat-free mass and adiposity that can alter with age, as fat-free mass is ~70-80 % water (Institute of Medicine, 2005) and muscle mass possibly diminishes with age if there is inactivity. Therefore, giving guidance to participants for their fluid intake would be a prudent precaution and to check this with specific gravity of urine measurements to ensure the variable is controlled for.

Although in many of these studies Hb, Hct and RBCs were the considered blood variables only two studies (Meurrens, et al., 2016 and Ziegler, et al., 2014) considered the iron storage protein, ferritin. Approximately 25 % of the body's iron is stored as ferritin (NHS, 2017) and repeated blood donations decrease its levels, as shown in the Meurrens, et al. (2016) study where 3 blood donations (taken 3 months apart from one another) were observed. The results saw ferritin begin prior to any donation at 55 ± 8 $\mu g \cdot l^{-1}$ to then reduce to 40 ± 6 $\mu g \cdot l^{-1}$ ($p < 0.01$) before the second donation. This result provides a Cohen's *d* value of 2.18 and thus is a large effect. Finally, a value of 41 ± 5 $\mu g \cdot l^{-1}$ ($p < 0.001$) was observed prior to the third donation, which against the initial value gives a Cohen's *d* value of 2.04 and is again a large effect. Thus if it is not known in some of the studies if the participants are new or regular donors, iron status according to ferritin levels could aid to indicate normal levels (41 – 400 $\mu g \cdot l^{-1}$ and borderline 16 – 40 $\mu g \cdot l^{-1}$ (NHS, 2017)). This might be important to help provide insight on differing results between studies. For example, Ziegler, et al. (2014) points out that the $\dot{V}O_{2peak}$ was back to baseline values 14 days after donation, the reason cited was iron status was normal before the study and yet table 3.6 shows distinct variations between studies, which could be due to the iron level. Supporting the importance of iron; a study from Friedmann, et al. (2001) investigated young elite athletes with low serum ferritin but normal Hb. Oral iron supplementation was given over 12 weeks and in that period $\dot{V}O_{2max}$ saw significant increases, as did the ferritin levels. Further evidence demonstrated that oral iron supplementation was associated with a decrease in time taken to complete a 15 km time trial and the authors (Hinton, et al.,

2000) attributed this to mainly serum ferritin level as opposed to Hb after substantiation via linear regression analysis. Patel and contemporaries (2013) suggest that Hb screening is a poor predictor of iron store and ferritin tests should be used, especially if the Hb is 12-13.5 $g \cdot dL^{-1}$.

Further consideration when comparing studies should be given to the heterogeneous mix of participants. It was previously stated in the results section that $\dot{V}O_{2max}$ results varied, this could be due to different training statuses. However, a study should be prepared to disclose more detail on the participants in order for conclusions to be made, for example a paper declares the participants to be trained athletes (Gordon, et al., 2013), but fails to mention what they were trained in. Another study reported to include physically active and healthy participants (Burnley, et al., 2006), but no quantification of this was provided. Quantification could come in the form of analysing individual $\dot{V}O_{2max}$ results, for example by using ACSM (2014) guidelines and observing the combined values for Gordon and colleagues (2013) study reveals the participants were of 'excellent fitness' and the participants for Burnley and associates (2006) were of 'good fitness'. However, athletes will have differing values such as the sprinter and the endurance runner. For example, both athletes maybe trained in their own right, but, the endurance runner will rely on a combination of $\dot{V}O_{2max}$, running economy and lactate threshold (Midgley, McNaughton and Jones, 2007), unlike the sprinter whose sport is based on explosiveness from the ATP-PCr energy system. As the research involved in this review were blood donation studies, the relevancy relates back to the type of participant, thus, generally the fitter and more aerobically developed an individual is the larger the blood volume (BV) (Maughan, 2013). Indeed, Martino, Gledhill and Jamnik (2002), show correlation coefficients (r) of 0.83 between $\dot{V}O_{2max}$ and BV. BV is additionally affected by issues such as metabolism, physical activity, body positioning, sleep, temperature, age, sex, musculature, body proportionality and mass (Yurkon, 1965), as reported in chapter 2.2.

This review had the potential to include 20 papers, but this was deemed inadmissible due to quality issues, leaving only nine papers considered to be of good quality, none of the papers were considered to be excellent quality. Reasons for exclusion were heterogeneous, but a reoccurring theme even in the good quality papers was a lack of power calculations, only the Meurrens, et al. (2016) and Judd, et al. (2011) studies that were in the final list reviewed provided this. Without power analysis, sample size could be too low, resulting in under power, unanswered questions, possible false inferences, no effect sizes and potentially less accuracy. Based on this there is a possibility that all the studies with the exception of Meurrens, et al. (2016) and Judd, et al. (2011) who had power

calculations and perhaps Ziegler, et al. (2014) who had 20 participants, but no power calculation were underpowered as the samples sizes ranged from 9-15 people. However, some studies justified their sample sizes, within the final reviewed list this included, Burnley, et al. (2006); Duda, et al. (2003); Gordon, et al. (2010, 2013); Hill, et al. (2013) Judd, et al. (2011) and Ziegler, et al. (2014), the remaining authors did not, which included Krip, et al. (1997). With the aid of statistical power online software (ClinCalc.com, 2017) and by using previous research (Gordon, et al., 2010) comparison of two means for Hb (14.23 and 13.1 $g{\cdot}dL^{-1}$) could be made. A recommended sample size of 14 can be calculated using a power of 80 %, critical value for normal distribution set at 1.96. The following formula was used from ClinCalc.com (2017):

$$N = \frac{\sigma^2(z_{1-\beta} + z_{1-\alpha/2})^2}{(\mu_0 - \mu_1)^2}$$

$$N = \frac{(1.52^2(0.84 + 1.96)^2}{(14.23 - 13.1)^2}$$

$$N = 14$$

Where n is the sample size (14), μ_0 is the population mean μ_1 is the mean of study population, σ^2 is variance in population, α is the probability of type I error (0.05) β is the probability of type II error (0.2) and $\mathcal{Z}$ is the critical $\mathcal{Z}$ vaule for a given α *or* β.

The only study in this review to use a randomized controlled trial (sham bleed) was that of Meurrens and colleagues (2016) and hence this study was not pooled into the relevant forest plots and subject of the meta-analysis. Thus, it can be thought with confidence that measures to reduce bias were adopted to test the effectiveness of the intervention (blood donation) in this study. The other studies involved opted not to operate in this manner, it is not known why, maybe because they thought it not appropriate, applicable or feasible. The Meurrens group blinded the participants and they all went through the process of donation; however, some were not aware that they had not donated blood (sham). They did have ethical approval, nevertheless the ethical implications of having an invasive procedure of a needle in the arm, but not donating blood could be considered inappropriate at some institutions or simply not possible. Conversely, the positive results that are associated with this type of approach should be appreciated. Blinding helps to ensure impartiality and that bias is eliminated or at least reduced.

With the exception of HR, if Meurrens and associates (2016) were to have been included in the meta-analysis clear statistical significance would have occurred across all variables and very large effect sizes, particularly with blood measures for example a Cohen's *d* - 6.01 for Hb. Hence, this study could have been considered as a possible outlier. However, leave-out analysis was performed to assess the influence of this study on the mean effect sizes for Hct, Hb, and RBCs. The results showed mean effect sizes remained large, however, pooling of robust high quality randomized controlled studies with lower quality uncontrolled designs is not recommended and would possibly lead to type I errors. Generally, this study appeared to be a very good research paper, although one of its flaws was a lack of hypothesis. Additionally, the reader was not told how the sample was selected, for example, opportunistic methods and finally hydration was not adequately controlled for.

3.5 Conclusion

In summary, it is clear from this systematic review that there are not enough high-quality papers to draw any definitive conclusions regarding cardiorespiratory outcomes. Although we have an understanding from individual studies that $\dot{V}O_{2max}$ is reduced from donation, pooled results of the raw data show that $\dot{V}O_{2max}$ is not significantly reduced from blood donation 24-48 h post donation. Additionally, sub maximal data is limited for substantial comparatives, suggesting that there is a need for more high-quality studies of this nature to be implemented. Data was also limited for HR and W_{max}. Blood variables showed more definitive results and so conclusions could be made, but only four studies were compared regarding Hb and only three studies could be compared regarding Hct and two studies for RBCs.

Moreover, there is a need for further exploration regarding post blood donation health and well-being in athletes and non-athletes alike. Generally, the papers reviewed in this instance portrayed the participants as active/athletic and additionally they were all young, but this does not necessarily reflect the general public (who donate blood). Lattimore, et al. (2015) reported that the median age for new and repeat donors in England and North Wales in 2011 were 29 and 47 years respectively. Thus, this avenue needs to be investigated further encompassing a more reflective cohort of the population. Although it does appear to be the athletic population that are concerned with the effects of donation on their performance and not necessarily the less active population. However, the advice from the NHS is limited regarding exercise and BD. The NHS Blood and Transplant (2016b) state:

> “You can still exercise, but don't do anything more strenuous than usual: this applies before and after donating blood.”

Blood Plasma Donating Tips (2017) advise to avoid strenuous exercise 12 - 24 hours after donation. The American Red Cross (2017) advises:

> “Do not do heavy exercise or lifting for five hours” and “student athletes should wait about 12 hours or more before resuming strenuous exercise.”

However, curiously despite there being various forms of advice across different countries, it appears there is no scientific supporting evidence to avoid exercise after donation, a conclusion also reached by Mora-Rodriguez, et al. (2012). Moreover, the advice regarding exercise is generic and does not even consider the disproportionate effect between two individuals of vastly different BVs. Thus, to conclude, pending superior quality studies, the current evidence cannot provide definitive answers regarding cardiorespiratory variables, but it can report the reductions in the blood variables investigated, which potentially impact sporting ability.

Chapter 4: General Methods

The following methods chapter contains the equipment that was used for the laboratory based experimental chapters.

4.1 Gas Analysis System – MetaLyzer® 3B

The MetaLyzer® 3B (Figure 4.1) is a metabolic stress test/CPX system with ergospirometry (Cortex MetaLyzer® 3B, UK) that is used for collecting gas variables ($\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$ and RER) on a breath-by-breath basis and has the ability to measure volume continuously. With the values during the test on continuous display for scrutiny having been transmitted to a PC (operating with Microsoft Windows), which allows for the visualisation of specific key points such as anaerobic threshold.

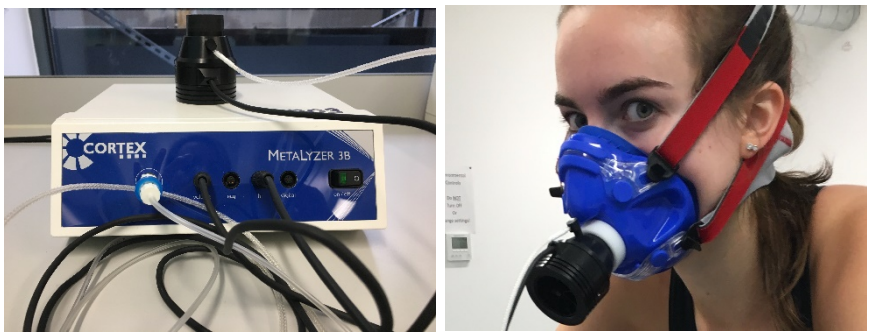

Figure 4.1: The MetaLyzer® 3B with turbine volume transducer and capillary line (left picture). On the right is the facemask, which is attached to the digital turbine volume transducer and capillary line for gases. (Permission from participant granted for use of the photo.)

A participant wears a facemask (Figure 4.1), which is attached to a digital turbine volume transducer and a capillary line for gases. The turbine volume transducer can be disassembled and re-assembled for cleaning and maintenance purposes. To disassemble a half turn left will disengage the electronics from the housing, which will allow the turbine to be slid out of the housing to be checked for damage or cleaned. Information to the main unit is relayed via the digital turbine volume transducer and a capillary line. CO_2 and O_2 are measured via infrared and a galvanic fuel cell respectively. CO_2 absorbs infrared radiation (in dual beam format) and is passed through a reference cell and parallel sample cell holding the test gas at a continual flow rate. The dual beams are interrupted by a mechanical gyratory chopper, which gives an oscillating signal, with the magnitude of the oscillations being proportional to the differences in concentrations of the test and reference gas. A stable current comparative to the partial pressure of O_2 is created via a semi-disposable electrochemical galvanic fuel cell to calculate O_2, which gives response times

of < 200 msec in the analysis (Macfarlane, 2001). The online gas analysis system uses the Haldane transformation (Haladane, 1897/1898; Poole and Whipp, 1988) to compute the expired gases (O'Hara, et al., 2017), which considers the ratio of inspired to expired nitrogen to calculate oxygen use.

In tests from Meyer, et al. (2001), 15 participants were recruited to test the retest reliability for the MetaLyzer® 3B by performing two ramp tests to exhaustion on a cycle ergometer. The outcome was excellent intra-class coefficients of correlation; $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}E$ were 0.969, 0.964 and 0.953 respectively and additionally Bland-Altman plots indicated narrow reference lines (±1.96 SD), showing the variables have 95 % confidence intervals. Unfortunately, the paper does not provide specific mean bias scores, but the plots on observation appear to show values just below zero for $\dot{V}O_2$, $\dot{V}CO_2$ and just above zero for $\dot{V}E$. According to the manual of the MetaLyzer® there is ± 0.1 Vol % accuracy for CO_2 and O_2. However, to maintain accuracy calibration should be performed. The manufacturers guide suggests that a 45 minute warm-up period should be adhered to before gas-calibration and also 15 minutes is required prior to pressure and volume calibration in order for the system to be entirely operative (Cortex MetaLyzer® 3B manual guide). The reliability of the MetaLyzer® 3B in situ at Anglia Ruskin University in Cambridge was tested using tidal volumes (flow rate) of 0.5, 1 and 3 L/s, known gas concentrations (ambient air, which contains ~21 % O_2 and Cranlea gas (Cranlea, Birmingham, UK), 17.05 % O_2, 4.98 % CO_2). The results can be seen in Table 4.1 and what can be observed is that the deviation for CO_2 and O_2 is less than 0.1 Vol % (in accordance with the manufacturer).

Table 4.1: Calibration comparisons on the MetaLyzer® 3B

	Test 1: 24 hours post $\dot{V}O_{2max}$ test	Test 2: Calibration (20 min) prior to $\dot{V}O_{2max}$ test	Test 3: (15 mins) Post $\dot{V}O_{2max}$ test	Test 4: 3 hours post $\dot{V}O_{2max}$ test
Barometric Pressure (mBar)	1020	1019	1021	1020
O_2 (Vol %)	16.99 ± 0.01	16.98 ± 0.02	16.96 ± 0.04	17.04 ± 0.06
CO_2 (Vol %)	4.96 ± 0.04	4.97 ± 0.03	4.95 ± 0.05	4.96 ± 0.04
Flow rate 0.5 L/s	2.88	2.86	2.86	2.87
Flow rate 1 L/s	2.89	2.90	2.91	2.90
Flow rate 3 L/s	2.98	2.96	2.96	2.97

mBar = millibar, Vol = volume

4.1.1 MetaLyzer® 3B Operation Procedure

For chapters 5 to 8 prior to participant arrival calibration of the MetaLyzer® 3B was performed. Following the warm up of the MetaLyzer® 3B gas and flow sensor calibrations were made. The participants personal details (mass, height and date of birth) were logged into the software accordingly and the sensor adjusted in preparation for data acquisition. Prior to exercise commencing the face mask (Hans Rudolph, USA) was placed on the participant and checked for fit to ensure that the mask was sealed to the skin appropriately. The turbine volume transducer was then attached and the participant was allowed to become accustomed to wearing the unit for approximately 2 minutes before the start of exercising.

4.2 Cycle Ergometer – Lode Excalibur Sport

This ergometer has a workload range of 8-2500 watt and is versatile due to its ability to change the workload in small adjustments of 1 watt or larger and with a low start up load of 7 watts. The workload accuracy is <100 W ± 2 W, 100 – 1500 W ≤ 2 % and <1500 W ≤ 5 % (Lode, n.d).

To adapt to frame sizes the handlebars adjust in length and height, as does the saddle with an angle adjustment too. These values for handlebar and seat positioning were stored for each participant and used again between tests to help with reliability and repeatability of the experiment. The participants positioning was according to comfort and stature.

Earnest and associates (2005) examined the reliability of the Lode Excalibur Sport in a test re-test experiment beginning at 100 W and increasing by 50 W every three minutes to exhaustion. No significant differences were found across outcomes of time to exhaustion, peak power output, peak heart rate and ventilatory thresholds. There were also low CV (≤ 5 %) at each test stage. However, this sequence of testing by Earnest and associates (2005) is more likely concerning participant reliability of how individuals can maintain the requested cadences, more than ergometer reliability. This is also the case for other studies such as Driller (2012), where the Lode ergometer is used and the outcome is it is a reliable method, but again the testing appears to be more about the participants maintaining cadences than ergometer reliability.

After contacting Lode directly, an email response from a product specialist informed that regrettably the company did not have any publications about the reliability and validity of

the Excalibur and reported the following details. The company report that the Excalibur is extensively used as the standard for Ergometry due to being furnished with an eddy current braking mechanism and Lode is producing and using dynamic calibration (unlike competitors). Dynamic calibration measures the load at the crank axle of the ergometer and considers mechanical losses that during a static calibration will never be measured (static calibrations remove electronic drift within factory specifications). Thus, dynamic calibration demonstrates the behaviour of the load at the crank axle at all different pedalling speeds whereas static calibration only provides good impression of electronics. The advantage to using an eddy current braking system accompanied with a copper disk is that a built-in feedback signal with strain gauges (to check brake torque) is not required. The feature of the used copper disk will not change at all and its behaviour is stored into the CPU board of the ergometer. Temperature may affect braking therefore during static calibration temperature sensors are checked. Lode calibrates every ergometer that leaves the company before shipment (and calibrations are performed at regular intervals by Lode). Load is measured on the crank axle and by using this method the workload measured is the workload the test subject has to cycle (and there is no time lag in adjustment of workload). Lode's unique selling point is that it is RPM independent in the full pedalling speed range.

4.3 Cardiac Function – Physioflow®

The Physioflow® (PF05L1, Manatec, Petit-Ebersviller, France) uses trans-thoracic bio impedance technology to determine (aortic) blood flow and rate through the heart. Trans-thoracic bio impedance technology computes mechanical activity as opposed to electrical activity (ECG). The Physioflow® is non-invasive and by using trans-thoracic bio impedance in association with ECG, cardiac function information is provided. A low magnitude (4.5 mA peak to peak) high frequency alternating current (66 kHz) is transmitted and received through the thorax area to monitor functions such as heart rate, stroke volume and cardiac output at a sampling rate of 1000 Hz. To achieve this, six electrodes (Skintact® FS50) creating a three-dimensional triangulation around the heart are placed near the carotid artery (two at the left side of the neck), mid sternum, the rib nearest to the left ventricle, left of the spine in line with the xyphoid process and another directly below this one. The signals are transmitted to a computer for data acquisition and analysis. Prior to the electrode placement, skin should be prepared with an abrasive gel. To ensure test retest reliability not only is skin preparation essential (shaving of the area and abrasive gel) but electrode gel should be used and placement of pads should be measured and marked to allow for the subsequent test. Additionally, to ensure test reliability calibration is needed

for signal stability. Calibration begins after electrode placement with the participant resting, still and calm. Part of this process includes blood pressure input, which is used for blood pressure profile during exercise and as a reference to cardiac output ($\dot{Q}$) computation (Physioflow®, 2016). Where haemodynamic parameters are concerned blood is the most conductive tissue in the thorax, and thoracic electrical impedance is sensitive to (pulsatile) blood volume and velocity changes in the aorta (in association with ventricular function) (Physioflow®, 2016).

Varying authors have researched the reliability of the Physioflow®. Ruddy and colleagues (2001) observed $\dot{Q}$ on 12 participants, each performed two 1 minute step incremental exercise tests. Findings showed a mean difference of -0.009 $l \cdot min^{-1}$ and 95 % confidence intervals of -4.2, 4.2 $l \cdot min^{-1}$. When compared with the direct Fick method (obtained from the second exercise test) correlation coefficient was $r = 0.94$ ($P < 0.01$). A constant exercise test comparing $\dot{Q}$ (Physioflow®) and the CO_2 rebreathing technique produced high correlation coefficients between the two techniques $r = 0.85$ and a mean difference of 0.06 $l \cdot min^{-1}$ (Tordi at al., 2004). Welsman and associates (2005) recruited twenty children to cycle to volitional exhaustion three times, separated by a week apart each time to test the Physioflow® reliability. For peak $\dot{V}O_2$, $\dot{Q}$ and stroke volume the coefficient of variation was 4.1, 9.3 and 9.3 % respectively, with the authors concluding that the Physioflow® reliability fell between that exhibited by CO_2 rebreathing method and echocardiography. Additionally, it offered advantages of lower expense, portability and less technical expertise being required. Methods for measuring $\dot{Q}$ such as dye dilution, thermodilution, doppler-echo-cardiography and CO_2 rebreathing require experienced operators in comparison to Physioflow® and furthermore the first two methods mentioned are invasive.

4.3.1 Physioflow® Operation Procedure

For chapters 6 and 7, in accordance with the manual (Physioflow®, 2016), on arrival to the laboratory (or prior to arrival) the participant had the areas for electrode placement shaved if applicable. The electrode sites were wiped thoroughly with an alcohol swab, dried and then the skin rubbed with an abrasive gel (Nuprep, Weaver, USA). The skin turned pink and this is required, as it shows good skin preparation. The placement of the electrodes were as previously described in the equipment specification section (4.3) and as depicted in the photos shown (Figure 4.2). The gel pads should contact the skin first and by pushing down firmly air pockets should be eliminated. The cables that connect to the electrodes were firmly attached to each participant by sports injury type tape and elastic bandage around the torso if needed to prevent movement whilst cycling.

Figure 4.2: Electrode colour and placement for Physioflow® operation, as depicted by the top two photos. The bottom three photos are the actual electrodes in position. Permission from participants was granted for use of the photos.

To calibrate the participant stayed relaxed, still and silent whilst sitting on the ergometer. The calibration was performed over 30 heartbeats and following this blood pressure values were taken for continuation of calibration. For reliable blood pressure proceedings refer to page 71, section 4.7.1.

Acceptable calibration values (Figure 4.3) should be between 64.6 (low) and 120 ml (high) for stroke volume, 50-90 bpm for heart rate, 4.6-7.8 $l \cdot min^{-1}$ for cardiac output ($\dot{Q}$), 2.5-4.2 $l \cdot min \cdot m^2$ for cardiac index (CI), 0-67 % for early diastolic filling ratio (EDFR), 1337-2483 $dyn \cdot s/cm5 \cdot m^2$ systemic vascular resistance index (SVRi) and 724-1345 $dyn \cdot s/cm5$ systemic vascular resistance (SVR). Once calibration was satisfactory, real-time monitoring was ready for data acquisition, with the main default screen presenting SV, HR, $\dot{Q}$ and CI (which is $\dot{Q}$/body surface area) (Figure 4.4).

Figure 4.3: Acceptable Physioflow® calibration values recorded in the laboratory at ARU.

Figure 4.4: Main Physioflow® default screen post calibration.

4.4 Haemoglobin & Haematocrit Analysis – EKF Hemo Control Analyzer

The EKF Hemo Control Analyzer (EKF Hemo Control, EKF diagnostic, Germany) is fundamentally a photometer, which measures solutions colour intensity and uses the cyanmethemoglobin reference method. This is founded on the transformation of Hb to a stable coloured complex, which is azide-methemoglobin (Vanzetti, 1966). Azide-methemoglobin has an almost identical absobance spectrum to haemiglobincyanide (HiCN), which is the chosen reference method of the International Committee for Standardisation in Haemotology (ICSH) for measuring haemoglobin (ICSH, 1996). All new Hb screening methods are judged against this committee.

Capillary blood (~10 µl) is collected onto a disposable cuvette, which acts as a receptacle for the reactions to take place. The method involves sodium desoxycholate dissolving the RBC walls, which makes Hb obtainable for analysis. Sodium nitrite then oxidises Hb (Fe^{2+}) to methhaemoglobin (Fe^{3+}). Following this sodium azid ions and methhaemoglobin form azide-methemoglobin, a stable coloured complex. It is this complex that is measured via photometrics in the spectrometer (Hemo Control) at a wavelength of 570 nm (EKF, Hemo Control Analyser Presentation, n.d). Haematocrit values on the Hemo Control are obtained from the Hb result with the factor 2.94 (this represents that Hct is 2.94 times the value of Hb).

The Hemo Control is factory calibrated, but in order to check standards control cuvettes and liquid materials that detect low, normal and high readings are used, which are made of bovine hemolysate. The manufacturer advises that although not compulsory the control cuvette can be used as quality control prior to use each time. The liquid low, medium and high controls should be checked weekly and they provide standards of 8.0 ± 0.3, 11.9 ± 0.5 and 16 ± 0.6 $g{\cdot}dL^{-1}$ respectively. Once open the liquid controls have a shelf life of 30 days. For accuracy and repeatability in-house testing (at Anglia Ruskin University in Cambridge) with each control being tested 10 times. This produced the following results: low control, 8.0 ± 0.08 $g{\cdot}dL^{-1}$, CV 1%; medium control 11.98 ± 0.06 $g{\cdot}dL^{-1}$, CV 1% and high control 15.97 ± 0.08 $g{\cdot}dL^{-1}$, CV 1%.

In order to confirm reliability of the device and the tester two volunteers had venous blood drawn. This was to avoid having fingers pricked multiple times, which would be painful and invasive, and also as the capillaries from fingers could be drawn from arterial or venous origins, which could convey varying results. Immediately blood from the collections was pipetted onto a surface to simulate a capillary sample. Each sample was tested 10 times

using the cuvettes supplied. The results were 14.14 ± 0.07 $g \cdot dL^{-1}$, CV 0.00 and 14.98 ± 0.06 $g \cdot dL^{-1}$, CV 0.00.

The device correlates (≥ 0.98) with the National Committee for Clinical Laboratory Standards and claims to have imprecision of CV < 2 %. In recent evaluations by Singh and co-workers (2015) the Hemo Control was shown to have 86.8 % sensitivity compared to 98.1 % for DiaSpect Hb T system and 71.1 % for NBM-200. In terms of intraclass correlation coefficients Hemo Control was 0.77 and DiaSpect Hb T system and NBM-200 were 0.78 and 0.43 respectively.

Using a spectrophotometer (Jenway 6305, Bibby Scientific, UK) in house at ARU a calibration curve (Figure 4.5) was prepared using Drabkin's reagent (a solution consisting of sodium bicarbonate, potassium ferricyanide and potassium cyanide) and the bovine hemolysate control liquids (of known values 8.0 ± 0.3 (low), 11.9 ± 0.5 (medium) and 16 ± 0.6 (high) $g \cdot dL^{-1}$) for the colorimetric determination of Hb concentration. Each specimen for the calibration curve was prepared according to the product information leaflet for Drabkin's reagent (Sigma-Aldrich, Saint Louis, USA) and triplicate readings of absorbance were recorded from the spectrophotometer and averaged.

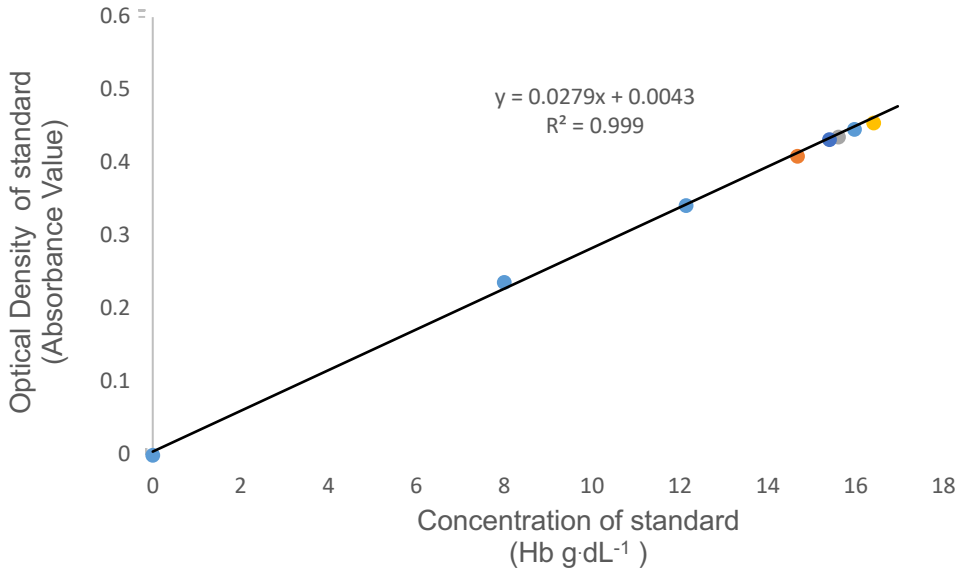

Figure 4.5: The calibration curve for colorimetric determination of Hb concentration is shown with the blue circles. The purple, red, turquoise and green circles represent the venous samples taken from the volunteers and placed onto the calibration curve.

Venous blood was withdrawn from four volunteers to obtain Hb readings from the Hemo Control and additionally mixed with Drabkin's solution accordingly for absorbance values.

Triplicate readings of absorbance values were recorded and averaged to determine Hb concentration from the calibration curve. Rearrangement of the trendline equation to establish the Hb value allowed for comparisons of the value of Hb given from the Hemo Control. The volunteers' values for Hb from the Hemo Control followed by the respective trendline equation value from absorbency were 14.66 and 14.51, 15.6 and 15.46, 16.4 and 16.17, 15.4 and 15.34 $g \cdot dL^{-1}$. All the pairs of values are very comparable with the largest percentage change being 1.40 %. The mean value for the Hemo Control results and the corresponding trendline equation values were 15.51 ± 0.62 $g \cdot dL^{-1}$ and 15.37 ± 0.59 $g \cdot dL^{-1}$ respectively, these values gave a Cohen's *d* effect size of 0.23. This small effect size indicates that the in house Hemo Control unit at ARU produces very comparable results to the calibration curve, indicating reliability.

For definitions of chemicals used in the EKF Hemo Control Analyzer see in section for definitions (page xvi).

4.4.1 Haemoglobin & Haematocrit Sampling Procedure

In chapter 5, capillary blood was examined prior to the incremental test and or 1-2 hours prior to BD and 3 hours post BD. In the further experimental chapters, capillary blood was examined prior to the commencement of all tests and on the morning prior to BD.

Using pre-calibrated equipment (EKF Hemo Control, EKF diagnostic, Germany) capillary blood from the 3rd finger (right hand) was examined for haemoglobin and haematocrit levels, with the participants first relaxed and seated for 10 minutes to gain equilibrium and avoid postural pseudoanaemia issues. Niwinski, (2009) suggests the recommended site for capillary collection is the end of the third or fourth finger and slightly off centre. These fingers tend to be chosen, as the little (5^{th}) finger has thinner tissue and the index (2^{nd}) finger and thumb can be prone to calluses, which may hamper blood collection. The sides and tips should be avoided, as the tissue is half as thick compared to the centre. The procedure for capillary blood collection was as follows: with the participant sitting, the chosen finger was cleaned with 70 % isopropyl alcohol (alcohol/disinfectant swab packets) and allowed to air dry. The first 2 - 3 blood drops were wiped away with a dry tissue/gauze to remove the platelet plug and tissue fluid. The blood was collected with the tip of the cuvette, ensuring that the blood drop filled the cuvette in one continuous process. If there was excess blood on the outside of the cuvette, the outside was wiped.

4.4.2 Pilot Study – Determining Optimal Time Frame for Hb Decrement

Prior to data collection for chapter 5 a pilot study was conducted to establish the optimal time that would display the most significant Hb decrement following BD. Three female volunteers (35.3 ± 15.0 years, 60.9 ± 4.7 kg, 169.2 ± 6.5 cm) who donated blood at Cambridge Donor Centre had capillary samples taken from their fingertips using the Hemo Control (EKF diagnostic, Germany) every hour for 7 hours immediately post BD.

The pilot results were as follows; prior to BD Hb values were 14.77 ± 0.67 $g{\cdot}dL^{-1}$, post BD values were, 13.63 ± 0.90 (1 h), 12.80 ± .1.31 (2 h), 12.75 ± 0.21 (3 h), 12.77 ± 1.55 (4 h), 13.60 ± 1.06) (5 h), 12.53 ± 0.23 (6 h) and 13.10 ± 0.36 (7 h) $g{\cdot}dL^{-1}$. The mean value for pre-BD against the post BD values gave Cohen's *d* effect sizes of 1.35 (1 h), 1.83 (2 h), 3.93 (3 h), 1.62 (4 h), 1.24 (5 h) and 4.33 (6 h). All are large effects with greatest effects seen post 3 and 6 hours. Based on the results, it was decided that the three-hour post BD values would be used. Although the six-hour window had a slightly larger effect, for ease of the participants and logistics of visits to ARU the three-hour window was deemed to be most suitable.

4.5 Lactate Analyser – EKF Biosen C-Line

For lactate analysis, 20 µl of blood is drawn from the fingertip for assessment of capillary blood. The sample is drawn up through a micro-capillary tube and placed into a pre-filled (with haemolysis solution) "safe lock" reaction cup (1000 µl) (Eppendorf, Germany), which eliminates manual dilution. The sample is gently agitated in order to mix and haemolyse the sample. Once placed into the analyser the sample is converted enzymatically, following a short flush the system is ready for another sample. Calibration is automatic with a solution of a known concentrate (12 mM), which is used as base for unknown concentrations (EKF, 2013). The results are available in 20-45 seconds. The lactate analyser has a measuring range of 0.5 – 40 mM. The manufacture claims lactate imprecision of CV ≤ 1.5 % (12 mM) (EKF, 2013).

The Biosen C-Line lactate analyser works on the principle of the enzymatic-amperometric method using chip-sensor technology, with the chip sensor being responsible for converting the sample concentration into an analysable, electrical signal (EKF, 2013). An amperometric biosensor uses the current created when an oxidation/reduction reaction ensues at an electrode. The current response produced in that reaction is proportionate to the strength of that species (lactate) in the sample, with concentrations determined relative

to a calibration solution (of known concentration) (EKF, 2013; Rathee, et al., 2016). Thus, functioning is from measurement of the current potential from the working and reference electrodes, resulting from electrocatalytic oxidation or reduction of the electroactive species involved. The current magnitude is directly correlated to the concentration of the product in the enzymatic reaction. The enzyme in enzymatic-amperometric should have the facility to catalyse a reaction encompassing the analyte with the consumption of an electroactive reactant or/and the construction of an electroactive product. The process then gives a measurement of the analyte strength (Rathee, et al., 2016). The products of the reaction (for lactate) are pyruvate and hydrogen peroxide, with the hydrogen peroxide detected at the electrode. Following each measurement, the chip sensor is automatically cleansed, which washes out the old sample matter and thus prepares for the subsequent sample (EKF, 2013).

4.5.1 Lactate Sampling Procedure

Using pre-calibrated equipment (Biosen C-Line, EKF diagnostic, Germany) capillary blood from a finger was examined for lactate levels.

Resting lactate

At the commencement of all laboratory testing resting blood lactate was taken at the same time as the Hb and Hct. The lactate sample was taken immediately after the Hb and Hct sample, by drawing the blood into a 20 µl micro-capillary tube (containing haemolyzing solution). The filled tube was placed into a reaction cup for analysis.

During and Post exercise end lactate

Immediately upon test termination for chapter 5 the tester wore surgical gloves and the finger of the participant was cleaned and punctured suitably. The sample was taken accordingly for analysis. The wound was covered and pressure applied to the wound and contaminated equipment disposed of accordingly.

For chapters 6 and 8 blood lactate was collected at the beginning of baseline and at the end of each 6 minute trial and at the end of all HIIT trials. The procedure for taking the blood lactate was as previously described.

4.6 Hydration Analysis – Eclipse Refractometer

Urine specific gravity is the density of a urine sample paralleled with the density of water (Oppliger and Bartok., 2002). Specific gravity conformity is established by using a pre-calibrated refractometer (Eclipse, Bellingham and Stanley, UK) (Figure 4.6). It is used with urine samples to check hydration levels. The refractometer is pre-calibrated with the use of distilled water and has a range of 1.000-1.040 U_{sg}. Refractometry passes a beam of light through a urine sample and measures how much the beam becomes refracted, thus the concentration/colour of the sample influences the result (Oppliger and Bartok., 2002). According to Casa, et al. (2000) a position statement from National Athletic Trainers' Association (NATA) a value of ≤1.020 is the upper range of acceptable.

Figure 4.6: The Eclipse refractometer.

4.6.1 Specific Gravity Conformity

Upon arrival to the laboratory for each experimental visit, a sample bottle was provided to the participants. The participant provided a mid-flow sample of urine (2nd void of the day) for testing of hydration status. The refractometer (Eclipse, Bellingham and Stanley, UK) was pre-calibrated to zero with the use of distilled water and sharp focus of the instrument was ensured prior to taking readings by rotating the eyepiece. While wearing gloves the operator pipetted a small sample of urine onto the glass window of the refractometer. If the reading when looking through the eyepiece was not sharp, then the volume of the sample may have been insufficient, a larger volume of urine will rectify this problem. The detection of normal hydration range (1.002 to 1.030 U_{sg} (Dirckx, 2001)) was desired. Following analysis, the sample bottle was disposed of in a bio-hazard bag and the refractometer cleaned and sterilized.

4.7 Blood Pressure and SpO_2 Measurement – Nonin 2120

Blood pressure (BP) and SpO_2 were measured in all experiments with the Nonin 2120 (Nonin, Medical Inc, USA). The Nonin 2120 is a portable non-invasive BP monitor and pulse oximeter with LED display. Its functions can be used simultaneously or individually (Nonin, 2014). The Nonin 2120 uses an oscillometric method whereby oscillation pulses in the artery wall are filtered and amplified to create oscillometric waveforms (Nonin, 2017).

BP measurement is non-invasive and provides insight into the pressure exerted on the blood vessels. Using an oscillatory monitor (on the upper arm), the cuff inflates to ~20 mmHg above systolic pressure for each individual. Once the cuff reaches the required inflation, no blood can flow through the artery. As deflation occurs, moving below systolic pressure, blood once again begins to flow, which produces vibrations in the arterial wall. When the cuff pressure falls below the diastolic pressure, blood resumes to a smooth flow without any vibrations. Vibrations transpire when cuff pressure is high enough to make the blood push the arterial wall in order to be able to flow within the artery. Vibrations are conveyed from the arterial wall to the air in the cuff and then to a transducer in the monitor, which transfigures quantities into electrical signals (Berger, 2001).

There are issues that can occur when taking BP, as it can be influenced by things such as stress, incorrect body positioning and poorly fitting cuffs. Thus, a period of rest should occur prior to measurements being taken and any conversation avoided to ensure complete relaxation with the participant seated upright in a chair, ideally with both feet on the floor. Furthermore, the arm should be supported (on a surface) so the muscles in the arm can be relaxed. Although not an issue in this thesis as BP was not taken during exercise it should be noted that it can pose additional challenges due to the movement of the body and the difficulty in stabilising/locking the arm while exercise is taking place.

Peripheral oxygen saturation, also known as arterial haemoglobin saturation value (SpO_2) is an estimate of O_2 in the blood, as sensed by a pulse oximeter. Utilising a sensor placed on a digit, pulse oximetry is non-invasive and continuous way to measure oxygen saturation. The sensor uses two wavelengths of light (red at 660 nm and Near IR at 940 nm (Chan, Chan and Chan., 2013)) that correspond to saturated and unsaturated oxyhaemoglobin and a photodetector (Kilbourn, 1989). Pulses of infrared and visible red light are emitted and the oxygenated and deoxygenated blood absorb varying light quantities; deoxygenated haemoglobin absorbs more red light while oxygenated haemoglobin absorbs more infrared light. A ratio is calculated by the machine, thus

providing an arterial oxygen saturation (SpO_2) estimate (Andrews, 2008), which refers to the quantity of oxygenated haemoglobin.

Normal SpO_2 is reflected as 97 ± 2 % (Goldberg et al, 2012) when PO_2 is at sea level, which is 100 mmHg. Thus, SpO_2 readings will be affected by atmospheric pressure, which is a function of barometric pressure. For example, at altitude the saturation value is lower due to a decrement in barometric pressure.

There are factors that may give inaccurate readings; these include the following according to World Health Organisation (WHO (2011a)):

- Nail varnish, as the colour may absorb the light emitted.
- Pigment in the digit, as the pigment will block the signal, although a pulse will be detected.
- Bright light on the probe, which may interfere with the light detector. Covering the sensor with opaque material can be done or simply avoid direct bright light. However, Fluck and colleagues (2003) are of the opinion that ambient light has no statistical significant effect.
- Patient movement (movement artefact) this could be from issues such as the probe not secured or from the patient shivering.
- Poor perfusion is usually due to peripheral vasoconstriction from the cold or hypovolaemia, warming can help alleviate this problem. Schramm, Bartunek and Gilly (1997) reported that cold digits (< 20˚C) might read 99 – 100 % SpO_2 and warm digits (> 30˚C) can read 96 – 97 % SpO_2 despite the individual having the same PaO_2. This is probably due to arterio-venous shunts dependent on temperature in the periphery.
- Carbon monoxide poisoning cannot always be detected by an oximeter and the SpO_2 has a tendency to be exaggerated.

Pulse oximeter manufacturers validate their accuracy by being calibrated empirically on normal, warm (normothermic), healthy, young adult volunteers during desaturation research (induced hypoxia), and the claims are submitted to the Food and Drug Administrator (FDA) (Masimo, 2007). The accuracy of a device will be according to its clinical trials for example the Avant Nonin 2120 accuracy data was calculated using root-mean-squared values for all participants according to ISO 80601-2-61 standards (Nonin, 2014). It has a saturation accuracy (% SpO_2 + 1SD) when in the 70 – 100 % range of ± 2 % in adults and paediatrics under the conditions of motion, no motion and low perfusion.

This testing was performed under the gold standard for accuracy testing with the OSM-3 Multi-wavelength Co-oximeter (Nonin, 2004).

Calibration is via CO-oximeter and uses arterial blood samples taken at exact times as indication values for the pulse oximeters calibration. There is however bias, as CO-oximeters may differ by up to 2-3 %, for this very reason there may be variability between pulse oximeters depending on which CO-oximeter was used for calibration. Also, each manufacturer will have their own specification due to different sensors and oxygen saturation. This results in variability amongst manufactures between 1-3 % (Nonin, 2016). The accuracy has to be reported via root mean squared (A_{rms}) in accordance with the FDA (Masimo, 2007).

4.7.1 Blood Pressure and SpO_2 procedure

With the participant sat on the cycle ergometer, after ~5 minutes of relaxation upon arrival at the laboratory, a resting assessment of blood pressure (BP) (Nonin, 2120, USA) post Hb capillary blood sampling was taken. BP was measured using the left arm, with the arm resting on a firm surface and elbow approximately at heart level. The cuff was wrapped around the arm snugly with the artery marker pointing to the brachial artery. Three measurements were taken with 60 s rest between each one and the average value recorded.

SpO_2 (Nonin, 2120, USA) was recorded at the same time as BP (but on the right-hand side of the body). Caution was taken to avoid movement artefact and electromagnetic interference from equipment such as mobile phones. The participant's hands were clean and fingernails free from nail varnish, to avoid value interference (WHO, 2011a). Additionally, if the participant hands were cold, fingers were warmed (by having hands placed into a bucket of warm water) to induce blood flow prior to the sensor being placed on a digit (WHO, 2011a).

4.8 Near-Infrared Spectroscopy (NIRS) – Portamon

NIRS relies on the relative transparency of tissue to light/photons in the near-infrared region (NIR) range and oxygenation dependent light absorbing characteristics of Hb. The Portamon (Artinis Medical Systems, Netherlands) (Figure 4.7) is a wearable, wireless NIRS unit for muscle research, which uses the modified Beer-Lambert law and spatially resolved spectroscopy (SRS). The modified Beer-Lambert law is where photon scattering

in tissues is corrected by a pathlength factor (often a fixed value of 4), because biological tissue is not consistent (Jones, et al., 2016) to calculate relative concentration changes in Hb concentrations. SRS is used to calculate absolute concentrations (Artenis, 2017), it is a multi-distance method that uses multi-distance algorithms to measure from two or more tissue depths and encompass the diffusion equation to deliver a percentage absolute tissue saturation index (TSI %) calculation, also referred to as tissue oxygenation index (TSI) (Jones, et al., 2016).

NIRS is a non-invasive method to observe changes in oxy and de-oxy Hb/myoglobin, but it is not possible to distinguish between Hb and myoglobin (chromophores), due to the spectrum, overlap (Shadgan, et al., 2009). In physiology, the main chromophores of study are oxygenated Hb and de-oxygenated Hb (O_2Hb & HHb), with both showing different absorption characteristics, as does myoglobin. According to Ferrari, Mottola and Quaresima (2004) the Hb measurement is not affected by myoglobin as the contribution from it is minimal. Evidence of the physiological aspects of the muscle chosen for investigation can be observed in the chromophores from performing movement, contracting the muscle or from inducing an occlusion.

The Portamon uses three paired light emitting diodes (also called optodes) that are positioned 30, 35 and 40 mm from the detector (the source detector distance) and of which have standard waves lengths of 760 & 850 nm for NIR light source. Light transmits through the skin when the Portamon is placed on the epidermis; it then disperses back and is acquisitioned by the receiver. The sampling rate is 10 Hz for data acquisition, which is connected to a computer (for visual display) via Bluetooth technology with a ~30 metre broadcast range.

Figure 4.7: The Portamon device, which measure 83 X 52 X 20 mm. The left hand picture shows the 3 light emitting diodes/optodes and the detector/receiver.

Skinfold thickness at a site should be determined prior to probe application to the participant. This is to ensure that the measurement depth is roughly half the distance between the transmitter optode and receiver. Monitoring the area of choice depends on how the NIR penetrates the tissues, the NIR forms a banana/elliptical type (Strangman, Li and Zhang, 2013) shaped curve between the emitter and the sensor (Figure 4.8).

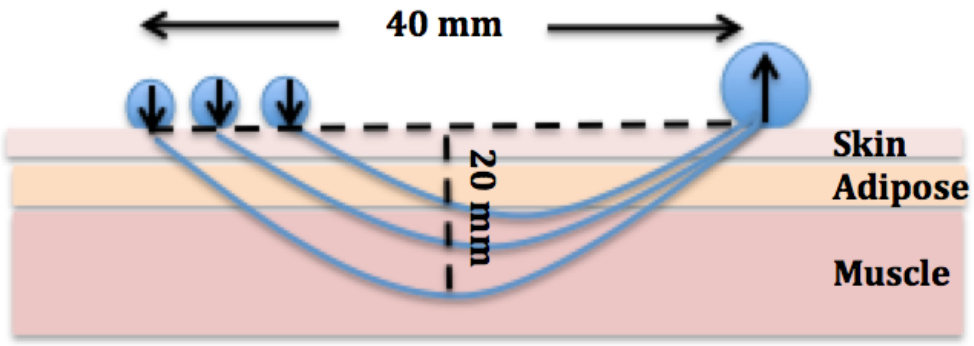

Figure 4.8: A schematic of the elliptical curves from NIRS. The trio of blue circles (left) represent the light emitting diodes (with the black arrows indicating light transported into the tissue). The larger circle (right) represent the detector (with the black arrow indicating light transported out of the tissue). The values shown illustrate the penetration depth of NIRS is limited to half the distance between diodes and detector i.e. 40 mm gives a penetration depth of 20 mm and thus 30 mm would provide 15 mm penetration depth. It should be noted that the limited penetration depth lends experiments and investigations to be completed on participants with minimal adiposity (Van Beekvelt, et al., 2001), as subcutaneous fat affects absolute readings. Hamaoka, et al. (2011) imply that 5 mm of overlying fat diminishes the signal concentration by approximately 20 %, with a light source to detector separation of 30-40 mm.

Values for oxy Hb/myoglobin concentration (O_2Hb + Mb) and deoxy-Hb/myoglobin concentration (HHb + Mb) are reported as a change from baseline in micromolar-centimeter units (μM.cm). Baseline is a measurement taken by the NIRS device for two-minutes as recommended by the manufacturer (30 s averaging). The variables of interest are O_2Hb, HHb and total haemoglobin (tHb), which results from the sum of O_2Hb and HHb. Variations in tHb reflect variations in BV from NIRS. Haemoglobin difference (Hb_{diff}) is another variable obtained via subtracting HHb from O_2Hb, it is used to acquire a better signal to noise ratio and is often referred to as oxygenation index (TSI) (Van Beekvelt, 2002).

Comparisons with other measures such as electromyography (Yada, et al., 2008) or phosphorous magnetic resonance spectroscopy (Sako, et al., 2001) summarise the validity of NIRS. Van Beekvelt (2002) suggests that most studies cannot be paralleled with each other, due to the large diversity in protocols, such as varying exercises and intensity, and the time that $m\dot{V}O_2$ is determined. Although the Portamon is the instrument of choice for many studies, work on the validity and reliability of the Portamon appears to be elusive.

However, a study that assessed the reproducibility of NIRS using the Portamon during isotonic multi-joint resistance exercise demonstrated intra-test reliability with intra-test CV and intra class correlation coefficients ranging from 1.8 – 25.3 % and 0.75-0.98 respectively (Scott, et al., 2014).

4.8.1 Blood Loss and NIRS

Sufficiency of tissue oxygenation is a criterion of aerobic metabolism and it is possible to measure local oxygen availability instantaneously using NIRS. It has also been suggested that NIRS can be used as an alternative transfusion trigger during medical situations. Normovolaemic haemodilution is a technique for conserving blood, whereby blood is removed from the patient shortly after being anesthetised (Shander and Silvergleid, 2020), BV is maintained with solutions and the blood is restored post operation. In 30 patients acute normovolaemic haemodilution was used and by utilising NIRS significant ($P < 0.001$) falls in cerebral and peripheral (gastrocnemius) oxygenation were observed (Torella, Haynes and McCollum, 2002). In this particular research, 450 ml of blood were removed via acute normovolaemic haemodilution over 30 minutes reducing Hb from 14.5 ± 1.36 to 10.8 ± 0.94 $g \cdot dL^{-1}$. Oxygen demand in striated muscle reduces during anaesthesia (Torella, Haynes and McCollum, 2002); conversely under exercising conditions oxygen demand in striated muscle increases. Exercise is proposed to be used in studies within this thesis therefore it is possible that the outcome of detecting statistical significance via NIRS may be different as the oxygen demand is converse to Torella and associates (2002) research. Additionally, these patients were sick (thus undergoing surgery), but work from Torella and contemporaries (2002) saw statistical significance ($P < 0.001$) in 10 healthy individuals pre to post (10 minutes) BD (470 ml) while monitoring the gastrocnemius using NIRS. Furthermore, the studies observed pre to post BD within a short time frame, whereas this thesis has an intervention period post BD of 24 h or more.

NIRS appears to play an important role regarding medical critical care, such as in heart surgery (Edmonds, 2006) and in resuscitation for tissue hypoxia a precursor to shock (Ward, et al., 2006). Away from the field of medicine NIRS has also been used to study the muscle oxygenation in various sports such as football (Brocherie, Millet and Girard, 2015), skating (Born, et al., 2014), running (Buchheit, et al., 2011) and many more. However, it appears that studies using NIRS concerning exercise together with BD are illusive, therefore providing scope for research, as it would be useful in identifying any changes in oxy and de-oxy Hb/myoglobin following BD.

4.8.2 NIRS procedure

Prior to arrival at the laboratory or upon arrival at the laboratory, the area for NIRS application was clean-shaven. Using Harpenden skinfold calipers (British Indicators Ltd, Burgess Hill, UK), skinfold thickness at the site of the NIRS optodes placement was determined prior to probe application to confirm that the measurement depth was approximately half the distance between the transmitter optode and receiver. The value was acquired and divided by two to establish the adipose tissue thickness (fat + skin = ATT) (Van Beekvelt, et al., 2001).

Prior to probe use to ensure waterproofing and protection from humidity the Portamon was wrapped in clingfilm (transparent food wrap). The Portamon was placed in the same position at every test, on the right gastrocnemius muscle, on the right-hand side of the muscle belly and at the point of the largest circumference of the muscle. After placement of the device permanent maker pen was drawn onto the skin surrounding the Portamon to enable duplication of the location for further testing and the area checked post testing for sliding (no sliding/movement was noted during testing). The participant was asked to maintain the pen marks for the duration of the testing period. The probe was covered by dark material to avoid corruption from ambient light and elastic bandage held the device in place.

For chapters 6 to 8 data was collected simultaneously. After Portamon placement, a rest period of approximately 3-4 minutes took place while the participant was positioned at rest with their right leg extended on the ergometer, during which time blood pressure readings were being taken by other equipment. Following this time, data acquisition began; a baseline value was recorded with the participant's leg in the extended position for a period of 2 minutes. The participant then commenced cycling for 4 minutes at an unloaded wattage (15 W). The Portamon continued monitoring throughout all square wave transitions, rest periods (of unloaded wattage) and HIIT trials.

The variables recorded included O_2Hb, HHb and total haemoglobin (tHb). TSI (TSI= O_2Hb-HHb) was also calculated for comparison between subjects (Jones, et al., 2016). NIRS data was gathered with Oxysoft (Artinis Medical Systems, Netherlands) with a frequency of 10 Hz and expressed relative to baseline. Data was placed into 1 s time bins followed by 10 s moving averages.

4.9 Maximal Exercise Testing ($\dot{V}O_{2max}$)

Maximal exercise testing ($\dot{V}O_{2max}$) procedure was used for chapters 5 and 6. Prior to the incremental test, the participants completed a self-selected warm-up. Each participant completed an incremental graded exercise test to volitional exhaustion, on a pre-calibrated electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) to determine $\dot{V}O_{2max}$. With the participant choosing a constant cadence between 75 and 95 rpm, the test increased linearly at a rate of 0.42 $W \cdot s^{-1}$, commencing at a constant workload of 100 W (males) or 50 W (females) for the first 60 s. The test concluded when the cadence declined by > 5 rpm from that which was self-selected or when volitional exhaustion was reached. Verbal encouragement was regulated and only provided when RER was ≥ 1.0.

A pre-calibrated metabolic cart/CPX system with ergospirometry (Cortex MetaLyzer 3B, UK) collected gas variables ($\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$ and RER) on a breath-by-breath basis. Heart rate (HR) was recorded continuously telemetrically (Polar 810s, Electro, Kemple, Finland).

Following a 5-minute active recovery subsequent to the incremental test, a verification procedure (Thoden, 1991) ensured a maximum effort was given from the participant. A lower $\dot{V}O_2$ should be produced than the incremental test, Midgely, McNaughton and Carroll (2006) suggest that the verification value should be ≤ 2 %. The verification starts at 25 W higher than the final incremental test wattage until exhaustion. A principle criterion for $\dot{V}O_{2max}$ is a plateau in the $\dot{V}O_2$ despite an increasing workload. Maximum heart rate (220-age ± 10 $b \cdot min^{-1}$), RER ≥ 1.10 and peak blood lactate (pBLA) ≥ 8.0 mM are secondary criteria (Howley, Basset and Welch., 1995) used to assist determination of maximal exertion. Additionally, $\dot{V}O_{2max}$ plateau was confirmed over the final minute by the Δ $\dot{V}O_2$ over the final two consecutive 30 breaths sample periods, accepting the plateau as ≤ 1.5 $ml \cdot kg^{-1} \cdot min^{-1}$ (Doherty, Nobbs and Noakes, 2003; Lucia, et al., 2006).

4.10 Determining Gas Exchange Threshold (GET)

GET was determined using three methods. Firstly, when $\dot{V}CO_2$ surpassed $\dot{V}O_2$ (also known as the excess carbon dioxide method) and can be communicated as, ($\dot{V}CO_2^2$ / $\dot{V}O_2$) – $\dot{V}CO_2$ (Volkov, Shirkovets and Borilkevich, 1975). Secondly, GET was ascertained via the intensity responsible for the initial increase in $\dot{V}E/O_2$ without a concurrent rise in $\dot{V}E/CO_2$ (Davis, Whipp and Wasserman, 1980). Thirdly, the V-slope method (Beaver, Wasserman and Whipp, 1986) was also adopted. When $\dot{V}O_2$ is plotted against $\dot{V}CO_2$ there is a point when the $\dot{V}CO_2$ increase is greater than the increase of $\dot{V}O_2$ (a representation of this can

be seen in Figure 4.9). The values of each method should be within 3 %; by combining these methods firm agreement can be attained (Gaskill, et al., 2001), as was the case in this thesis.

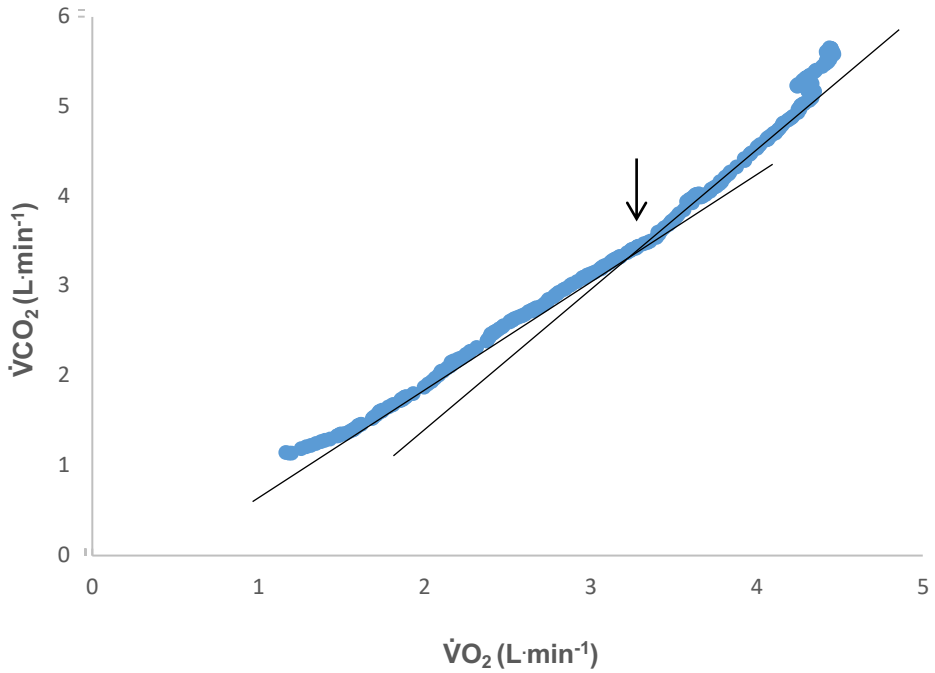

Figure 4.9: V-slope method, the arrow indicates the point of GET.

4.11 O_2 Kinetics Trials

The $\dot{V}O_2$ on-kinetics trials comprised three square-wave transitions of workload. Baseline pedalling was at 15 W, sustained for 4 minutes, to reduce noise in the breath-by-breath data, as this time allowed for participant adaptation to the ergometer and mouthpiece. Two transitions were applied in the moderate intensity domain and a third was in the heavy intensity domain.

4.11.1 Moderate Intensity Domain O_2 Kinetics Testing

The moderate domain was calculated as Δ50 % (50 % of the difference between $\dot{V}O_2$ at rest and GET). The participants exercised as follows:

1. Cycle for 4 minutes unloaded at 15 W to provide baseline data.
2. The square wave transition was then applied (this is time point zero), which took the participant from 15 W instantly to the moderate domain wattage. They cycled at this wattage for 6 minutes.
3. Recovery then took place for 6 minutes at 15 W.

4. The participant continued to cycle for 4 minutes again, as in step number 1.
5. Steps 2 to 4 were repeated.

4.11.2 Heavy Intensity Domain O_2 Kinetics Testing

The heavy domain was calculated as ∆20 % (20 % of the difference in $\dot{V}O_2$ between GET and $\dot{V}O_{2max/peak}$).

Continuing immediately on from step number 3 & 4 in the moderate domain step number 2 repeated again, but with the wattage for the heavy domain. Recovery followed step 3.

Figure 6.2 in chapter 6 (page 112) visually describes these testing processes.

4.12 Repeated Sprint Testing

Immediately following the O_2 kinetics testing 20 minutes rest ensued. Özyener, et al. (2001), demonstrated that it took up to ~20 minutes for $\dot{V}O_2$ to return to pre-exercise values following heavy intensity kinetics.

The repeated sprints comprised 90 s of unloaded (15 W) pedalling followed by 15 s of all out sprinting efforts. This was repeated 4 times and the load was set at a resistance of 7 % (0.07 $kg \cdot kg^{-1}$) of body mass.

Chapter 5: 2nd Experimental Chapter

The effects of Body Composition and Markers of Cardiorespiratory Fitness on Haematological Responses Post Blood Donation

5.1 Introduction

Donating a unit (~ 470 ml (NHS choices, 2014)) of blood removes 236 mg of iron in men and 213 mg in women (Abdullah, 2011). This is a 4 – 10 % decrease of iron levels, thus 10 % of red blood cells are lost (Rosvik, Ulvik and Wentzel, 2009), resulting in lower Hb levels, which can take between 6 - 12 weeks to replenish (NHS blood and Transplant, 2018). Agreeing with this, Pottgiesser, et al. (2008) found that the total amount of Hb loss of 75 ± 15 g (8.8 ± 1.9 %) was recovered after a mean of 36 ± 11 days (range: 20-59 days), with a 550 ml donation. This would decrease the oxygen carrying capacity of the blood (Burnley, et al., 2006; Gordon, et al., 2013) and the immediate effects of this are increased breathing rate, stamina is decreased, as energy generation is impaired due to insufficient oxygen from the blood to mitochondria (Wasserman, et al., 2005) to sustain oxidative phosphorylation and recovery time is prolonged in exercise. Consequently, the advice from the NHS blood and Transplant (2016d) is to avoid vigorous exercise (although quantification or explanation is not defined) or heavy lifting before and after donation, light exercise like walking is fine.

However, what is not fully addressed in the NHS public guidelines is that donation recovery could plausibly alter according to an individual's body composition or fitness status. For example, the larger the individual the bigger the BV and thus Hb carrying capacity could be different compared to a smaller individual and yet every donor has the same amount of blood removed (~ 470 ml). For example, in terms of sexes, a male and female both 170 cm and 65 kg will have a BV of 4499 and 4083 ml respectively according to the Nadler formula (Nadler, Hidalgo and Bloch, 1962), however the percentage of BV reduction following a BD will be 10.5 % for the male and 11.5 % for the female. Another example representing extremes of body size is a male who is 200 cm and 110 kg will have a BV of 7080 ml (Nadler, Hidalgo and Bloch, 1962), compared to the male in the previous example this is an increase in BV of 57.4 %. Therefore, determining how much Hb decreases post donation will aid in establishing effects according to body composition. Although body composition is perhaps generally known to describe fat percentages, in this instance mass and height are also considered, as they are variables for the calculation of BV.

Due to the apparent lack of research on the influence of body composition and previous fitness levels on BD, the aim of this study was to determine the effects of body composition and markers of cardiorespiratory fitness on haematological responses as a consequence of donating a unit (470 ml) of blood.

Null hypotheses:
(H_{01}): Haematological responses will not be altered from donating a unit of blood.
(H_{02}): Body composition will have no significant relationship to haematological responses as a consequence of donating a unit of blood.
(H_{03}): Markers of cardiorespiratory fitness will have no significant relationship to haematological responses as a consequence of donating a unit of blood.

Experimental hypotheses:
(H_1): Haematological responses will be altered from donating a unit of blood.
(H_2): Body composition will have a significant relationship to haematological responses as a consequence of donating a unit of blood.
(H_3): Markers of cardiorespiratory fitness will have a significant relationship to haematological responses as a consequence of donating a unit of blood.

5.2 Methods

5.2.1 Participants

A statistical power calculation (using ClinCalc.com, 2017) determined participant numbers by using previous research (Gordon, et al., 2010) results to compare two means for pre BD (14.23 ± 1.52 $g{\cdot}dL^{-1}$) and post BD (13.1 $g{\cdot}dL^{-1}$) Hb. A recommended sample size of 14 was calculated using a power of 80 % and critical value for normal distribution set at 1.96.

$$n = \frac{\sigma^2(Z_{1-\beta} + Z_{1-\alpha/2})^2}{(\mu_0 - \mu_1)^2}$$

Where n is the sample size (14), μ_0 is the population mean μ_1 is the mean of study population, σ^2 is variance in population, α is the probability of type I error (0.05) β is the probability of type II error (0.2) and $\mathcal{Z}$ is the critical $\mathcal{Z}$ vaule for a given $\alpha \, or \, \beta$.

Subsequent to ethical approval (Anglia Ruskin University, Faculty Research Ethics Commitee), 28 participants of differing activity levels (Table 5.1), 19 male (age 37.9 ± 14.7

years, mass 82.1 ± 10.3 kg, height 178.3 ± 6.0 cm) and 9 female (age 39.8 ± 14.8 years, mass 68.9 ± 8.3 kg, height 169.0 ± 3.9 cm), aged 18 – 65 years (38.5 ±14.5) of varying statures and ethnicities volunteered to participate. The volunteers provided written informed consent and were directed on their right to withdraw at any point. Inclusion criteria were that the volunteers needed to be between 18 - 65 years old, able to donate blood (at an NHS donation centre), be regularly active with no contra-indications and able to exercise at maximal intensity. Exclusion from the study included the inability to donate blood and appearance of contraindications to the exercise such as a virus or anaemia. Participants were requested to avoid strenuous exercise (defined as great exertion) 24 hours prior to the $\dot{V}O_{2max}$ test and to arrive hydrated (having drank a recommended 500 ml of water an hour prior to testing) and eaten a balanced meal (containing carbohydrate, fats and protein) 2-3 hours prior to the test.

The participants were analysed as a whole group, as males and females, according to age (18 - 40 years, 41 - 65 years) and according to fitness rank of good to superior or very poor to fair, as distinguished from the ACSM guidelines (2014, pp.88-93).

Table 5.1: The activity/exercise levels of the 28 participants, stating their age, sex and if they had donated blood previously.

Participant number	Age (yrs) & sex (M/F)	New to BD	Weekly activity levels
1	44, F	No	Runs twice a week totalling 2-2.5 h & gym 1 h
2	22, M	No	2-3 h football, run 2-3 h & weights/gym1-2 h
3	58, M	Yes	Varied exercise classes totalling 5 h
4	30, M	No	Run 2-3 h & weights for 45 min, bike 1 h daily
5	60, M	Yes	Varied exercise classes 5-6 h
6	31, M	Yes	5 h football & 3 h gym
7	25, M	Yes	5 h of gym based exercise
8	20, M	No	3-4 h football & gym 4 h
9	54, M	No	1-2 h run and 2 gym sessions
10	19, M	Yes	Combination of running, gym and karate 6 h
11	64, F	Yes	4 h run
12	41, F	Yes	Walk to school and back with children
13	27, M	Yes	Tennis & pilates total 4 h
14	54, M	Yes	7-8 h run
15	23, M	Yes	8 h run, 30 min cycle a day
16	47, M	No	1-1.5 h run and karate 5 h
17	45, M	No	3-4 h football
18	31, F	No	5- 10 h rugby, 3 h weights
19	46, M	No	3 h gym
20	26, F	No	3-4 h dance per day
21	18, F	Yes	3-4 h dance per day
22	53, F	Yes	None, sits at a desk
23	36, M	No	2-3 h a day of spin, weights, polo
24	51, M	Yes	30 min walk with dog per day
25	51, F	No	45 min squash & 1 Legs Tums & Bums class
26	20, M	Yes	1-2 h per day row
27	30, F	Yes	3 h boot camp
28	53, M	No	3-4 h cycling

5.2.2 Study Design

All the participants were tested during the morning between the hours of 7.30 am and 11 am in order to reduce diurnal variation (Scheer, at al., 2010). Visit one served to establish

fitness to take part, body stature, haematological levels of the participants by capillary sampling, blood pressure, anthropometric measurements and cardiorespiratory fitness by using a $\dot{V}O_{2max}$ test. The second visit was 3 hours post BD in order to establish haematological levels only. If visit one and visit two could not be on the same day the participant had an extra visit to the laboratory, which enabled haematological readings to be taken on the same day prior to BD.

5.2.3 Hydration Analysis

Upon arrival for visit one, participants provided a urine sample (2nd void of the day), to establish specific gravity and conformity to fluid intake, by using a pre-calibrated refractometer (Eclipse, Bellingham and Stanley, UK). Refer to page 68, methods chapter 4.6.1.

5.2.4 Blood Pressure and SpO_2 Responses

With the participant sat down, after ~5 minutes of relaxation following arrival at the laboratory, a resting assessment of blood pressure (BP) (Nonin, 2120, USA), prior to Hb capillary blood sampling, was taken. Refer to page 71, methods chapter 4.7.1. SpO_2 (Nonin 2120, USA) was recorded at the same time as BP (but on the right-hand side of the body). Refer to page 71, methods section 4.7.1.

5.2.5 Haematological Responses

Each participant made a standard blood donation at a National Blood Transfusion Service within a few hours of haematological levels being established.

Using pre-calibrated equipment (EKF Hemo Control, EKF diagnostic, Germany), capillary blood (~10 µl) from a finger was examined for haemoglobin and haematocrit levels prior to the incremental test and or 1-2 hours prior to BD and 3 hours post BD. Refer to page 65, methods chapter 4.4.1.

BV was calculated using the Nadler formula (Nadler, Hidalgo and Bloch, 1962), for males the equation is $(366.9H(cm)^3) + (32.19W(kg)) + 604$, and for females the equation is $(356.1H(cm)^3) + (33.08W(kg)) + 183.3$, where H = height and W = body mass. Hb removed/lost from donating a unit of blood was calculated according to each individual's levels.

5.2.6 Lactate Responses

After calibration, blood lactate (BLa) was taken (Biosen C-Line, EKF diagnostic, Germany) via capillary blood from the fingertip prior to commencement of the incremental exercise test to gain a resting value and immediately at test termination. Refer to page 67, methods chapter 4.5.1.

5.2.7 Body Composition Analysis

Body composition analysis was performed by the principle researcher (DJ) to avoid variation. Harpenden calipers (Figure 5.1) and ISAK standards were used to asses body fat percentage. Executed on the right side of the body (ISAK, 2001), seven sites were used to gain body fat percentage for males, including triceps, biceps, subscapular, supraspinale, abdominal, front of thigh and medial calf, four sites were used for females (triceps, subscapular, supraspinale and medial calf) (Figure 5.2).

Figure 5.1: Harpenden skinfold caliper (British Indicators Ltd, Burgess Hill, UK).

The equations that ISAK use are from Withers, et al. (1987); a cross validated generalised equation (Garrett and Kirkendall, 2000) and thus there are equations for males and females, as follows: %BF (males) = 495/(1.0988-0.0004*∑7) – 450 (Withers, et al., 1987a) %BK (females) = 495/(1.17484-0.07229*∑4) – 450 (Withers, et al., 1987b). The equations incorporate the Siri Equation (1956) to derive body fat percentage, which is, % BF = (495/Body Density) – 450. For the males, Σ7 represents triceps, biceps, subscapular, supraspinale, abdominal, front of thigh and medial calf, for the females Σ4 represents triceps, subscapular, supraspinale and medial calf.

To ensure quality assurance, technical error of measurement (TEM) for intra-tester evaluation was performed on 10 volunteers measured over two days and their varying values recorded. The ISAK target for TEM is ≤ 10 % for skinfolds (Stewart and Sutton, 2012). Two measurements were obtained per site and a difference value obtained and the calculation is as follows (Perini, et al., 2005):

$$Absolute\ TEM = \sqrt{\frac{\Sigma\ di^2}{2n}}$$

Where:

$\sum d^2$ = sum of the deviation squared, n = volunteers, i = number of deviations.

The relative TEM was obtained by considering the variable average value (VAV), which is the mean of the first and second values of a skinfold of day 1 and then the same for day two. The values are added and divided by two, which gives an average of this skinfold. These values are then added up and divided by the number of subjects (10), which gives VAV. The calculation is:

$$relative\ TEM(\%) = \frac{TEM}{VAV} X\ 100$$

An example of figures obtained for the triceps can be seen in Table 5.2 with the equations for the triceps TEM and relative TEM shown below. The relative TEM is 0.50 % and thus within the 10 % guidance range required from ISAK.

$$0.07 = \sqrt{\frac{0.1}{2 * 10}} \qquad\qquad 0.50 = \frac{0.07}{14.11} X\ 100$$

Table 5.2: Results of triceps skinfold thickness performed by the same person. SKF = skinfold, V = volunteer.

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
Average of day 1 SKF	16.8	14.0	10.1	15.0	29.7	8.0	18.6	12.3	9.9	7.1
Average of day 2 SKF	16.7	14.2	9.9	14.9	29.8	7.9	18.6	12.3	9.9	7.0
Average of day 1 and 2 SKF	16.8	14.1	10.0	15.0	29.8	7.93	18.6	12.3	9.9	7.1
Deviations	0.10	-0.20	0.20	0.10	-0.10	0.10	0.00	0.00	0.00	0.10
Deviations ^{2}	0.01	0.04	0.04	0.01	0.01	0.01	0.00	0.00	0.00	0.00
$\sum$ Deviations (V1- V10) ^{2}	0.12									
Absolute TEM	0.08									
VAV	14.15									
Relative TEM %	0.50									

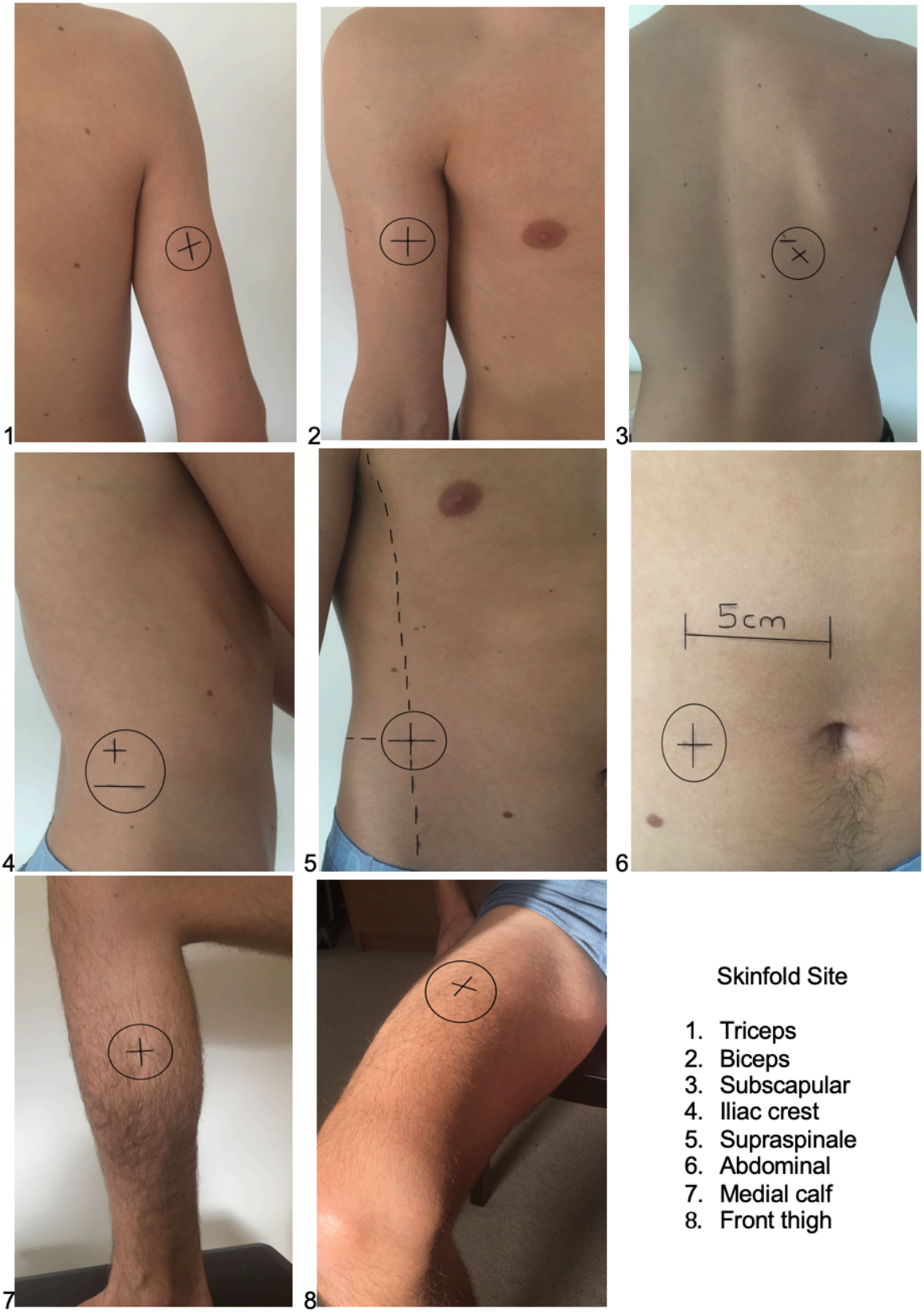

Figure 5.2: Identified sites for skinfold measurements. Photos taken by principle researcher (DJ).

5.2.8 $\dot{V}O_{2max}$ Protocol

Each participant completed an incremental graded exercise test to volitional exhaustion, on a pre-calibrated electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) to determine $\dot{V}O_{2max}$ (refer to page 75-76, chapter 4.9) and GET (refer to page 76, chapter 4.10).

5.2.9 Data and Statistical Analysis

Means and standard deviation for all data were calculated. Data was checked for normality using a Kolmogorov-Smirnov test and paired samples *t* tests were used (or Wilcoxon Signed Ranks test if normality was not present) to test the hypothesis that blood donation will have an effect on Hb & Hct. Paired sample *t* tests were additionally adopted to record the lactate response pre to post $\dot{V}O_{2max}$ test. Where *t* tests were not adopted, correlations (Pearson or Spearman if not normally distributed) were used to assess relationships. Cohens *d* (standardized difference in means) were calculated to show effect sizes (evaluated as 0.8 – > 2.0 large, 0.3 - 0.5 medium, < 0.2 small) where appropriate (Cohen, 1988). Cohens *d* (standardized difference in means) is expressed as the difference between two (raw) means divided by the pooled standard deviation (SD) of the groups (M_1 – M_2 / SD) (Cohen, 1988). Statistical analysis was completed using SPSS version 20 (SPSS, Chicago, IL). Statistical significance level was set at $P < 0.05$.

5.3 Results

5.3.1 Haematological and Lactate Responses

5.3.1.1 Haemoglobin (Hb) and Haematocrit (Hct)

Table 5.3 summarises mean and SD values for the amount of Hb that is lost in a 470 ml BD for all of the participants. Table 5.4 summarises the haematological responses mean and SD values pre and post BD, the delta value and effect size value. Post BD there were significant Hb concentration reductions and percentage decrements, with values as follows: For all 28 participants 6.51 % (*P* = 0.000), females 6.85 % (*P* = 0.000), males 6.31 % (*P* = 0.000), 18 - 40 years 5.50 % (*P* = 0.001), 41 - 65 years 6.51 % *(P* = 0.002). Similarly, this was also the case for Hct concentration, values were as follows, for all 28 participants 6.37 % (*P* = 0.000), females 5.81 % (*P* = 0.020), males 6.62 % (*P* = 0.000), 18 - 40 years 6 % (*P* = 0.001), 41- 65 years 6.74 (*P* = 0.006). The Cohens *d* showed large effect sizes across all groups with values ranging from -0.62 to -3.64. Figure 5.3 represents the female

and male cohorts pre to post BD Hb levels, with all participants showing decrements with the exception of one male participant.

Table 5.3: Mean ± SD Hb value that is lost in a 470 ml BD for all 28 participants, females, males, 18-40 and 41-65 years. Values derived from each participants Hb results per 100ml of blood.

Participant type	Hb ($g \cdot dL^{-1}$) lost in 470 ml BD
All 28	70.03 ± 6.12
Female (n = 9)	63.76 ± 2.91
Male (n = 19)	73.00 ± 4.85
18 - 40 yrs (n = 14)	70.10 ± 4.69
41 - 65 yrs (n = 14)	69.96 ± 7.47

Table 5.4: Mean ± SD haematological values for all 28 participants, females, males, 18-40 and 41-65 years, before and after blood donation and the delta value.

Haematological component	Pre BD	Post BD	Δ Value	Cohens *d* & CI
All 28 Participants				
Hb ($g \cdot dL^{-1}$)	14.90 ± 1.30	13.93 ± 1.24 ***	0.96 ± 0.71	-0.76 [-1.31, -0.22]
HcT (%)	43.74 ± 4.13	40.95 ± 3.75 ***	2.79 ± 2.42	-0.71 [-1.25, -0.17]
Female Participants (n = 9)				
Hb ($g \cdot dL^{-1}$)	13.57 ± 0.62	12.64 ± 0.28 ***	0.92 ± 0.28	-1.93 [-3.04, -0.83]
HcT (%)	39.40 ± 2.65	37.11 ± 1.83 *	2.29 ± 1.90	-3.64 [-5.10, -2.18
Male Participants (n = 19)				
Hb ($g \cdot dL^{-1}$)	15.53 ± 1.03	14.55 ± 0.98 ***	0.98 ± 0.85	-0.97 [-1.65, -0.30]
HcT (%)	45.79 ± 2.94	42.76 ± 2.96 ***	3.03 ± 2.65	-1.03 [-1.70, -0.35]
Participants between 18 – 40 years inclusive (n = 14)				
Hb ($g \cdot dL^{-1}$)	14.91 ± 1.00	14.09 ± 1.15 ***	0.82 ± 0.35	-0.76 [-1.53, 0.00]
HcT (%)	44.00 ± 2.91	41.36 ± 3.23 ***	2.64 ± 0.93	-0.86 [-1.63, -0.09]
Participants between 41 – 65 years inclusive (n = 14)				
Hb ($g \cdot dL^{-1}$)	14.89 ± 1.59	13.92 ± 1.39 **	1.11 ± 0.94	-0.72 [-1.48, 0.05]
HcT (%)	43.47 ± 5.18	40.54 ± 4.30 **	3.22 ± 3.06	-0.62 [-1.37, 0.14]

Hb haemoglobin, Hct haematocrit
** P < 0.05 and **P < 0.01 and ***P < 0.001 denotes a significant difference between Pre BD and Post BD.*

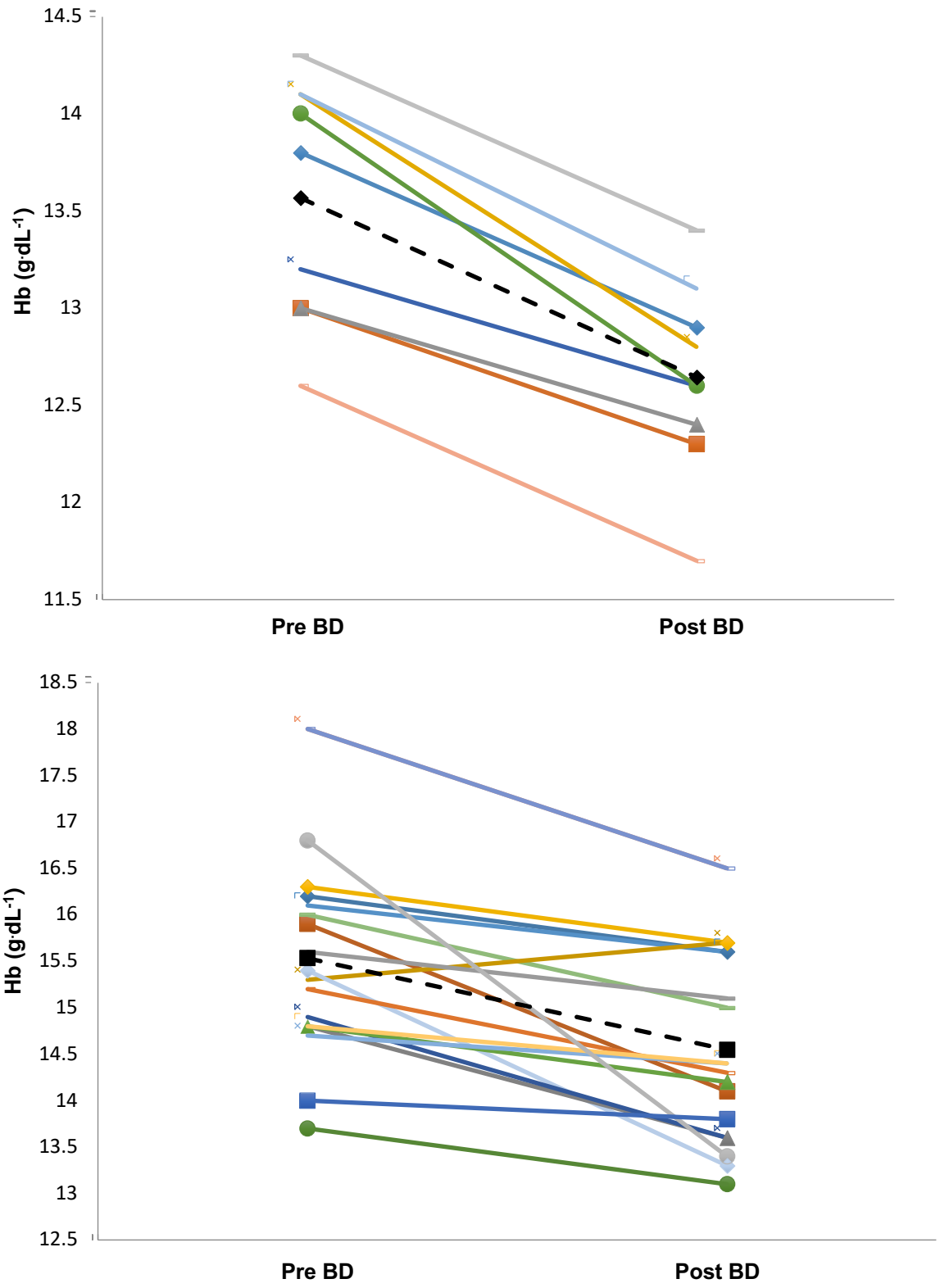

Figure 5.3: Individual participants Hb ($g \cdot dL^{-1}$) values pre and post BD. The top graph represents the female cohort and the bottom graph represents the male cohort. The dashed black like is the average.

5.3.1.2 Blood volume

Table 5.5 summarises the mean and SD values pre and post BD for BV, the percentage decrease and the effect size. Although these are paired samples, a *t* test cannot be computed, as the standard error of the difference is zero. Large effect sizes across all groups can be observed with values ranging from Cohens *d* -0.65 to -1.45. The BV values of the various participant groups when correlated against respective delta Hb values (pre to post BD) revealed generally weak or moderate correlations and non-significant relationships (Table 5.6). Table 5.7 shows the BV values of the various participant groups when correlated against the respective values for Hb ($g{\cdot}dL^{-1}$) lost in 470 ml BD. Some large correlational values are represented where all 28 participants are shown and this is also the case for the older age cohort, with significance seen across both these groups too. A representative scatter graph with a positive strong correlation that represents the cohort 41 – 65 years is shown in Figure 5.4 for BV (ml) against Hb ($g{\cdot}dL^{-1}$) lost in 470 ml BD.

Table 5.5: Mean ± SD for BV values, percentage decrease and Cohens *d* for all 28 participants, females, males, 18-40 and 41-65 years. With an additional column showing mean ± SD for BV removed per kg of body mass, which is a representation of the donated 470 ml of blood.

Participant type	BV pre BD (ml)	BV post BD (ml)	% Decrease	Cohens *d* & CI	BV removed per kg of body mass
All 28	4963.57 ± 681.03	4493.57 ± 681.03	9.65 ± 1.39	-0.69 [-1.23, -0.15]	6.16 ± 0.89
Female (n = 9)	4183.33 ± 322.72	3713.33 ± 322.72	11.29 ± 0.86	-1.45 [-2.49, -0.43]	6.90 ± 0.77
Male (n = 19)	5333.16 ± 448.16	4863.16 ± 448.16	8.87 ± .75	-1.05 -1.73, -0.37]	5.81 ± 0.72
18 - 40 yrs (n = 14)	4995.71 ± 665.49	4525 ± 665.49	9.57 ± 1.30	-0.71 [-1.47, 0.06]	6.16 ± 0.83
41 - 65 yrs (n = 14)	4931 ± 719.85	4461.43 ± 719.85	9.73 ± 1.51	-0.65 [-1.41, 0.11]	6.16 ± 0.97

Table 5.6: BV and BV removed per kg of body mass against Δ Hb value correlation coefficient and coefficient of determination results of the varying participants groups.

Participant type	Correlation coefficient results (r) & coefficient of determination (R^2)	
	BV against Δ Hb Value	BV removed per kg of body mass against Δ Hb Value
All 28	r = .18, p > .05 R^2 = 0.03	r = -.25, p > .05 R^2 = 0.06
Female (n = 9)	r = .13, p > .05 R^2 = 0.02	r = -.07, p > .05 R^2 = 0.00
Male (n = 19)	r = .27, p > .05 R^2 = 0.07	r = -.34, p > .05 R^2 = 0.11
18 - 40 yrs (n = 14)	r = -.36, p > .05 R^2 = 0.13	r = .27, p > .05 R^2 = 0.07
41 - 65 yrs (n = 14)	r = .41, p > .05 R^2 = 0.17	r = -.44, p > .05 R^2 = 0.19

Table 5.7: BV and BV removed per kg of body mass against Hb ($g \cdot dL^{-1}$) lost in 470 ml BD value correlation coefficient and coefficient of determination results of the varying participants groups.

Participant type	Correlation coefficient results (r) & coefficient of determination (R^2)	
	BV against Hb ($g \cdot dL^{-1}$) lost in 470 ml BD	BV removed per kg of body mass against Hb ($g \cdot dL^{-1}$) lost in 470 ml BD
All 28	r = .62, p < .05*** R^2 = 0.38	r = -.56, p < .05** R^2 = 0.31
Female (n = 9)	r = .42, p > .05 R^2 = 0.18	r = -.45, p > .05 R^2 = 0.20
Male (n = 19)	r = .03, p > .05 R^2 = 0.00	r = -.19, p > .05 R^2 = 0.04
18 - 40 yrs (n = 14)	r = .30, p > .05 R^2 = 0.09	r = -.08, p > .05 R^2 = 0.00
41 - 65 yrs (n = 14)	r = .82, p < .05*** R^2 = 0.67	r = -.82, p < .05*** R^2 = 0.67

** P < 0.05 and **P < 0.01 and ***P < 0.001 denotes a significant difference.*

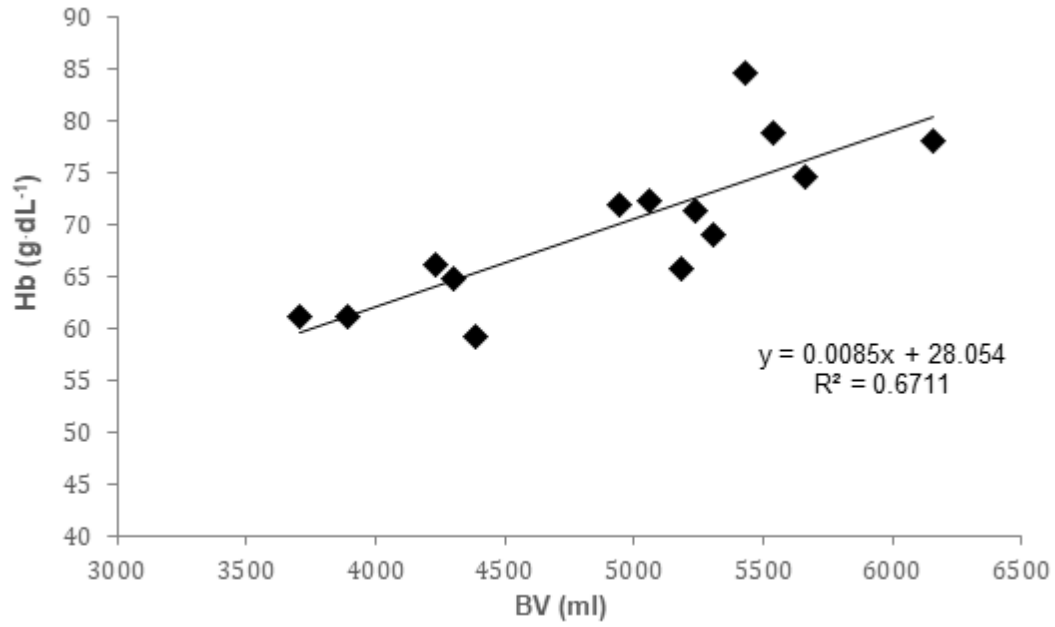

Figure 5.4: BV (ml) against Hb ($g \cdot dL^{-1}$) lost in 470 ml BD. Showing a positive strong correlation for the group that represents 41 – 65 years (n = 14).

5.3.1.3 Lactate Responses

Lactate responses (Table 5.8) pre to post $\dot{V}O_{2max}$ test showed increases across all participants. Resting values ranged from 0.54 to 1.93 mM and peak values ranged from 7.09 to 15.88 mM. Large effect sizes (Cohens *d* 4.46 to 5.06) across all groups can be observed.

Table 5.8: Mean ± SD lactate values for all 28 participants, females, males, 18-40 and 41-65 years, age categories before and after the $\dot{V}O_{2max}$ test. Additionally, the delta and Cohens *d* values are recorded.

Participant	rBLa (mM)	pBLa (mM)	Δ Value	Cohens *d* & CI
All 28	1.18 ± 0.69	10.30 ± 2.53***	9.12 ± 2.52	4.92 [3.88, 5.96]
Female (n = 9)	1.27 ± 0.88	9.49 ± 2.32***	8.22 ± 2.34	4.68 [2.96, 6.41]
Male (n = 19)	1.14 ± 0.61	10.69 ± 2.60***	9.55 ± 2.54	5.06 [3.78, 6.34]
18 - 40 yrs (n = 14)	1.10 ± 0.36	11.32 ± 2.42***	10.22 ± 2.54	5.03 [3.55, 6.51]
41 - 65 yrs (n = 14)	1.26 ± 0.89	9.29 ± 2.21***	8.03 ± 1.96	4.45 [3.10, 5.80]

rBLa resting blood lactate, pBLa peak blood lactate.
****P < 0.001denotes a significant difference between resting and peak blood lactate*

5.3.2 Cardio-Respiratory Responses

Of the 28 participants 20 had plateau responses and thus achieved $\dot{V}O_{2max}$, according to the criteria set out in the methods section. Those that did not achieve plateau, achieved $\dot{V}O_{2max}$ either according to secondary criterion or according to verification. All participants were thus used for further analysis.

5.3.2.1 Maximal Oxygen Uptake

The $\dot{V}O_{2max}$ values shown in Table 5.9 were heterogeneous and values ranged from 23.59 $ml \cdot kg^{-1} \cdot min^{-1}$ to 56.12 $ml \cdot kg^{-1} \cdot min^{-1}$ across all participants. The $\dot{V}O_{2max}$ values of the various participant groups when correlated against respective delta Hb values (pre to post BD) revealed generally very weak, weak or moderate correlations and non-significant relationships (Table 5.10). A representative scatter graph with a negative moderate correlation that represents the cohort 18 - 40 years is shown in Figure 5.5. Table 5.11 shows the $\dot{V}O_{2max}$ values of the various participant groups when correlated against the respective values for Hb ($g \cdot dL^{-1}$) lost in 470 ml BD. Some large correlational values are represented in both the fitness rank groups and significance is seen across both these groups too (P = 0.009 for fitness rank good to superior and P = 0.035 for fitness rank very poor to fair). A representative scatter graph with a positive moderate correlation that represents the cohort fitness rank good to superior is shown in Figure 5.6 for fitness rank good to superior against Hb ($g \cdot dL^{-1}$) lost in 470 ml BD.

Table 5.9: The mean ± SD values for $\dot{V}O_{2max}$ ($ml \cdot kg^{-1} min^{-1}$) across all participants and various groups. The fitness ranks are according to ACSM (2014, p.88-93).

Participant type	$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)
All 28	40.14 ± 8.77
Female (n = 9)	33.41 ± 7.61
Male (n = 19)	43.33 ± 7.51
18 - 40 yrs (n = 14)	44.31 ± 7.01
41 - 65 yrs (n = 14)	35.97 ± 8.56
Fitness rank good to superior (n = 13)	47.04 ± 5.96
Fitness rank very poor to fair (n = 15)	35.47 ± 5.28

Table 5.10: $\dot{V}O_{2max}$ against Δ Hb value correlation coefficient and coefficient of determination results of the varying participants groups.

Participant type	$\dot{V}O_{2max}$ against Δ Hb value correlation coefficient results (r) & coefficient of determination (R^2)
All 28	r = -.10, p > .05 R^2 = 0.01
Female (n = 9)	r = .23, p > .05 R^2 = 0.05
Male (n = 19)	r = -.22, p > .05 R^2 = 0.05
18 - 40 yrs (n = 14)	r = -.39, p > .05 R^2 = 0.15
41 - 65 yrs (n = 14)	r = .11, p > .05 R^2 = 0.01
Fitness rank good to superior (n = 13)	r_s = .39, p > .05 R^2 = 0.15
Fitness rank very poor to fair (n = 15)	r_s = .10, p > .05 R^2 = 0.01

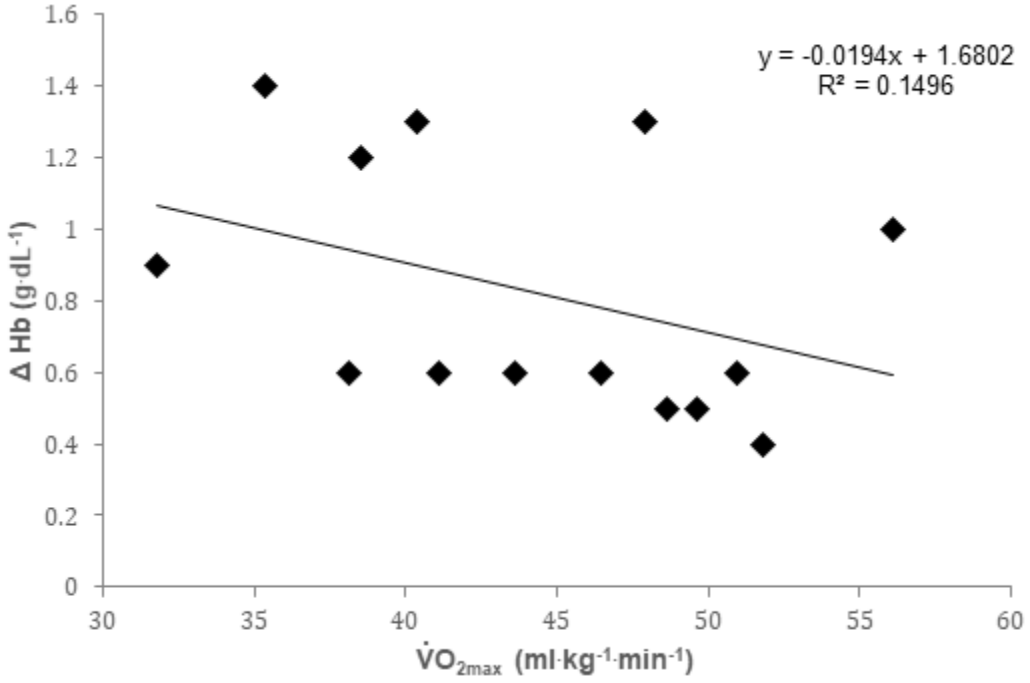

Figure 5.5: $\dot{V}O_{2max}$ against Δ Hb Value. Showing a negative moderate correlation for the group that represents 18 – 40 years (n = 14).

Table 5.11: $\dot{V}O_{2max}$ against Hb ($g \cdot dL^{-1}$) lost in 470 ml BD value correlation coefficient and coefficient of determination results of the varying participants groups.

Participant type	$\dot{V}O_{2max}$ against Hb ($g \cdot dL^{-1}$) lost in 470ml BD Correlation coefficient results (r) & coefficient of determination (R^2)
All 28	r = .26, p > .05 R^2 = 0.07
Female (n = 9)	r = .10, p > .05 R^2 = 0.01
Male (n = 19)	r = -.30, p > .05 R^2 = 0.09
18 - 40 yrs (n = 14)	r = .30, p > .05 R^2 = 0.09
41 - 65 yrs (n = 14)	r = .28, p > .05 R^2 = 0.09
Fitness rank good to superior (n = 13)	r = .69, p < .05 R^2 = 0.48**
Fitness rank very poor to fair (n = 15)	r = .55, p < .05 R^2 = 0.30*

** P < 0.05 and **P < 0.01 denotes a significant difference.*

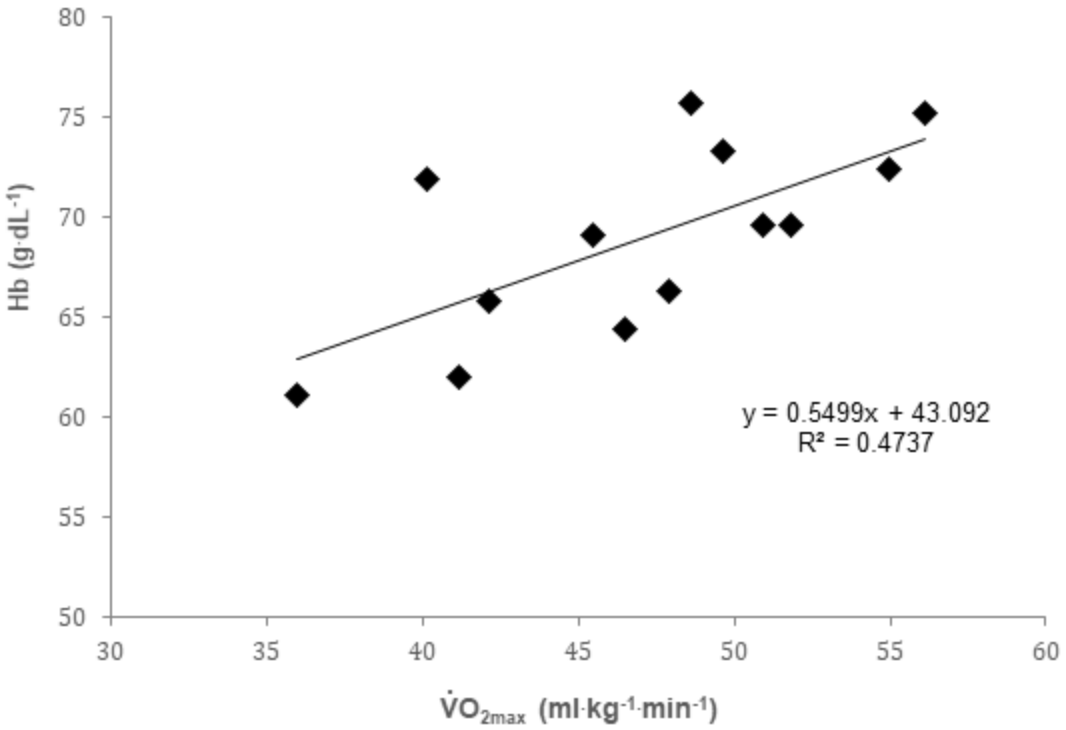

Figure 5.6: $\dot{V}O_{2max}$ against Hb ($g \cdot dL^{-1}$) lost in 470 ml BD value. Showing a positive moderate correlation for the group that represents fitness rank good to superior (n = 13).

5.3.2.2 Heart Rate, Gas Exchange Threshold and Oxygen Pulse

HR_{max} ranged from 151.6 to 197.2 $b \cdot min^{-1}$, GET ranged from 31.65 to 77.48 % $\dot{V}O_{2max}$ and O_2 pulse ranged from 9.62 to 29.05 $ml \cdot beat^{-1}$. The mean and SD values are presented in Table 5.12. The HR_{max}, GET and O_2 pulse values of the various participant groups when correlated against respective delta Hb values (pre to post BD) revealed generally very weak, weak or moderate correlations and non-significant relationships (Table 5.13). Table 5.14 shows the HR_{max}, GET and O_2 pulse values of the various participant groups when correlated against the respective values for Hb ($g \cdot dL^{-1}$) lost in 470 ml BD. Large correlational values are represented in the female group for HR_{max} and when regarding O_2 pulse in the older cohort, with a medium correlation observed for all 28 participants. Additionally, significance can be seen in O_2 pulse for all 28 participants (P = 0.018) and the older cohort (P = 0.009).

Table 5.12: Mean ± SD values across varying participant categories for HRmax, GET and O_2 pulse.

Participant type	HR_{max} ($b \cdot min^{-1}$)	GET (%$\dot{V}O_{2max}$)	O_2 Pulse ($ml \cdot beat^{-1}$)
All 28	173.37 ± 12.33	61.53 ± 10.23	18 ± 4.59
Female (n = 9)	175.55 ± 14.70	67.52 ± 9.05	13.10 ± 3.01
Male (n = 19)	172.35 ± 11.34	58.69 ± 9.71	20.47 ± 3.02
18 - 40 yrs (n = 14)	178.52 ± 11.03	61.03 ± 13.14	19.43 ± 4.43
41 - 65 yrs (n = 14)	168.23 ± 11.71	62.03 ± 6.65	16.76 ± 4.50

Table 5.13: HR, GET and O_2 pulse against Δ Hb value correlation coefficient and coefficient of determination results of the varying participant groups.

Participant type	Correlation coefficient results (r) & coefficient of determination (R^2)		
	HR_{max} against Δ Hb Value	GET against Δ Hb Value	O_2 Pulse against Δ Hb Value
All 28	r = .10, p > .05 R^2 = 0.01	r = -.04, p > .05 R^2 = 0.00	r_s = -.08, p > .05 R^2 = 0.01
Female (n = 9)	r_s = .41, p > .05 R^2 = 0.17	r = -.24, p > .05 R^2 = 0.06	r = .10, p > .05 R^2 = 0.01
Male (n = 19)	r = .07, p > .05 R^2 = 0.00	r = .01, p > .05 R^2 = 0.00	r = -.09, p > .05 R^2 = 0.01
18 - 40 yrs (n = 14)	r = -.04, p > .05 R^2 = 0.00	r = .37, p > .05 R^2 = 0.14	r = -.43, p > .05 R^2 = 0.18
41 - 65 yrs (n = 14)	r = .32, p > .05 R^2 = 0.10	r = -.39, p > .05 R^2 = 0.15	r = .25, p > .05 R^2 = 0.06

Table 5.14: HR, GET and O_2 pulse against Hb ($g \cdot dL^{-1}$) in 470 ml BD value correlation coefficient and coefficient of determination results of the varying participant groups.

Participant type	Correlation coefficient results (r) & coefficient of determination (R^2)		
	HR_{max} against Hb ($g \cdot dL^{-1}$) lost in 470 ml BD	GET against Hb ($g \cdot dL^{-1}$) lost in 470 ml BD	O_2 Pulse against Hb ($g \cdot dL^{-1}$) lost in 470 ml BD
All 28	r = .02, p > .05 R^2 = 0.00	r = -.31, p > .05 R^2 = 0.10	r_s = .44, p < .05 R^2 = 0.19*
Female (n = 9)	r_s = .63, p > .05 R^2 = 0.39	r = .07, p > .05 R^2 = 0.00	r = .13, p > .05 R^2 = 0.02
Male (n = 19)	r = .06, p > .05 R^2 = 0.00	r = -.05, p > .05 R^2 = 0.00	r = -.23, p > .05 R^2 = 0.05
18 - 40 yrs (n = 14)	r = -.10, p > .05 R^2 = 0.01	r = -.33, p > .05 R^2 = 0.11	r = .26, p > .05 R^2 = 0.07
41 - 65 yrs (n = 14)	r = .09, p > .05 R^2 = 0.01	r = -.41, p > .05 R^2 = 0.17	r = .67, p < .05 R^2 = 0.45**

** P < 0.05 and **P < 0.01 denotes a significant difference*

5.3.3 Body Composition

Body mass ranged from 60.25 to 104.80 kg, body fat (BF) ranged from 9.8 to 32.8 %, lean body mass ranged from 46.5 to 84.2 kg and BMI ranged from 21.2 to 31.7 Kg/m^2, the mean

and SD values are presented in Table 5.15. Mass, BF, lean body mass and BMI values of the various participant groups when correlated against respective delta Hb values (pre to post BD) revealed generally very weak, weak or moderate correlations and non-significant relationships (Table 5.16). Table 5.17 shows mass, BF, lean body mass and BMI values of the various participant groups correlated against the respective values for Hb ($g \cdot dL^{-1}$) lost in 470 ml BD. Moderate and large correlational values are represented generally across all groups with only a few exceptions. Significance can be seen regarding mass (P = 0.002) and lean body mass (P = 0.002) for all 28 participants, this is also the case for the older cohort (P = 0.009 and P = 0.001 respectively). The older cohort also shows significance regarding BMI (P = 0.004). When regarding BF and the 18-40 groups there is also significance (P = 0.03).

Table 5.15: Mean and SD values across varying participant categories for body mass, BF, lean body mass and BMI.

Participant type	Body Mass (kg)	BF (%)	Lean Body Mass (kg)	BMI (kg/m^2)
All 28	77.87 ± 11.45	18.4 ± 7.1	63.5 ± 10.1	25.48 ± 3.30
Female (n = 9)	68.91 ± 8.26	24.6 ± 5.1	51.7 ± 4.5	24.68 ± 3.55
Male (n = 19)	82.12 ± 10.34	15.4 ± 5.9	69.1 ± 6.5	25.85 ± 3.21
18 - 40 yrs (n = 14)	77.63 ± 10.36	16.4 ± 6.8	65.0 ± 10.7	25.39 ± 3.17
41 - 65 yrs (n = 14)	78.11 ± 12 83	20.4 ± 7.1	62.0 ± 9.6	25.56 ± 3.54

Table 5.16: Mass, BF, lean body mass and BMI against Δ Hb value correlation coefficient and coefficient of determination results of the varying participant groups.

Participant type	Correlation coefficient results (r) & Coefficient of determination (R^2)			
	Body Mass against Δ Hb	BF against Δ Hb	Lean Body Mass against Δ Hb	BMI against Δ Hb
All 28	r = .27, p > .05 R^2 = 0.07	r_s = .39, p > .05 R^2 = 0.15	r = .09, p > .05 R^2 = 0.01	r = .21, p > .05 R^2 = 0.04
Female (n = 9)	r = .07, p > .05 R^2 = 0.00	r = -.02, p > .05 R^2 = 0.00	r = .13, p > .05 R^2 = 0.02	r = -.33, p > .05 R^2 = 0.11
Male (n = 19)	r = .33, p > .05 R^2 = 0.11	r_s = .37, p > .05 R^2 = 0.14	r = .09, p > .05 R^2 = 0.01	r = .32, p > .05 R^2 = 0.10
18 - 40 yrs (n = 14)	r = -.28, p > .05 R^2 = 0.08	r = .19, p > .05 R^2 = 0.04	r = -.32, p > .05 R^2 = 0.10	r = -.26, p > .05 R^2 = 0.07
41 - 65 yrs (n = 14)	r = .45, p > .05 R^2 = 0.20	r = .24, p > .05 R^2 = 0.06	r_s = .34, p > .05 R^2 = 0.11	r = .39, p > .05 R^2 = 0.15

Table 5.17: Mass, BF, lean body mass and BMI against Hb ($g \cdot dL^{-1}$) in 470 ml BD value correlation coefficient and coefficient of determination results of the varying participant groups.

Participant type	Correlation coefficient results (r) & Coefficient of determination (R^2)			
	Body Mass against Hb ($g \cdot dL^{-1}$) lost in 470 ml BD	BF against Hb ($g \cdot dL^{-1}$) lost in 470 ml BD	Lean Body Mass against Hb ($g \cdot dL^{-1}$) lost in 470 ml BD	BMI against Hb ($g \cdot dL^{-1}$) lost in 470 ml BD
All 28	r = .55, p < .05** R^2 = 0.30	r_s = -.23, p > .05 R^2 = 0.05	r = .55, p < .05** R^2 = 0.30	r = .35, p > .05 R^2 = 0.12
Female (n = 9)	r = .48, p > .05 R^2 = 0.23	r = .31, p > .05 R^2 = 0.10	r = .39, p > .05 R^2 = 0.15	r = .20, p > .05 R^2 = 0.04
Male (n = 19)	r = .23, p > .05 R^2 = 0.05	r_s = .44, p > .05 R^2 = 0.19	r = -.18, p > .05 R^2 = 0.01	r = .40, p > .05 R^2 = 0.16
18 - 40 yrs (n = 14)	r = .08, p > .05 R^2 = 0.01	r = -.58, p < .05* R^2 = 0.34	r = .32, p > .05 R^2 = 0.10	r = -.27, p > .05 R^2 = 0.07
41 - 65 yrs (n = 14)	r = .80, p < .05 R^2 = 0.64**	r = .10, p > .05 R^2 = 0.01	r_s = .67, p < .05** R^2 = 0.45	r = .71, p < .05** R^2 = 0.50

** P < 0.05 and **P < 0.01 denotes a significant difference*

5.3.4 Blood Pressure and SpO2 Responses

Blood pressure ranged from 91/56 to 156/96 mmHg and SpO_2 ranged from 96 to 99 % across all 28 participants. The mean and SD values were 121.7/70.3 ± 13.8/9.3 mmHg for all 28 participants, 118.8/70.2 ± 14.8/9.6 mmHg for females, 123.1/70.4 ± 13.5/9.4 mmHg for males, 119.4/67.5 ± 14.9/8.7 mmHg for 18 – 40 years and 124.0/73.1 ± 12.7/9.2 mmHg for 41 – 65 years.

5.3.5 Hydration Status Responses

Hydration ranged from 1.001 to 1.024 U_{sg}. The mean and SD values were 1.010 ± 0.008 U_{sg} for all participants, 1.010 ± 0.008 U_{sg} for females, 1.011 ± 0.009 for males, 1.010 ± 0.009 for 18 – 40 years and 1.011 ± 0.008 for 41 – 65 years.

5.4 Discussion/Conclusions

The *a-priori* hypothesis was that haematological responses would be altered from donating a unit of blood and the effects of body composition and markers of cardiorespiratory fitness will have a significant relationship to haematological responses as a consequence of donating a unit of blood. Haematological responses altering from donating a unit of blood is a logical postulation as after a small time frame (not necessarily acutely, as plasma and RBCs are lost together) fluid is moved into blood vessels (extracellular) from tissues, which decreases Hb as the blood becomes more diluted. When reflecting if the effects of body

composition and markers of cardiorespiratory fitness have a significant relationship to haematological responses as a consequence of donating a unit of blood, the results do not fully support experimental hypothesis 2 and 3 although many significances were seen across all variables deliberated with the exception of GET and HR_{max} when studied against the amount of Hb lost following BD.

When regarding pre to post BD Hb and Hct significances were seen across all groups. Therefore, experimental hypothesis 1 was accepted and that haematological responses will indeed be altered from donating a unit of blood. The older age category showed slightly less significance in pre to post BD Hb compared to the other groups, also the largest percentage decrease in Hb was seen in females. If effect size was also to be considered, the largest effect was observed in the females, indicating that a more profound effect on Hb levels was had from BD on this group. Furthermore, with the confidence intervals of the effect not passing through zero, then the significance is supported. However, no significant difference in Δ Hb levels was observed between different sex and age categories, which possibly indicates sex and age have no relevance.

Individually, all participants had a reduction in Hb and Hct with the exception of one male, which can be seen in Figure 5.3. The reason for this is possibly linked to his hydration status. However, all participants were hydrated prior to the exercise test (1.010 ± 0.008 U_{sg}, normal ranges from 1.002 to 1.030 U_{sg} (Dirckx, 2001)), but the hydration levels were not checked after BD and prior to the final capillary blood samples, as the participant was not required to exercise again post BD. Hydration levels are important when it concerns maintaining plasma volume (especially after donating blood). Donor centre guidelines advise drinking 500 ml of fluid prior to donating, but to additionally keep hydrated after BD, however the donation service do not check hydration status of the donor other than verbally and the donor is solely responsible for fluid intake. Hb and Hct levels will emerge higher if a person is dehydrated when compared to normovolemic conditions and if too much fluid has been consumed the opposite is true (Billett, 1990). This can be explained accordingly, Hct is typically ~3 times the value of Hb (Quinto, et al., 2006) therefore if one changes so does the other. When regarding dehydration, plasma is dependent on water thus Hct and Hb will be elevated as there will be water loss from intra and extra cellular spaces, which increases blood viscosity as plasma demises. Evidence of this can be observed in a study on fire-fighters (Holsworth, Cho and Weidman, 2013), investigating the effects of a fire fighting simulation followed by rehydration. The values prior to interventions were Hb 14.8 ± 1.2 $g \cdot dL^{-1}$, Hct 43.7 ± 3.1 %; following the simulation the

values were Hb 15.4 ± 0.9 $g \cdot dL^{-1}$, Hct 46.1 ± 2.3 %; post simulation and 45 minutes after rehydration the values were Hb 14.5 ± 0.9 $g \cdot dL^{-1}$, Hct 44 ± 2.7 %.

An additional issue is the fact that Hb values will alter throughout the day, thus the value taken in the morning will be different to a value 3 hours later due not only to ingestion of fluids, but also to food and body movement. Postural pseudoanaemia affects PV and when an individual is (semi) reclined after 10-15 minutes, there is an alteration in PV (Jacob, et al., 2005). Therefore, if the male with a higher Hb reading post BD (compared to pre BD) firstly was not adequately hydrated, but also had been standing resulting in blood pooling from gravity, blood pressure and heart rate will be elevated and as such will cause plasma (intravascular fluid) to move to interstitial compartments, this would also result in Hb (and Hct) to increase and PV to decrease. This participant is possibly deemed an outlier; except this could have been normal for him and as such the data was left in. Additionally, there could have been error in the measurements taken both pre and post BD by the operator. To ameliorate this problem for future work it would be wise to allow for a period of rest and to check hydration levels of the participants with urine specific gravity measurements prior to the Hb checks being made.

Regarding BV, large effect sizes were seen across all groups, but the most profound effect was observed in the female category, with an 11 % reduction of BV post BD, Cohens *d* of -1.45 and confidence interval (-2.49, -0.43) supporting the significance. This finding possibly is not surprising given that BV will alter for differing statures and compositions and males BV will be divergent to females, due to characteristics such as males having more muscle, which generates more BV and females that naturally carry more body fat, which does not generate BV. This divergence between males and females is evident in Table 5.15, where it can be seen that the mean values regarding BF for males are 37.3 % less than females, and males have 25 % more lean body mass than females.

Regarding BV against Δ Hb, weak or moderate correlations were observed and no significance. However, the correlation coefficient results regarding BV against the amount of Hb that is lost in a standard BD revealed strong positive correlations and high significance in all 28 participants (r = .62, *P* = .002) and the 41 – 65 year age group (r = .82, *P* = .000). This was also the case when correlating the amount of Hb lost against BV removed per kg of body mass, only a strong negative correlation occurred. It could be concluded from these results that the higher the BV the larger the amount of Hb lost in 470ml becomes, but this is possibly because the individuals have a larger absolute Hb value to start with. Also, individuals who have more BV removed per kg of body mass have

a lower amount of Hb lost in a standard 470 ml donation because the individual has a lower Hb value to begin with, thus they are negatively affected as they lose more BV per kg of body mass as they are smaller than a larger individual.

When considering stature, two moderate correlations were observed in body mass against Δ Hb, r = .33 and r = .45, which were for males and the older age category respectively, the other correlations were weak. There were also two moderate correlations in BF against Δ Hb, r_s = .39 and r_s = .37 for all 28 participants and the males respectively, the other correlations were weak. Similarly, two moderate correlations in Lean Body Mass against Δ Hb were seen, r = -.32 and r_s = .34, which were for the younger and older age groups respectively, the other correlations were weak. For BMI against Δ Hb there were three moderate correlations of, r = -.33, r = .32 and r = .39, which were for females, males and the older age categories respectively. No significance was observed for any of these variables however; when regarding the correlations of mass and lean body mass against Hb lost there were strong correlations and significance seen for all 28 participants (*P* = .002). This was also the case for the older cohort with significance of *P* = .001 and *P* = .009 for mass and lean body mass respectively. The positive correlations indicate that as lean muscle mass (and mass) increase so does the Hb content. It could be that in the older cohort they had a higher significance because they were leaner and as such had more musculature, which by its nature has a higher Hb content. The older cohort also displayed significance (*P* = .004), and a strong correlation regarding BMI against Hb lost following BD. However, BMI itself is contentious, as amongst other issues, such as poor representation of anatomical variation in different ethnic groups (Romero-Corral, et al., 2008), poor representation of trained athletes or the physically athletic and poor identification of intermediate (overweight) category individuals. It also cannot differentiate between lean body mass (LBM) and non-lean body mass (adipose) (De Schutter, et al., 2013; Romero-Corral, et al., 2008).

In order to reflect cardiovascular fitness of the participants, two extra categories were placed into the $\dot{V}O_{2max}$ tables ranking them good to superior or very poor to fair. The fitness ranks are according to normative data concurring to age and sex from the ACSM guidelines (2014, p.88-93). The results revealed weak or moderate correlations when observing the relationship between $\dot{V}O_{2max}$ and Δ Hb. The highest moderate correlation (r_s = .39) was in the very good to superior fitness rank group, but was not significant. With $\dot{V}O_{2max}$ sharing 15 % of the variability in delta Hb (0.39 ^ 2 = 0.15). The correlation was the same (r = -.39) for the 18 - 40 year category, but with a negative direction. When regarding the good to superior fitness rank group generally a school of thought is that the more

cardiovascular developed an individual is the more the Hb increases, as there are more red bloods cells and haemoglobin circulating in athletes compared to sedentary individuals with exercise/training stimulating erythropoiesis (Mairbäuri, 2013). What the correlation implies is that the higher the $\dot{V}O_{2max}$ the larger the Δ Hb, this could indicate that a fitter individual is more susceptible to a larger change in Δ Hb, although this is speculative. With the correlation being negative in the 18 - 40 year group (which can be seen in Figure 5.4), it appears that the higher the $\dot{V}O_{2max}$ the smaller the Δ Hb. Why this is the case is not clear, particularly when 57 % of this 18 - 40 year cohort are in the good-superior fitness group and the outcome is opposite to that groups correlation. One of the issues here is that it is difficult to make direct comparisons between these two groups as one result was obtained via non-parametric testing due to the data not being normally distributed and the other was parametric and it is possible to have a situation where Pearson coefficient is negative while Spearman is positive (Hauke & Kossowski, 2011).

Regarding the relationship between $\dot{V}O_{2max}$ and Hb lost there was significance seen and moderate correlations in both of the fitness ranked groups. The highest significance (*P* = .009) of the two groups was observed for the good to superior data set. Interpretation of the correlation indicates that a higher $\dot{V}O_{2max}$ has a relationship with a higher amount of Hb, as $\dot{V}O_{2max}$ increases so does the Hb values. An enlarged Hb concentration increases the amount of O_2 that can be distributed to tissues. Past authors have shown strong correlations (r = 0.81) between total Hb mass and $\dot{V}O_{2max}$, (Schmidt & Prommer, 2010) hence the total Hb mass effects $\dot{V}O_{2max}$ by altering O_2 transport capacity, i.e. Hb.

When considering HR_{max} against Δ Hb only the female and older age category showed moderate correlations (of r_s = .41 and r = .32 respectively). All other correlations were weak. The GET against Δ Hb were all weak correlations and the O_2 pulse against Δ Hb correlations were all weak with the exception of the 18 - 40 year category that showed a moderate correlation of r = -.43. O_2 pulse against Hb lost following BD showed significance, this was regarding all 28 participants (*P* = .018) and the older cohort (*P* = .009), with moderate and strong positive correlations observed respectively. O_2 pulse ($\dot{V}O_2$/HR) is an indirect indicator of stroke volume (SV) and $\dot{V}O_2$ equates to cardiac output (SV x HR) multiplied by arteriovenous oxygen difference (a-vO_{2diff}), however increasing a-vO_{2diff} will depend on Hb concentration and arterial oxygen content (Wasserman, et al., 2005). If Hb is unchanged then oxygen content should be unchanged, the correlations increase though indicating that as Hb content increases so does the O_2 pulse.

When contemplating each individuals Hb lost following BD thus making the data proportional, followed by checking correlations, exposed significance in all variables with the exception of HR_{max} and GET. Indeed all 28 participants collectively showed significance and high correlations in the variables of lean body mass, mass, BV and BV removed per kg of body mass. Also, the older age cohort had significance and strong correlations in many variables such as of O_2 pulse, lean body mass, mass, BV and BV removed per kg of body mass. It could be that there are more associations with this age group and the Hb lost in 470 ml BD, because Hb diminishes with age. The average age in the older cohort was 51.5 ± 6.49 (years), 51.9 ± 4.95 (years) for males and 50.5 ± 10.34 (years) for females. Hawkins, Speck and Leonard (1954) noted that Hb in males declined progressively after the 5^{th} decade (this did not appear so in females). Similarly, a more recent study (Mahlknecht & Kaiser, 2010) of 1,724 hospitalised patients showed that Hb ($g \cdot dL^{-1}$) began at ~5.5 between the age or 18-19 years and then showed a decline to ~14.8 (40 - 49 years), ~14.5 (50 - 59 years), ~14 (60 - 69 years), ~13 (70 - 79 years) and ~12.2 (80+ years) for males. For females, the values remained approximately between13.3 and 13.7 up to the age of 60 - 69 years at which point it dipped to 12.8 and continued to dip down for the next 3 decades.

Overall, when the assorted variables are computed against the Δ Hb there appears to be generally no significance or large correlations across the varying participant groups. This possibly indicates that for future work it would be acceptable to use a general pool of participants, as the results highlight that physicality/body composition has little bearing when calculating the Δ Hb value against pre donation values. Although when comparing cardiovascular parameters following BD it may be that stature is of relevance.

This specific study is narrative and acted as a starting point to establish if physiology and stature had an impact regarding BD. It has revealed that significance and strong correlations can be observed, but to fully understand in greater depth parameters such as cardio-respiratory responses, GET, O_2 pulse, HR_{max}, $\dot{V}O_2$ maximally and sub-maximally, they have to be observed before and after BD. This therefore demonstrates the need for further work on the cardio-respiratory responses. Additionally, due to the female cohort being most affected regarding the Hb decreases, this category should be included in future work.

Chapter 6: 3rd Experimental Chapter

Influence of a Standard UK Blood Donation (~470 ml) on Moderate and Heavy Intensity Cycle Exercise Over a Period of 24-96 Hours; Part A – Oxygen Uptake Kinetics

6.1 Introduction

More than a century has passed since Krogh and Lindhard's (1913) pioneering work observed O_2 uptake (and $\dot{V}O_2$ post exercise) and results were obtained that are consistent with more modern experiments. Following this Hill, Long and Lupton (1924) demonstrated the exponential nature of oxygen uptake at the onset of exercise prior to a steady state being achieved. At the commencement of exercise O_2 intake and CO_2 output rise quickly and as demonstrated by Hill and companions the achievement of steady state is dependent on the exercise intensity, with O_2 supply meeting demand at moderate levels of exercise much more readily than at higher levels. A square-wave transition occurs in ATP demand in order to sustain work at the onset of exercise and the desired rate of O_2 uptake is delayed reaching steady state as supply does not immediately match demand. At moderate and heavy intensities steady state is not achieved until 2-3 minutes (Jones and Poole, 2005) and ~6-8 minutes respectively (Gordon, et al., 2010), thus creating an O_2 deficit, a term adopted in 1950 by Hohwü, Christensen and Högberg, which describes the differences of $\dot{V}O_2$ and steady state values post exercise on-set.

There are three phases commonly associated with O_2 uptake kinetics. Phase I concerns cardio-dynamics, phase II concerns muscle $\dot{V}O_2$ and phase III is reaching steady state. Phase I is swift due to the immediate increase in $\dot{V}O_2$ chiefly from the prompt rise of cardiac output ($\dot{Q}$) (Hughson, 2009) pushing blood through the lungs (Whipp and Wasserman, 1972) with little variation in a-vO_{2diff} (Hughson, 2009) as blood returning to the lung has not been exposed to augmented O_2 extraction via the muscle (Burnley and Jones, 2007). Phase II occurs prior to steady state occurring and comprises the exponential rise of $\dot{V}O_2$, as $\dot{Q}$ and a-vO_{2diff} will increase, reflecting the kinetics of muscle O_2 uptake (Burnley and Jones, 2007). Phase III occurs at steady state and includes the slow component when exercising beyond GET and comprises a further increase in $\dot{V}O_2$.

It appears that BD studies fixate on $\dot{V}O_{2max}$ and how it decreases post BD (Balke, et al., 1954; Burnley, et al., 2006; Gordon, et al., 2013; Hill, Vingren and Burdette, 2013). However, people generally work-out/exercise in a sub-maximal nature and thus it is

possibly naïve not to appreciate this area further. In the past studies have been limited to determine the effects of BD on O_2 uptake kinetics. One study appears to exist regarding whether exercise 24 hours post BD adjusts whole body O_2 on-kinetics in the moderate and heavy domains (Gordon, et al., 2010) and another from Burnley and colleagues (2006) report on $\dot{V}O_2$ kinetics pre to post BD (450 ml) in the severe domain. Furthermore, as reported in chapter 3 there are minimal studies that report the acute effects of BD beyond that of 24-48 hours in the sub-maximal domains. Additionally, work from Gordon and colleagues (2010) suggest that it would be interesting to conduct O_2 kinetic analysis accompanied by Near Infrared Spectroscopy (NIRS) to establish if there is a difference pre to post BD within the muscle beds. Therefore, to develop the work of Gordon and colleagues (2010) and to provide additional sub-maximal data as shown to be required in the review by Johnson and colleagues (2019), the aim of this study was to determine the effects of BD (~470 ml) over a period of up to 96 hours on on-transient O_2 uptake kinetics in moderate and heavy exercise domain whilst reviewing metabolic and cardiorespiratory markers and Hb concentration changes in the muscle.

Null hypotheses:

(H_{01}): The phase II response of on-transient O_2 uptake kinetics will not be altered in the moderate domain from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_{02}): The phase III response of on-transient O_2 uptake kinetics will not be altered in the moderate domain from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_{03}): The phase II response of on-transient O_2 uptake kinetics will not be altered in the heavy domain from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_{04}): The phase III response of on-transient O_2 uptake kinetics will not be altered in the heavy domain from donating a unit of blood after 24 hours and across the subsequent 96 hours.

Experimental hypotheses:

(H_1): The phase II response of on-transient O_2 uptake kinetics will show a significant change in the moderate domain from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_2): The phase III response of on-transient O_2 uptake kinetics will show a significant change in the moderate domain from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_3): The phase II response of on-transient O_2 uptake kinetics will show a significant change in the heavy domain from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_4): The phase III response of on-transient O_2 uptake kinetics will show a significant change in the heavy domain from donating a unit of blood after 24 hours and across the subsequent 96 hours.

6.2 Methods

6.2.1 Participants

A statistical power calculation (ClinCalc.com, 2017) determined participant numbers by using previous oxygen kinetics research (Gordon, et al., 2010) results to compare two means for pre BD (14.2 ± 1.5 $g \cdot dL^{-1}$) and post BD (13.1 $g \cdot dL^{-1}$) Hb. A recommended sample size of 14 was calculated using a power of 80 % and critical value for normal distribution set at 1.96.

$$n = \frac{\sigma^2 (Z_{1-\beta} + Z_{1-\alpha/2})^2}{(\mu_0 - \mu_1)^2}$$

Where n is the sample size (14), μ_0 is the population mean μ_1 is the mean of study population, σ^2 is variance in population, α is the probability of type I error (0.05) β is the probability of type II error (0.2) and $\mathcal{Z}$ is the critical $\mathcal{Z}$ vaule for a given $\alpha \, or \, \beta$.

Subsequent to ethical approval (Anglia Ruskin University, Faculty Research Ethics Panel (number FST/FREP/17/727)) and NHS Blood and Transplant approval 15 volunteers of varying statures and ethnicities agreed to participate (age 30.8 ± 10.8 years, mass 80.8 ± 14.7 kg, height 175.3 ± 9.2 cm), however due to unforeseen circumstances at BD and an injury 3 participants were withdrawn. Thus, analysis was based on the remaining 12 participants of differing fitness levels (Table 6.1), 9 male (31.2 ± 12.6 years, mass 84.3 ± 11.1 kg, height 178.3 ± 6.2 cm) and 3 female (age 34.7 ± 10.3 years, mass 67.0 ± 8.54 kg, height 167.1 ± 4.2 cm), aged 22 – 61 years. All procedures conformed to the World Medical Association Declaration of Helsinki (2013, statement 26) and the volunteers provided written informed consent and were directed on their right to withdraw at any point.

Inclusion criteria were that the volunteers needed to be between 18 - 65 years old, able to donate blood (at a NHS donation centre) be regularly physically active, which for adults

between 19 - 64 years can be defined as at least 150 minutes of moderate aerobic activity weekly and strength exercises for 2 days a week or 75 minutes of vigorous aerobic activity weekly and strength exercises for 2 days a week or moderate and vigorous activity for 150 minutes weekly and strength exercises for 2 days a week (NHS, 2018). Additionally, participants should have no contra-indications to exercise and be able to exercise at maximal, moderate and heavy intensities. Exclusion from the study included the inability to donate blood and appearance of contra-indications to the exercise such as a virus or anaemia. Participants were requested to avoid strenuous exercise (defined as exercise that would require more than a day for recovery) 24 hours prior to the $\dot{V}O_{2max}$ test although they were allowed to maintain normal training/activity that would generally be part of their weekly routine. Additionally, participants were asked to arrive hydrated (having consumed a recommended 500 ml of water an hour prior to testing) and eaten a balanced meal (containing carbohydrate, fats and protein) 2-3 hours prior to the test.

Table 6.1: The activity/exercise levels of the participants, stating their age in years, sex, $\dot{V}O_{2max}$ and the number of blood donations (No BD) prior to being a participant.

Participant number	Age & sex (M/F)	No BD	$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	Weekly activity levels
1	32, F	6	44.00	Rugby training 2 x week (90 minutes each session), weights 2 x week (90 minutes each session), 1 run a week, 1 rugby match 1 x week.
2	26, M	2	57.94	Strength training (weights/body exercises) and conditioning (running, boxing or rowing) 4-6 x weekly (45-90 minutes depending on activity)
3	26, M	0	32.44	Weights 5 x week (60-90 minutes each session), cardio as a warm up pre-weights.
4	22, M	0	44.32	Cycle 4 x week (30 minutes each session), run 5 x week (3 miles each session), weight/resistance training 1 x week (80 minutes)
5	23, M	0	38.68	Run 2 x week (20 minutes each session), gym/weight/resistance training 2 x week (60 minutes)
6	33, M	0	54.17	Run 4 x week (2 x 20 and 2 x 50 minutes ~30km per week), weight/resistance training 5 x week (60 minutes), hockey 1 x week (45-70 minutes)
7	46, F	25	41.86	Run 2-3 x week (50-60-minute sessions), spin 1 x week (45 minutes), legs/tums/bums class 1 x week (1 hour), circuits 1 x week (1 hour)
8	20, M	0	43.3	Weight/resistance training 3 x week (90-110 minutes), 1 x 5km run (sub 30 minutes), walking 9-12km throughout the week
9	26, F	0	30.07	Treadmill run 4-5 x week (30-45 minutes each)
10	61, M	10	48.55	Spin 3 x week (45 minutes), legs/tums/bums class 1 x week (1 hour), circuits 1 x week (1 hour), body blast/pump x 2 (60 minutes)
11	32, M	8	41.73	Cycle 1 x week (3 hours) and 1 x week (1 hour), weights 1 x week (45 minutes)
12	38, M	10	52.69	Run 6 x week (45-90 minutes) weight and core training 2-3 x week (30-45 minutes)
Mean ± SD	32.1 ± 11.2	6 ± 7.2	44.15 ± 8.31	

6.2.2 Study Design

All the participants were tested during the morning between the hours of 7.30am and 11am in order to reduce diurnal variation (Scheer, at al., 2010). This occurred over the period of two weeks with a gap of one-two week's in-between, which served as a rest period to avoid a training effect. Week one involved testing prior to BD and week two was testing post BD. Visit one (day 0) of week one served to establish baseline levels in order to take part, haematological levels of the participants by capillary sampling, blood pressure, cardiorespiratory fitness by a $\dot{V}O_{2max}$ test, which served to establish GET in order for intensity levels for the moderate and heavy domains for later visits to be decided. Subsequent visits during week one were for moderate and heavy domain testing, with

week two being almost identical with the exception of the participant donating blood (~470 ml) on the Monday (day 0) of week two. Figure 6.1 depicts the activity according to days. The study design is an experimental before and after self-controlled design, that is the participants acted as their own controls following exposure to BD.

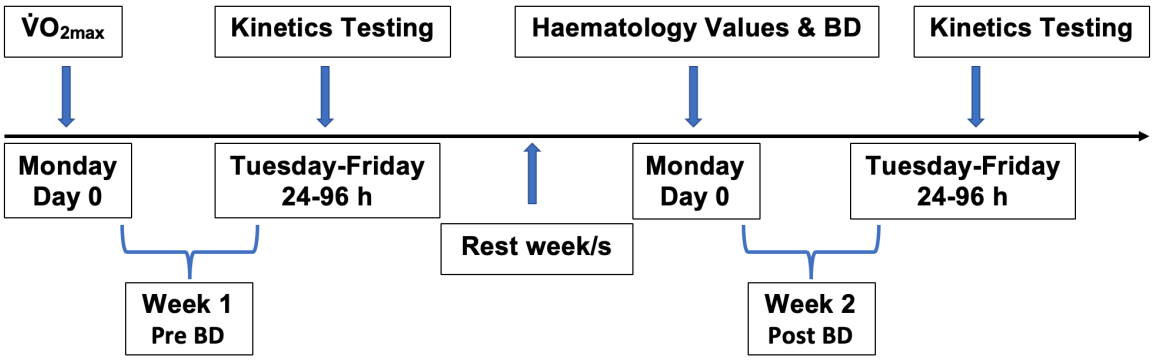

Figure 6.1: Timeline of kinetic testing.

6.2.3 $\dot{V}O_{2max}$ Protocol

All exercise was performed on a Lode Excalibur Sport, pre-calibrated electronically braked cycle (Lode, Groningen, Netherlands). For $\dot{V}O_{2max}$ the participant was instructed to choose a constant cadence between 75 and 95 rpm that they could also maintain throughout the kinetics trials. Refer to page 75-76, chapter 4.9 for more detail.

To collect the gas variables ($\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$ and RER) on a breath-by-breath basis the MetaLyzer® 3B with ergospirometry (Cortex MetaLyzer® 3B, UK) was used. Refer to page 56-57, chapter 4.1 for detail on this piece of equipment.

6.2.4 Determination of Gas Exchange Threshold

Refer to page 76, chapter 4.10 for detail on determining GET.

6.2.5 On-Kinetics Trial – *Pilot Study*

A pilot study aided to establish achievable intensities for the square wave transitions of moderate and heavy domains. Two participants, a male competitive cyclist and runner, (38 years, mass 64.6 kg, height 168 cm, $\dot{V}O_{2max}$ 61.82 $ml \cdot kg^{-1} \cdot min^{-1}$) and a female professional dancer, (19 years, mass 61.6 kg, height 166 cm, $\dot{V}O_{2max}$ 30.91 $ml \cdot kg^{-1} \cdot min^{-1}$) were recruited for this, and conformed to doing more than the physical active guidelines as set by the NHS (2018). Following $\dot{V}O_{2max}$ testing and in accordance with previous work from Gordon

and colleagues (2010) the intensities for the moderate domain were calculated as Δ 80 % (80 % of the difference between rest and GET) and the heavy domain was calculated as Δ 50 % (50 % of the difference between GET and $\dot{V}O_{2max}$). However, although the male participant managed (but felt that it was extremely hard) the female participant found these intensities exceptionally challenging and was not able to complete the moderate domain or heavy domain testing, thus a different strategy was adopted, as the intensities had to be achievable for all participants. Both participants returned for a second day, and the moderate domain was subsequently calculated as Δ 50 % (50 % of the difference between rest and GET) and the heavy domain was calculated as Δ 20 % (20 % of the difference between GET and $\dot{V}O_{2max}$). Both participants successfully completed both domain tests and thus the intensities were adopted for the main trials. It is acknowledged that exercise/fitness ability may have influenced the outcome, however it is also important not to infer that the result was due to gender, but more likely due to the disparity in the demands of their chosen sports, as each sport (cycling/running and dancing) will have different aerobic and anaerobic demands, and as such both participants were "fit" at their own discipline. As previously mentioned, the intensities had to be achievable for all participants and thus using two participants of different sporting abilities allowed for any differentiation required.

6.2.6 On-Kinetics Trials

Subsequent to establishing the domains from the pilot study the $\dot{V}O_2$ on-kinetics trials comprised three square-wave transitions of workload. Baseline pedalling was at 15W sustained for 4 minutes to reduce noise in the breath-by-breath data, as this time allowed for participant adaptation to the ergometer and mouthpiece (Gordon, et al., 2010). Two transitions were applied in the moderate intensity domain (to aid with reliability) and a third was in the heavy intensity domain. The %Δ model is used to normalise exercise intensity (as GET will occur at a different % of $\dot{V}O_{2max}$ for all participants) and thus accounts for $\dot{V}O_2$ at GET and $\dot{V}O2_{max}$ (Jones and Poole, 2005, p.15). Figure 6.2 provides an overview of the testing for the domains and the procedure.

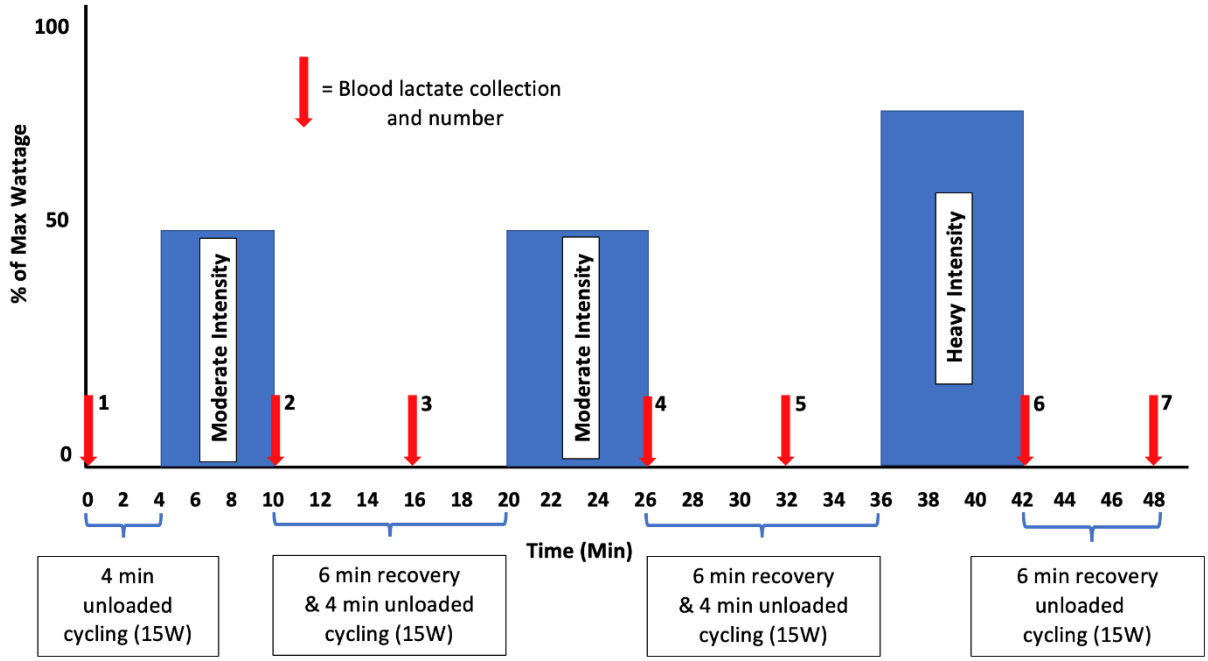

Figure 6.2: A schematic providing an overview of the domain testing activity.

6.2.7 Haematological Responses

Using pre-calibrated equipment (EKF Hemo Control, EKF diagnostic, Germany) capillary blood (~10 µl) from a finger was examined for haemoglobin and haematocrit levels prior to all fitness testing procedures. Refer to page 65, chapter 4.4.1 for detail on procedure. Additionally, each participant made a standard blood donation (~470 ml) at a National Blood Transfusion Service within a few hours of haematological levels being established on the Monday of the second week of testing.

6.2.8 Blood Lactate Responses

Blood lactate (BLa) was taken (Biosen C-Line, EKF diagnostic, Germany) via capillary blood from the fingertip prior to commencement of any exercise to gain a resting value. Additionally, it was taken at the end of each moderate or heavy domain exercise test and again at the end of the recovery period that followed each domain test (Figure 6.2). Refer to page 67, chapter 4.5.1 for detail on procedure.

6.2.9 Near Infrared Spectroscopy (NIRS)

The Portamon was used to observe changes in oxy and de-oxy Hb/myoglobin. Prior to arrival at the laboratory or upon arrival at the laboratory, the area for NIRS application was clean-shaven. Using Harpenden skinfold callipers (British Indicators Ltd, Burgess Hill, UK) skinfold thickness at the site of the NIRS optodes placement was determined prior to probe application to confirm that the measurement depth was approximately half the distance between the transmitter optode and receiver. The value was acquired and divided by two to establish the adipose tissue thickness (fat + skin = ATT) (Van Beekvelt, et al., 2001). The Portamon was placed in the same position at every test, on the right gastrocnemius muscle, on the right-hand side of the muscle belly and at the point of the largest circumference of the muscle. Refer to page 74-75, chapter 4.8.2. for further detail.

The variables recorded included oxygenated Hb (O_2Hb), de-oxygenated Hb (HHb), total haemoglobin (tHb) and tissue oxygenation saturation index (TSI%) (= O_2Hb-HHb). NIRS data was gathered with Oxysoft (Artinis Medical Systems, Netherlands) with a frequency of 10 Hz and expressed relative to baseline. Refer to page 74-75, chapter 4.8.2. for further detail.

6.2.10 Hydration Analysis

Upon arrival for each visit, participants provided a urine sample (2nd void of the day), to establish specific gravity and conformity to fluid intake, by using a pre-calibrated refractometer (Eclipse, Bellingham and Stanley, UK). Refer to page 68, chapter 4.6.1 for detail on procedure.

6.2.11 Blood Pressure and SpO_2 Responses

With the participant sat down, after ~5 minutes of relaxation after arrival at the laboratory, a resting assessment of blood pressure (BP) (Nonin, 2120, USA) prior to Hb capillary blood sampling was taken. SpO_2 (Nonin, 2120, USA) was recorded at the same time as BP (but on the right-hand side of the body). Refer to page 71, chapter 4.7.1 for detail on procedures.

6.2.12 Data Analysis

In accordance with the previous work of Gordon and colleagues (2010) for the analysis of O_2 kinetics, initially the $\dot{V}O_2$ (breath-by-breath) data was time-aligned to the onset of

exercise and smoothed with a 9-point rolling average for each moderate and heavy domain trial. Outlying values (as a consequence of issues such as coughing, sighing or talking) were eradicated if the value was ≥ than ± 2 SD from the mean of the rolling averages. For the modelling of the moderate and heavy kinetic trials subsequent to data smoothing, the following equation was used.

$$\dot{V}O_{2(t)} = \dot{V}O_{2(bsl)} + a_1 \oplus (1 - e^{-(t - TD_1)\tau_1}) + a_2 \oplus (1 - e^{-(t - TD_2)/\tau_2}) + a_3 \oplus (1 - e^{-(t - TD_3)/\tau_3})$$

Where $\dot{V}O_{2(t)}$ denotes the total velocity of oxygen utilization at any given time (t). $\dot{V}O_{2(bsl)}$ denotes the average velocity of oxygen uptake throughout the baseline recording (4 minutes). The other components denote, respectively, the amplitude (a_1), time delay (TD_1) and time constant (τ_1) of phase I; the amplitude (a_2), time delay (TD_2) and time constant (τ_2) of phase II; and the amplitude (a_3), time delay (TD_3) and time constant (τ_3) of the slow component (Gordon, et al., 2010). Furthermore the equation contains exponential terms/functions for example ($1 - e^{-(t - TD_1)\tau_1}$), with e representing the base for the natural logarithm (Whipp and Rossiter., 2005). Additionally, the symbol $\oplus$ represents a logical operation similar to that of addition and refers to direct sum. This three-compartment model was used as it has shown to be reliable in other research from the previous work of Gordon and colleagues (2010). To ensure modelling was as accurately performed as possible, two researchers agreed each kinetic data set generated.

Following modelling of the moderate data the values were combined and averaged in-order to gain one set of values as opposed to two. To determine if this was possible Bland Altman plots were used to assess bias and 95 % limits of agreement between the two sets of quantitative data. With no proportional bias detected a one sample *t*-test was used for further indication of data reliability.

The NIRS data (TSI, O_2Hb, HHb, tHb) was initially smoothed with a 1 s moving average (thus placed into 1 s time bins). To further reduce the “noise” in the data 10 s rolling averages were used. Values gathered were based upon the last 2 minutes of the first unloaded (15 W) section for each domain intensity and expressed relative to resting baseline. The baseline value obtained was an average of the full 2 minutes when the participant was completely inactive. Similarly, to the kinetics data prior to analysis, the moderate data values were combined and averaged to gain one set of data.

6.2.13 Statistical Analysis

All variables collected have values stated as mean ± SD. Following tests for normality (Shapiro-Wilk's) a combination of repeated measures *t*-tests, one-way repeated measures ANOVA (for differences in trial/week 1 (pre BD) or 2 (post BD) and two-way repeated measures ANOVA were utilised to compare within and between trials (time-by-trial). Mauchly's test was used to check sphericity and Greenhouse-Geisser correction applied where necessary. Additionally, Bonferroni correction was applied to avoid Type 1 Error for pairwise (pre to post) comparisons. Effect sizes were also considered; Cohen's *d* was adopted for *t*-tests and partial eta squared (η_p^2) for ANOVA results. Cohens *d* is evaluated as 0.8 - > 2.0 (large), 0.3 - 0.5 (medium) and < 0.2 (small), and conveys the standardized difference in means; expressed as the difference between two (raw) means divided by the pooled standard deviation (SD) of the groups ($M_1 – M_2 / SD_{pooled}$) (Cohen, 1988). Partial eta squared is evaluated as 0.14 (large), 0.06 (medium) and 0.01 (small), conveying the sum of squares of the effect with respect to the sum of squares of the effect in addition to the sum of squares of the error connected with the effect ($\eta_p^2 = SS_{effect} / SS_{effect} + SS_{error}$) (Lakens, 2013). A level of statistical significance was set at $P < 0.05$ and statistical analysis was accomplished using SPSS version 20 (SPSS, Chicago, IL).

6.3 Results

6.3.1 On-Kinetics Trials Responses

The steps taken for the O_2 kinetic modelling in both moderate and heavy trial kinetic responses can be seen in Figure 6.3. When comparing moderate to heavy trials significant differences ($P < 0.05$) can be seen in most components of the O_2 kinetics (Table 6.2). However, few significant differences were observed when comparing pre to post BD in moderate or heavy intensity exercise. Regarding *t*-tests in the moderate trial, tau_2 (time $constant_2$) showed significant difference when comparing pre to post BD values (*t*-test) at 24 h (32.88 ± 9.35 s, 24.88 ± 5.38 s; $P = 0.021$, Cohens $d = 1.10$ (CI, -4.19, 4.14)) and at 48 h (28.77 ± 8.26 s, 23.09 ± 5.29 s; $P = 0.031$, Cohens $d = 0.86$ (CI, -3.81, 3.80)). This was also the case for tau_3 (time $constant_3$) at 72 h (121.20 ± 15.45 s, 137.39 ± 12.52 s; P =0.012, Cohens $d = 1.20$ (CI, -9.94, 5.88)) and at 96 h (127.84 ± 11.58 s, 139.75 ± 20.46 s; $P = 0.32$, Cohens $d = -0.75$ (CI, -7.30, 10.83)) and for TD_3 (time $delay_3$) at 72 h (159.63 ± 23.49 s, 134.25 ± 18.06 s; $P = 0.009$, Cohens $d = 1.27$ (CI, -12.03, 11.48)) and at 96 h (148.54 ± 17.19 s, 130.75 ± 20.46 s; $P = 0.029$, Cohens $d = 0.98$ (CI, -8.74, 12.56)). T-tests in the heavy trial, tau_1 showed significance when comparing pre to post BD values at

72 h (10.76 ± 2.00 s, 9.29 ± 2.05 s; P = 0.038, Cohens d = 0.76 (CI, -0.37, 1.92)), this was also the case for TD_2 (time $delay_2$) at 72 h (17.08 ± 3.18, 14.75 ± 3.25; P = 0.038, Cohens d = 0.76 (CI, -1.04, 2.60)), tau_3 (time $constant_3$) at 24 h (89.77 ± 16.08 s, 100.12 ± 15.09 s; P = 0.21, Cohens d = -0.69 (CI, -9.79, 7.84)) and TD_3 (time $delay_3$) at 24 h (206.67 ± 25.82 s, 191.25 ± 22.94 s; P = 0.034, Cohens d = 0.66 (CI, -13.95, 13.64)) and at 48 h (191.58 ± 31.24 s, 201.67 ± 25.56 s; P = 0.010, Cohens d = -0.37 (CI, -18.04, 14.09)).

Two-way ANOVA results in the moderate domain showed significance between pre to post BD for pairwise comparisons when regarding tau_1 (P = 0.035, η_p^2 = 0.34 (CI, 0.06, 1.46), tau_3 (P = 0.019, η_p^2 = 0.41 (CI, -19.36, -2.14), TD_2 (P = 0.035, η_p^2 = 0.34 (CI, 0.10, 2.32) and TD_3 (P = 0.028, η_p^2 = 0.37 (CI, 2.04, 29.52). One-way ANOVA results for the moderate domain showed little significance for the different variables, however a_3 post BD showed significance (P = 0.010, η_p^2 = 0.29), with pairwise significance between 72 and 96 h (P = 0.025, (CI, 0.10, 2.32). Also, tau_3 was significant (P = 0.041, η_p^2 = 0.22) post BD, but with no pairwise significance between days. TD_3 equalled significance (P = 0.05, η_p^2 = 0.21) post BD, but had no pairwise significance between days and lastly pre BD a_{total} had a non-significant one-way ANOVA, but a significant pairwise comparison between 24 and 96 h (P = 0.043, (CI, -143.16, -1.87). Two-way ANOVA results in the heavy domain showed non-significance across all variables and only the variable a_2 post BD had significance in one-way ANOVA testing, showing significant pairwise comparisons between 24 and 72 h (P = 0.044, (CI, -260.23, -4.06) and 24 and 96 h (P = 0.026, (CI, -224.59, -17.54) despite a non-significant (P > 0.05) ANOVA.

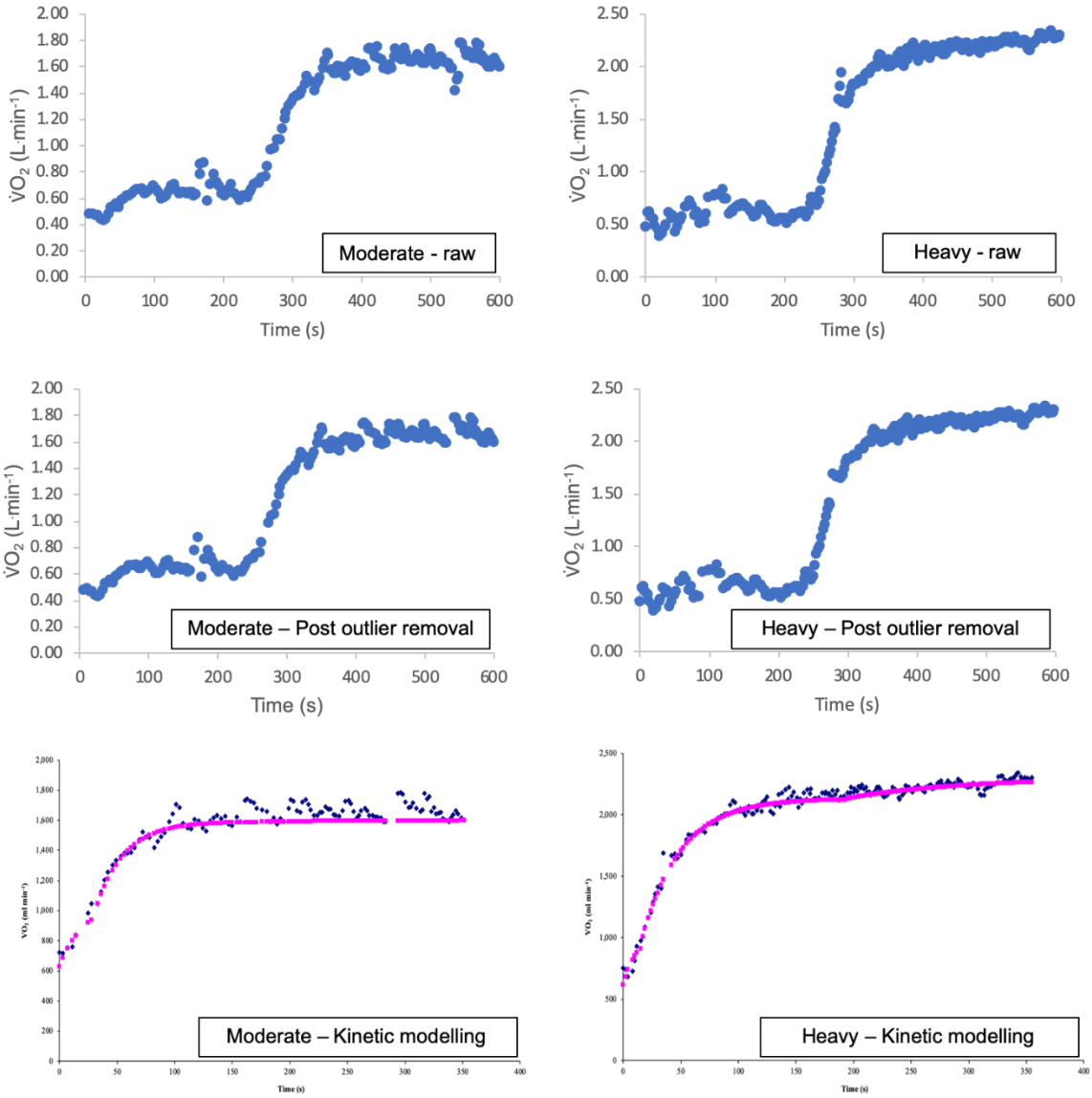

Figure 6.3: Representative outputs from a participant to show the steps taken for the oxygen kinetic modelling. With the top two graphs showing the raw data for moderate and heavy intensity, the middle graphs representing the data post outlier removal and the bottom graphs the kinetic modelling package where the dark blue diamond shapes are representative of the VO_2 and the pink output is the kinetic modelling.

Table 6.2: Mean ± SD for moderate and heavy oxygen uptake kinetics over four days across a two week time period that represented 24, 48, 72 and 96 h pre and post BD.

Variable	Pre BD Moderate Trial (Week 1)				Post BD Moderate Trial (Week 2)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
a_1 ($\dot{V}O_2$ml·min^{-1})	366.77 ±102.85	391.63 ± 115.03	429.00 ± 99.12	386.28 ± 115.17	422.99 ± 68.42	428.11 ± 156.94	378.41 ± 91.05	418.75 ± 180.14
tau_1 (seconds)	11.08 ± 2.39	10.89 ± 2.95	12.26 ± 2.57	10.74 ± 2.18	10.47 ± 2.39	10.87 ± 2.09	10.79 ± 2.05	9.79 ± 2.62
a_2 ($\dot{V}O_2$ml·min^{-1})	1168.77 ± 381.68	1166.08 ± 375.48	1113.55 ± 316.23	1223.04 ± 367.85	1098.41 ± 364.64	1085.05 ± 300.41	1152.06 ± 392.28	1163.94 ± 325.12
tau_2 (seconds)	32.88 ± 9.35	28.77 ± 8.26	31.77 ± 8.63	29.83 ± 7.64	24.88 ± 5.38*	23.09 ± 5.29*	27.88 ± 6.43	27.76 ± 6.59
TD_2 (seconds)	17.58 ± 3.80	17.29 ± 4.68	19.46 ± 4.08	17.01 ± 3.46	16.63 ± 3.80	17.25 ± 3.32	17.13 ± 3.26	15.54 ± 4.15
a_3 ($\dot{V}O_2$ml·min^{-1})	48.98 ± 23.86	42.38 ± 17.72	34.44 ± 18.58	47.72 ± 24.75	52.30 ± 20.82	32.07 ± 21.91	48.64 ± 18.46	35.09 ± 12.81
tau_3 (seconds)	121.43 ± 22.33	130.31 ± 13.77	121.20 ± 15.45	127.84 ± 11.58	128.99 ± 13.09	137.81 ± 17.31	137.39 ± 12.52*	139.75 ± 20.46*
TD_3 (seconds)	157.13 ± 37.93	143.58 ± 21.46	159.63 ± 23.49	148.54 ± 17.19	146.92 ± 19.55	133.83 ± 25.81	134.25 ± 18.06*	130.75 ± 20.46*
a_{total} ($\dot{V}O_2$ml·min^{-1})	1584.52 ± 404.74	1600.09 ± 403.11	1577.41 ± 391.72	1657.03 ± 402.05	1573.71 ± 411.04	1503.56 ± 476.41	1579.11 ± 413.83	1617.78 ± 433.11
	Pre BD Heavy Trial (Week 1)				Post BD Heavy Trial (Week 2)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
a_1 ($\dot{V}O_2$ml·min^{-1})	465.76 ± 163.25$^{\$}$	502.34 ± 213.15	529.18 ± 259.73	527.33 ± 197.90$^{\$\$}$	556.20 ± 203.04$^{\$\$}$	527.22 ± 209.74	464.90 ± 145.31	510.43 ± 153.06$^{\$}$
tau_1 (seconds)	9.87 ± 2.12$^{\$}$	10.08 ± 2.48	10.76 ± 2.00	9.40 ± 1.53$^{\$}$	9.08 ± 2.60	9.87 ± 2.25	9.29 ± 2.05*$^{\$}$	9.50 ± 3.02
a_2 ($\dot{V}O_2$ml·min^{-1})	1893.03 ± 572.03 $^{\$\$\$}$	1939.76 ± 541.60 $^{\$\$\$}$	1871.48 ± 571.13 $^{\$\$\$}$	1907.08 ± 467.53 $^{\$\$\$}$	1773.39 ± 418.45 $^{\$\$\$}$	1819.16 ± 485.93 $^{\$\$\$}$	1905.54 ± 558.79 $^{\$\$\$}$	1894.45 ± 462.88 $^{\$\$\$}$
tau_2 (seconds)	35.36 ± 10.14	37.86 ± 8.67$^{\$}$	35.71 ± 8.29	37.44 ± 7.50$^{\$\$}$	35.38 ± 7.49$^{\$\$}$	35.56 ± 6.23$^{\$\$\$}$	32.69 ± 9.36	35.24 ± 11.01$^{\$}$
TD_2 (seconds)	15.67 ± 3.37$^{\$}$	16.00 ± 3.93	17.08 ± 3.18	14.92 ± 2.43$^{\$}$	14.42 ± 4.12	15.67 ± 3.58	14.75 ± 3.25*$^{\$}$	15.08 ± 4.80
a_3 ($\dot{V}O_2$ml·min^{-1})	297.31 ± 110.39 $^{\$\$\$}$	294.58 ± 142.46 $^{\$\$\$}$	347.46 ± 163.05 $^{\$\$\$}$	299.63 ± 162.09 $^{\$\$\$}$	281.65 ± 149.35 $^{\$\$\$}$	271.18 ± 118.63 $^{\$\$\$}$	283.12 ± 111.55 $^{\$\$\$}$	345.11 ± 161.45 $^{\$\$\$}$
tau_3 (seconds)	89.77 ± 16.08$^{\$\$\$}$	100.07 ± 21.70$^{\$\$\$}$	100.49 ± 15.68$^{\$}$	95.60 ± 20.94$^{\$\$\$}$	100.12 ± 15.09 *$^{\$\$}$	97.12 ± 15.92$^{\$\$\$}$	93.50 ± 17.13$^{\$\$\$}$	94.71 ± 13.09$^{\$\$\$}$
TD_3 (seconds)	206.67 ± 25.82$^{\$\$\$}$	191.58 ± 31.24$^{\$\$\$}$	196.50 ± 25.14$^{\$\$}$	202.67 ± 31.53$^{\$\$\$}$	191.25 ± 22.94*$^{\$\$\$}$	201.67 ± 25.56*$^{\$\$\$}$	206.33 ± 27.05$^{\$\$\$}$	205.58 ± 21.31$^{\$\$\$}$
a_{total} ($\dot{V}O_2$ml·min^{-1})	2656.11 ± 665.56 $^{\$\$\$}$	2736.68 ± 683.99 $^{\$\$\$}$	2748.12 ± 682.40 $^{\$\$\$}$	2734.04 ± 619.52 $^{\$\$\$}$	2611.24 ± 562.36 $^{\$\$\$}$	2617.97 ± 623.44 $^{\$\$\$}$	2653.55 ± 620.33 $^{\$\$\$}$	2750.00 ± 652.60 $^{\$\$\$}$

** $P < 0.05$ denotes a significant difference from Pre BD to Post BD.*
$^{\$}$ $P < 0.05$, $^{\$\$}$ $P < 0.0$, $^{\$\$\$}$ $P < 0.001$ denotes a significant difference from moderate to heavy kinetics.

6.3.2 Haematological (Hb and Hct) Responses

Figure 6.4 depicts the heterogeneous values of Hb across participants and Figure 6.5 shows the mean Hb values across each week. Table 6.3 summarises the haematological responses pre and post BD, with two-way ANOVA pairwise comparisons for Hb between week 1 and 2 reporting significance ($P = 0.000$, $\eta_p^2 = 0.83$, (CI, 0.71, 1.31) between trials. Significant differences were absent in the one-way ANOVA pre BD (week1), but was present for post BD (week 2) ($P = 0.000$, $\eta_p^2 = 0.77$). Pairwise comparisons of each time point in Hb levels against 0 h (BD day) showed significance ($P = 0.000$); resulted in a 7.3

% Hb decrease from time 0 to 24 h, also a decrement from 24 to 48 h of 2.3 %, a further reduction of 2.1 % from 48 to 72 h and between 72 and 96 h a 1.5 % increase occurred. This was similar for Hct when moving through the time points as previously described for Hb, with the decrements of 7.2, 2.3, 2.6 % and an increase of 1.9 % when compared to baseline. T-test analysis was significant post BD at 24, 48, 72 and 96 h (P < 0.000 respectively). A comparison of the mean values of pre BD (14.48 ± 0.16 g·dL^{-1}) to post BD (13.47 ± 0.66 g·dL^{-1}) showed significance (P = 0.01, Cohens d = 2.20 (CI, 2.11, 2.57)).

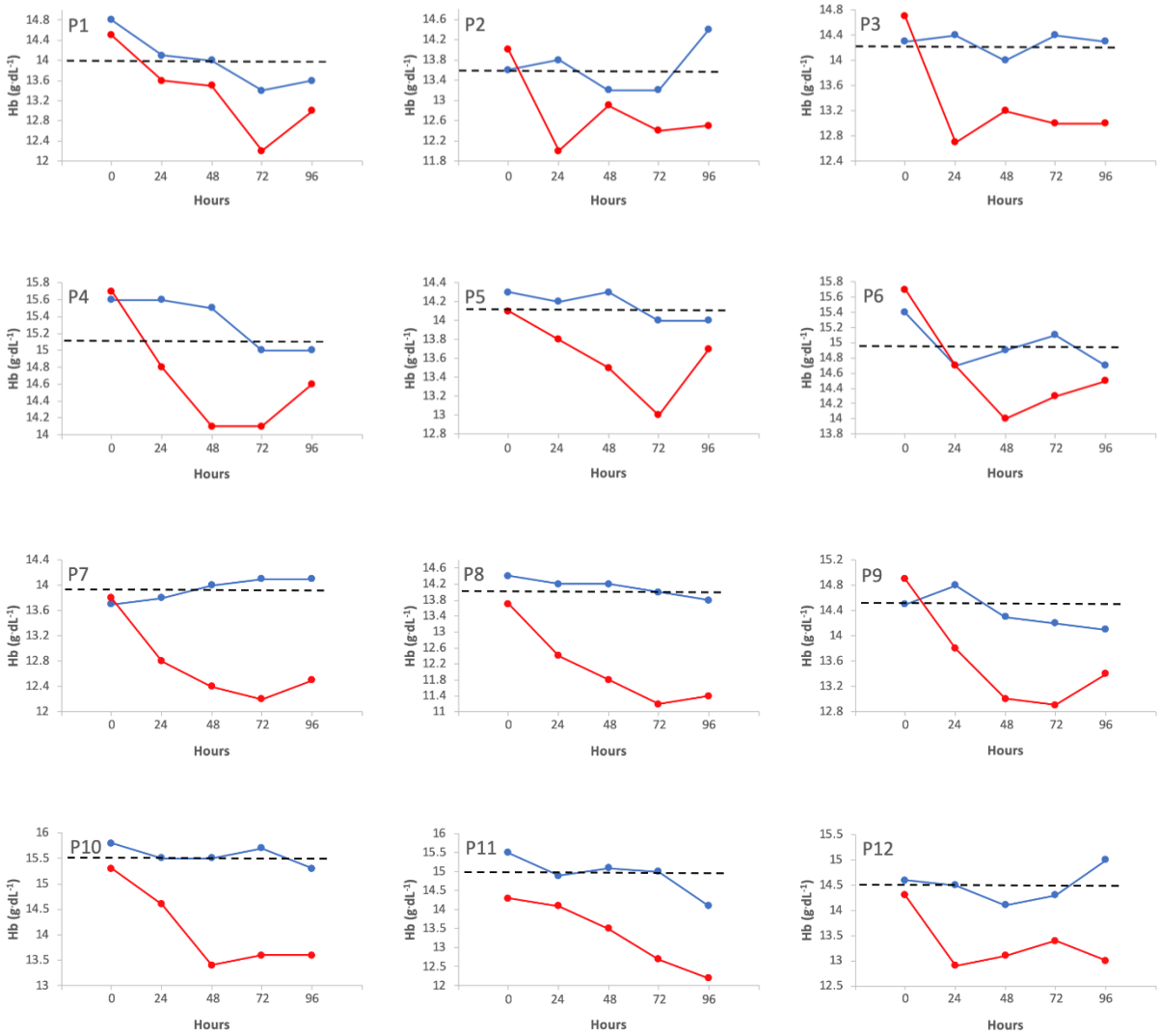

Figure 6.4: The Hb values for all participants throughout the testing period. With the blue line representing week 1 and the red line representing week 2. The black dotted line shows the average Hb for week 1. The zero on the x-axis represents day 0 (the Hb value obtained prior to BD).

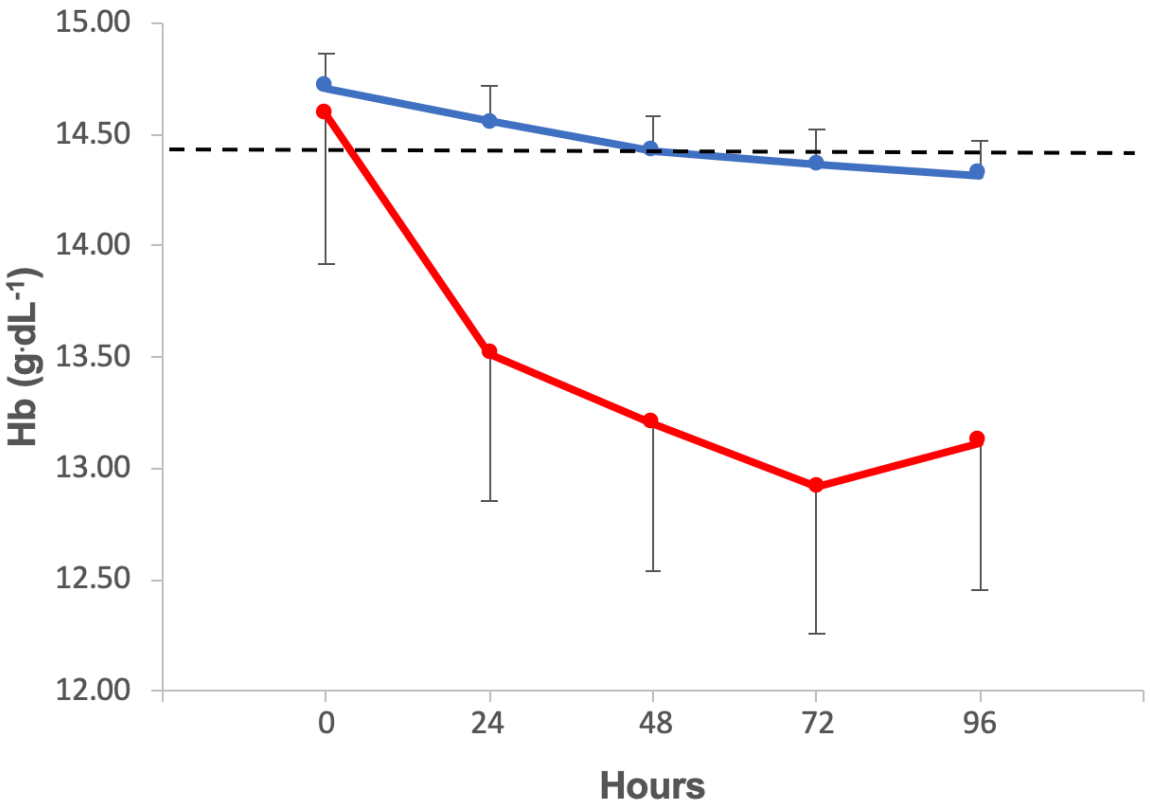

Figure 6.5: Mean pre and post BD Hb values over the two-week testing period. The blue Hb responses are the mean pre BD (week 1) values and the dotted black line represents the mean value for that week. Mean Hb values for post BD (week 2) is shown in red. The zero on the x-axis represents day 0 (the Hb value obtained prior to BD).

Table 6.3: Mean ± SD Hb and Hct values over the two-week testing period.

Time h	Hb (g·dL^{-1}) Wk 1(Pre BD)	Hb (g·dL^{-1}) Wk 2 (Post BD)	Hct (%) Wk 1(Pre BD)	Hct (%) Wk 2(Post BD)
0	14.71 ± 0.73	14.58 ± 0.69	43.25 ± 2.18	42.83 ± 2.04
24	14.56 ± 0.58	13.52 ± 0.95***	42.92 ± 1.78	39.75 ± 2.93***
48	14.43 ± 0.69	13.20 ± 0.64***	42.33 ± 2.02	38.83 ± 1.85***
72	14.37 ± 0.73	12.92 ± 0.87***	42.08 ± 2.11	37.83 ± 2.48***
96	14.32 ± 0.61	13.12 ± 0.92***	41.92 ± 1.78	38.58 ± 2.64***
Mean ± SD	14.48 ± 0.16	13.47 ± 0.66*	42.50 ± 0.57	39.57 ± 1.95*

****P < 0.001 denotes a significant difference from 0 h through to 96 h of Wk 2*

** P < 0.05 denotes a significant difference of the mean pre to post BD*

6.3.3 Correlational Responses Between Haemoglobin and Kinetic Variables

The correlation (r) values between Hb and the kinetic variables can be viewed in Table 6.4, with the largest effects generally being observed in a_3.

Table 6.4: Table 6.4: Correlations of Δ Hb (pre BD to 24, 48, 72 and 96h post BD) against Δ kinetic variable (e.g. the difference between Wk1, 24 h of tau_1 and Wk2, 24 h of tau_1).

Variable	Moderate Trial Correlation (r) (time post BD)				Heavy Trial Correlation (r) (time post BD)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
a_1 ($\dot{V}O_2$ml·min^{-1})	0.08	-0.05	-0.17	0.05	0.17	0.01	-0.34	0.17
tau_1 (seconds)	-0.24	0.02	-0.24	0.21	-0.39	-0.29	0.07	-0.31
a_2 ($\dot{V}O_2$ml·min^{-1})	0.26	0.03	-0.15	-0.26	-0.17	-0.17	0.13	-0.06
tau_2 (seconds)	-0.03	0.16	-0.29	0.09	0.16	-0.27	0.05	-0.09
TD_2 (seconds)	-0.24	0.02	-0.24	0.21	-0.39	-0.29	0.07	-0.31
a_3 ($\dot{V}O_2$ml·min^{1})	-0.18	-0.32	0.49	0.76	-0.38	-0.46	0.36	0.49
tau_3 (seconds)	0.39	-0.07	0.23	-0.23	0.17	-0.11	-0.05	-0.08
TD_3 (seconds)	-0.13	-0.01	-0.23	0.26	-0.32	-0.01	0.06	0.07
a_{total} ($\dot{V}O_2$ml·min^{-1})	0.39	0.00	-0.20	-0.21	0.39	-0.34	0.08	0.21

6.3.4 Blood Lactate Responses

Table 6.5 summarises the lactate responses pre and post BD and the mean lactate profile is shown in Figure 6.6. Pairwise comparisons between pre and post BD reported non-significance (P = .227) between trials. Significance was seen when moving from resting through the testing phases at each time point (as reported in Table 6.5), additionally the resting lactate appeared to significantly (P = .041, Cohens d = -0.92 (CI, -1.16, -0.67)) differ at the 24 h time points for pre (1.15 ± 0.42 mM) to post (1.53 ± 0.44 mM) BD and similarly, there was significant difference (pre BD=10.56 ± 2.08, post BD=11.39 ± 2.28; P = .027, Cohens d = -0.40 (CI, -1.57, 0.89)) immediately after the heavy kinetics trial (Table 6.5) at 48 h.

Table 6.5: Mean ± SD lactate values over the two-week period of the kinetics testing.

Hours	Wk 1 - Pre BD Lactate (mM)						
	Resting no 1	Post M1 no 2	Post M1R no 3	Post M2 no 4	Post M2R no 5	Post H no 6	Post HR no 7
24	1.15 ± 0.42	4.05 ± 1.26***	2.85 ± 1.24*	3.76 ± 1.50**	2.56 ± 1.38	10.94 ± 2.38***	10.01 ± 2.67***
48	1.34 ± 0.50	3.74 ± 1.18**	2.74 ± 1.24	3.61 ± 1.48*	2.43 ± 1.37	10.56 ± 2.08***	9.70 ± 2.76***
72	1.23 ± 0.37	3.74 ± 1.16***	2.62 ± 1.21*	3.47 ± 1.53**	2.50 ± 1.30	10.54 ± 2.12***	9.51 ± 2.73***
96	1.18 ± 0.32	3.79 ± 1.06***	2.77 ± 1.18**	3.68 ± 1.64**	2.68 ± 1.46	10.61 ± 2.40***	9.75 ± 2.83***
Mean ± SD	1.22 ± 0.09	3.83 ± 0.15***	2.74 ± 0.10*	3.63 ± 0.12**	2.54 ± 0.10	10.66 ± 0.19***	9.74 ± 0.20***
Hours	**Wk 2 - Post BD Lactate (mM)**						
	Resting no 1	Post M1 no 2	Post M1R no 3	Post M2 no 4	Post M2R no 5	Post H no 6	Post HR no 7
24	1.53 ± 0.44$	4.55 ± 1.28***	3.17 ± 1.21**	4.28 ± 1.74**	3.00 ± 1.39*	11.70 ± 2.35***	10.34 ± 2.48***
48	1.23 ± 0.45	3.99 ± 1.10***	2.72 ± 0.90**	3.88 ± 1.39**	2.55 ± 1.09*	11.39 ± 2.28$***	10.31 ± 2.68***
72	1.26 ± 0.41	3.93 ± 1.10***	2.67 ± 1.02**	3.64 ± 1.27**	2.39 ± 1.03	10.73 ± 2.07***	9.85 ± 2.48***
96	1.28 ± 0.48	3.81 ± 0.96***	2.64 ± 0.94*	3.73 ± 1.40**	2.61 ± 1.11*	10.57 ± 1.65***	9.87 ± 2.09***
Mean ± SD	1.32 ± 0.14	4.07 ± 0.33***	2.80 ± 0.25**	3.88 ± 0.28**	2.64 ± 0.26*	11.10 ± 0.53***	10.09 ± 0.27***

N.B. Post M1 denotes immediately after the 1st moderate kinetics trial, Post M1R denotes after the 1st moderate kinetics trial 6 mins recovery period, Post M2 denotes immediately after the 2nd moderate kinetics trial, Post M2R denotes after the 2nd moderate kinetics trial 6 mins recovery period, Post H denotes immediately after the heavy kinetics trial and Post HR denotes after the heavy kinetics trial 6 mins recovery period.

** P < 0.05 and **P < 0.01 and ***P < 0.001 denotes a significant difference between daily resting values and the various time points throughout the testing day.*

$ P < 0.05 denotes a significant difference between Pre BD and Post BD for the relative pair e.g. 24 h resting pre to 24 h resting post BD

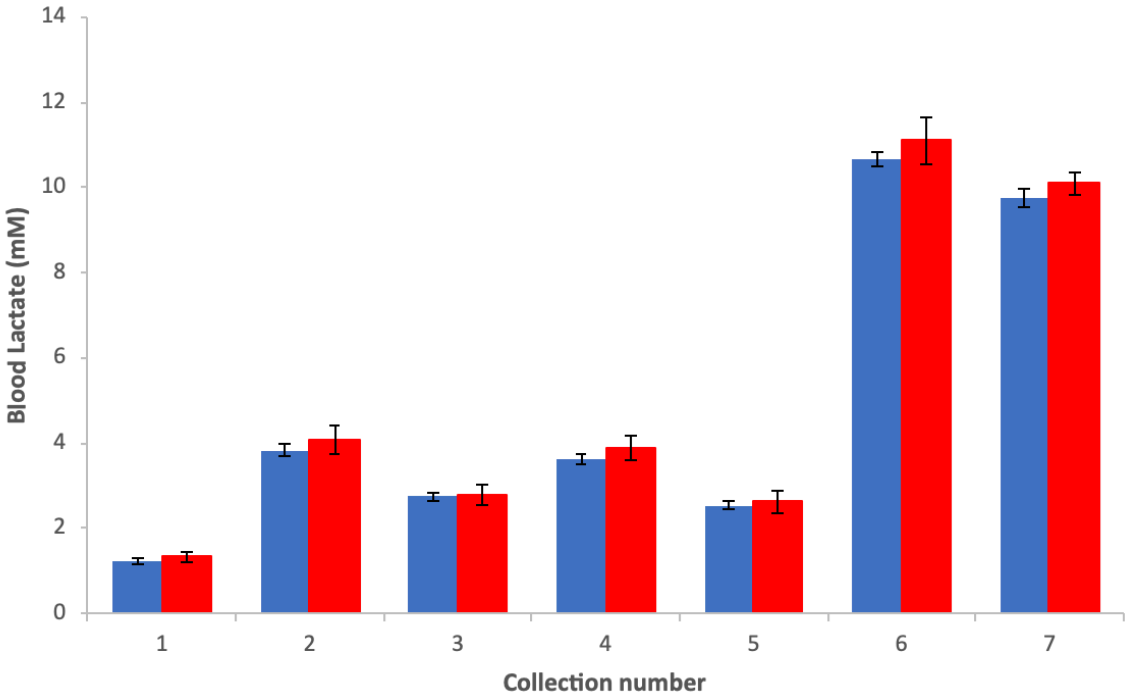

Figure 6.6: The mean lactate profile over the seven collection points (of resting, PostM1, PostM1R, PostM2, PostM2R, PostH & PostHR) throughout the kinetic trials, with the blue data being representative of week one (pre BD) and red representative of week two (post BD).

6.3.5 Near Infrared Spectroscopy (NIRS) Responses

Due to equipment failure, the results for this section are from 6 participants (1 female and 5 males) age 34.8 ± 14.2 years, height 175.2 ± 6.7 cm, mass 79.2 ± 9.7 kg. The acquired ATT obtained from all 6 participants was within the maximum tolerable 15-20 mm depth for optimum optode penetration, with the values ranging from 1.9 mm to 10.8 mm, with an average depth of 5.9 ± 3.1 mm. Figure 6.7 shows an example of a full NIRS response for a single participant pre and post BD. Table 6.6 represents results from TSI, O_2Hb, HHb and tHb.

Figure 6.8 shows that at each time point the TSI decreases from both baseline and baseline unloaded (15W cycling) in both exercise domains. In all cases except 96 h post BD TSI increases from baseline to unloaded cycling and decreases from moderate to heavy. Indeed, one-way ANOVA shows significant decreases in pairwise comparisons from moderate to heavy intensity in the following: pre BD 24 h (P = 0.013 (CI, 1.61, 10.11), pre BD 96 h (P = 0.028 (CI, 0.90, 12.50), post BD 24 h (P < 0.020 (CI, 0.98L 8.99), post BD 48 h (P = 0.020 (CI, 0.92, 8.69) and post BD 96 h (P = 0.005 (CI, 1.66, 6.64). Two-way ANOVA for TSI showed no significant difference between pre BD and post BD in pairwise comparisons. However, a *t*-test pre to post BD found a significant decrease in TSI at 96 h

during the moderate domain 65.58 ± 7.49 v 63.05 ± 8.03 % (*P* = 0.006, Cohens *d* = 0.36 (CI, -5.64, 6.78).

Figure 6.9 shows resting baseline O_2Hb values close to zero and also the decreasing nature of O_2Hb as the exercise intensity increases. One-way and two-way ANOVA tests showed no significant differences. However, *t*-testing of resting baseline between pre BD and post BD at 24 h showed a significant decrease 1.70 ± 1.74 v -0.35 ± 1.17 µM.cm (*P* = 0.039, Cohens *d* = 1.51 (CI, 0.12, 2.45)) and a significant decrease was seen at 48 h in the heavy domain -5.71 ± 8.42 v -2.01 ± 5.56 µM.cm (*P* = 0.046, Cohens *d* = -0.57 (CI-7.31, 3.88)). Contrasting to O_2Hb, HHb shows a cumulative nature as intensity increases from baseline unloaded cycling (Figure 6.10). One-way ANOVA showed a significant increase in a pairwise comparison from moderate to heavy intensity in pre BD at 48 h only (*P* = 0.049 (CI, -7.92, -0.02). Two-way ANOVA showed two significant differences in pairwise comparisons between pre BD and post BD at 24 h (*P* = 0 .028, η_p^2 = 0.65 (CI, -5.28 -0.45)) and at 96 h (*P* = 0 .048, η_p^2 = 0.58 (CI, -3.76 0.03)). Similarly to HHb, tHb increases from baseline unloaded cycling (Figure 6.11). One-way ANOVA tests showed significant increases in the following: pre BD at 48 h baseline unloaded cycling to moderate intensity (*P* = 0.023 (CI, -5.24, -0.49)), post BD at 48 h resting baseline to heavy intensity (*P* = 0.045 (CI, -16.10, -0.21) and baseline unloaded cycling to heavy intensity (*P* = 0.032 (CI, -12.07 -0.65)) and post BD at 72 h resting baseline to heavy intensity (*P* = 0.044 (CI, -7.92, -0.12)). Two-way ANOVA showed only one significant pairwise comparison between pre and post BD, which was for 48 h (*P* = 0 .023, η_p^2 = 0.68 (CI, -7.09 -0.80)). T-tests from pre to post BD showed a tendency toward a significant decrease at 24 h for unloaded cycling 1.32 ± 7.16 v -4.61 ± 5.63 µM.cm (*P* = 0.050, Cohens *d* = 1.01 (CI, -4.72, 5.51)), a significant increase at 48 h for moderate intensity 2.28 ± 5.19 v 7.82 ± 4.81 µM.cm (*P* = 0.018, Cohens *d* = -1.21 (CI, -5.37, 2.64)) and a significant increase at 48 h for heavy intensity 3.51 ± 5.98 v 9.68 ± 4.72 µM.cm (*P* = 0.038, Cohens *d* = -1.25 (CI-6.04, 2.52)).

Table 6.6: Pre and post mean ± SD values for the NIRS variables. With activity levels represented as follows: B = baseline resting values, UC = unloaded 15 W cycling, M = moderate intensity, H = heavy intensity.

Hour & Activity Level	TSI (%)		O_2Hb (µM.cm)		HHB (µM.cm)		tHB (µM.cm)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
24 - B	68.57 ± 3.82	67.98 ± 2.87	1.70 ± 1.74	-0.35 ± 1.17*	0.31 ± 1.02	-0.06 ± 1.63	2.01 ± 2.67	-0.42 ± 2.56
24 - UC	70.37 ± 5.08	70.26 ± 2.84	2.17 ± 3.85	-1.27 ± 3.74	-0.86 ± 3.40	-3.34 ± 2.77	1.32 ± 7.16	-4.61 ± 5.63*
24 - M	65.27 ± 9.68	66.01 ± 6.33	-0.12 ± 9.10	-2.93 ± 3.41	5.71 ± 9.45	1.77 ± 6.86	5.59 ± 7.39	-1.16 ± 7.19
24 - H	59.41 ± 11.35	61.02 ± 8.41	-4.23 ± 10.02	-5.80 ± 4.23	10.99 ± 13.03	6.33 ± 9.61	6.76 ± 8.78	0.53 ± 7.41
M – UC (Δ)	-5.10 ± 6.03	-4.25 ± 4.54	-2.30 ± 8.69	-1.66 ± - 5.16	6.57 ± 8.94	5.11 ± 5.58	4.27 ± 3.37	3.45 ± 2.59
H – UC (Δ)	-10.96 ± 7.69	-9.24 ± 6.40	-6.41 ± 10.53	-4.53 ± 7.06	11.85 ± 12.36	9.67 ± 8.41	5.44 ± 6.52	5.14 ± 3.88
48 - B	68.08 ± 3.21	68.22 ± 3.91	0.61 ± 1.51	0.53 ± 1.94	0.76 ± 0.80	0.99 ± 1.67	1.37 ± 1.84	1.53 ± 2.89
48 - UC	69.82 ± 3.73	70.30 ± 4.29	0.63 ± 2.38	2.97 ± 2.08	-1.21 ± 2.82	0.35 ± 2.43	-0.58 ± 4.86	3.32 ± 1.78
48 - M	64.51 ± 9.52	64.89 ± 8.18	-2.97 ± 8.34	1.14 ± 4.22	5.25 ± 10.28	6.68 ± 5.71	2.28 ± 5.19	7.82 ± 4.81*
48 - H	59.53 ± 10.52	60.09 ± 10.01	-5.71 ± 8.42	-2.01 ± 5.56*	9.22 ± 11.24	11.69 ± 8.79	3.51 ± 5.97	9.68 ± 4.72*
M – UC (Δ)	-5.31 ± 6.75	-5.40 ± 4.96	-3.60 ± 8.92	-1.83 ± 4.76	6.46 ± 9.27	6.33 ± 5.34	2.86 ± 1.38	4.50 ± 3.41
H – UC (Δ)	-10.29 ± 7.53	-10.21 ± 6.88	-6.33 ± 9.61	-4.98 ± 6.51	10.43 ± 10.33	11.34 ± 8.71	4.10 ± 5.18	6.36 ± 3.32
72 - B	69.16 ± 3.66	67.59 ± 2.72	0.32 ± 1.46	0.21 ± 1.67	0.10 ± 1.38	0.87 ± 1.09	0.42 ± 2.54	1.08 ± 2.15
72 - UC	69.86 ± 4.67	70.15 ± 3.27	-1.17 ± 2.85	1.31 ± 1.33	-0.70 ± 2.30	-1.04 ± 2.79	-1.87 ± 4.97	0.27 ± 3.96
72 - M	64.13 ± 10.17	63.94 ± 6.81	-2.54 ± 7.78	-0.42 ± 4.43	6.05 ± 9.16	4.58 ± 3.40	3.51 ± 6.92	4.16 ± 2.87
72 - H	60.47 ± 10.56	59.28 ± 9.27	-4.40 ± 8.26	-6.30 ± 4.81	9.97 ± 10.54	9.58 ± 6.60	5.57 ± 7.85	3.28 ± 6.02
M – UC (Δ)	-5.73 ± 6.31	-6.21 ± 5.02	-1.37 ± 7.11	-1.72 ± 3.95	6.75 ± 6.07	5.62 ± 4.45	5.38 ± 3.21	3.89 ± - 2.19
H – UC (Δ)	-9.39 ± 6.31	-10.88 ± 6.98	-3.24 ± 8.54	-7.61 ± 4.44	10.67 ± 9.15	10.62 ± 7.88	7.44 ± 6.05	3.02 ± 7.44
96 - B	68.91 ± 2.89	69.75 ± 4.94	-0.53 ± 2.09	0.30 ± 1.47	0.39 ± 3.46	0.80 ± 3.68	-0.14 ± 5.45	1.10 ± 3.18
96 - UC	70.20 ± 3.55	68.35 ± 9.07	-0.90 ± 2.05	0.17 ± 5.36	-1.79± 2.16	1.71 ± 4.13	-2.69 ± 3.63	1.87 ± 5.28
96 - M	65.58 ± 7.49	63.05 ± 8.03**	-1.78 ± 3.77	-2.94 ± 9.69	3.86 ± 6.14	7.35 ± 5.84	2.08 ± 3.57	4.40 ± 6.10
96 - H	58.90 ± 10.83	58.90 ± 8.58	-4.60 ± 8.60	-5.69 ± 10.53	9.85 ± 10.49	10.02 ± 6.27	5.25 ± 6.45	4.33 ± 7.32
M – UC (Δ)	-4.62 ± 4.76	-5.31 ± - 6.77	-0.87 ± 5.47	-3.11 ± 8.74	5.65 ± 5.83	5.64 ± 7.11	4.77 ± - 3.65	2.53 ± 2.87
H – UC (Δ)	-11.30 ± 7.97	-9.45 ± - 5.84	-3.70 ± 10.28	-5.86 ± 9.05	11.64 ± 10.34	8.31 ± 7.30	7.95 ± 7.54	2.46 ± 3.79

** P < 0.05 and **P < 0.01 denotes a significant difference between pre BD (week 1) and post BD (week 2).*

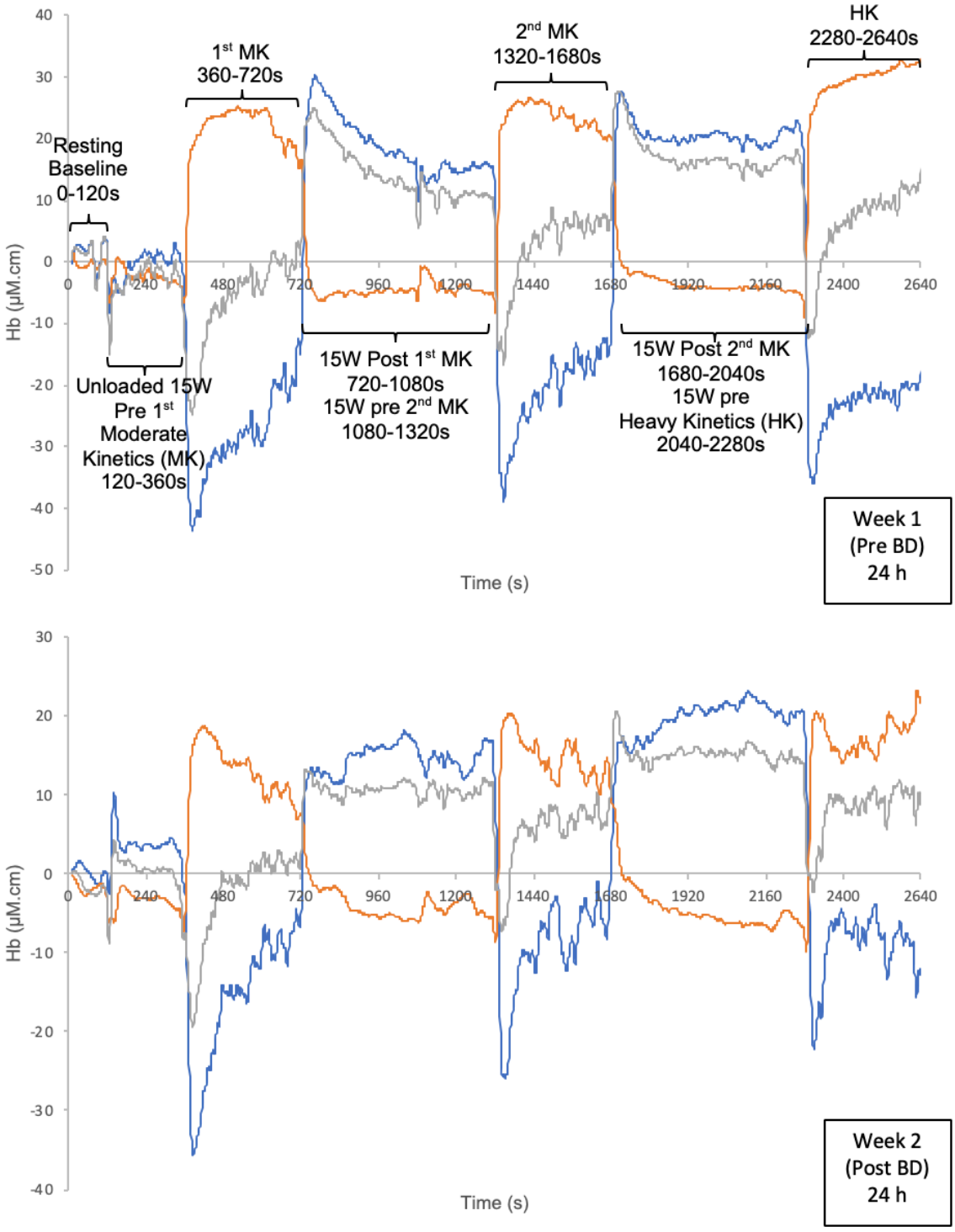

Figure 6.7: One participant's NIRS output for pre and post BD for a testing day (24 h), showing oxygenated Hb (O_2Hb) in blue, de-oxygenated Hb (HHb) in orange and total Hb (tHb) in grey. With the top graph annotated to show the various stages of testing.

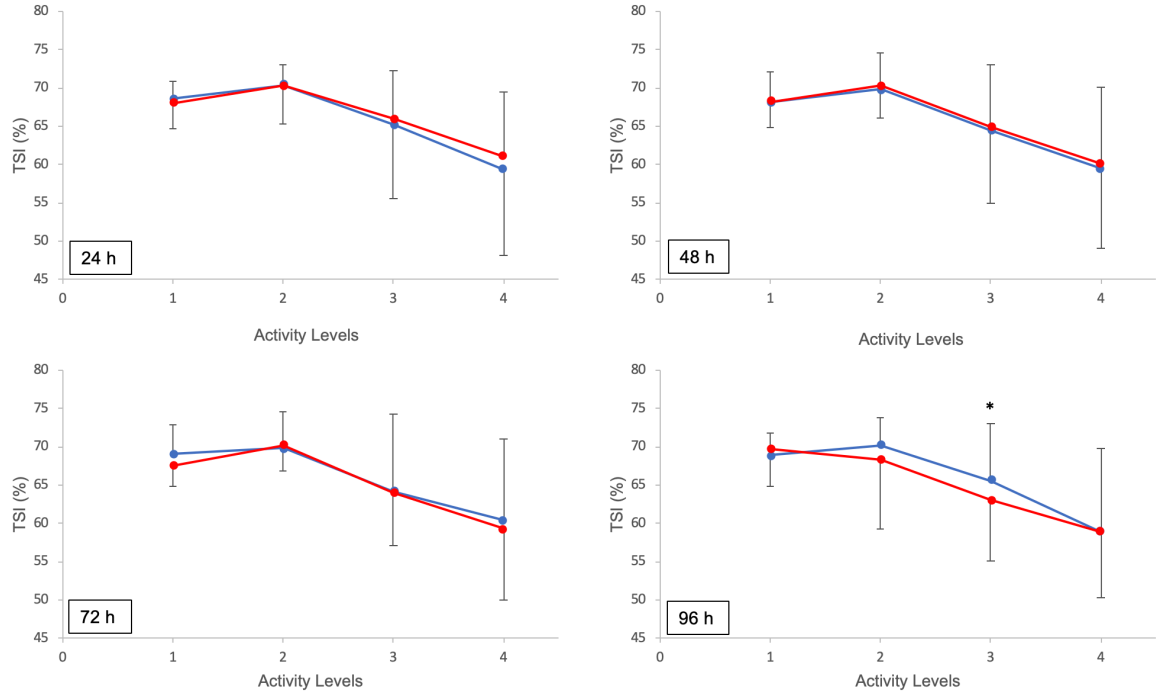

Figure 6.8: The TSI values throughout the course of the 96 h time period. With blue being representative of pre BD and red representative of post BD 4 activity levels. The activity levels equate to the following 1 = resting baseline, 2 = unloaded 15W cycling, 3 = moderate intensity and 4 = heavy intensity. The asterisk represents P < 0.05.

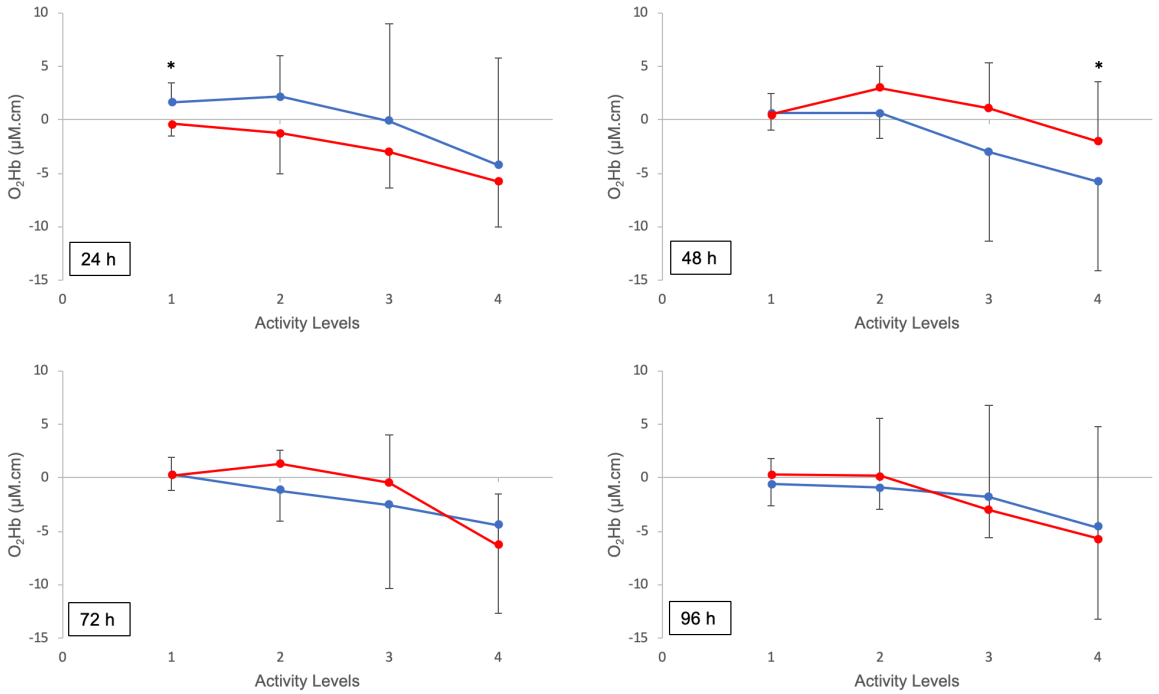

Figure 6.9: The O_2Hb values throughout the course of the 96 h time period. With blue being representative of pre BD and red representative of post BD 4 activity levels. The activity levels equate to the following 1 = resting baseline, 2 = unloaded 15W cycling, 3 = moderate intensity and 4 = heavy intensity. The asterisks represent P < 0.05.

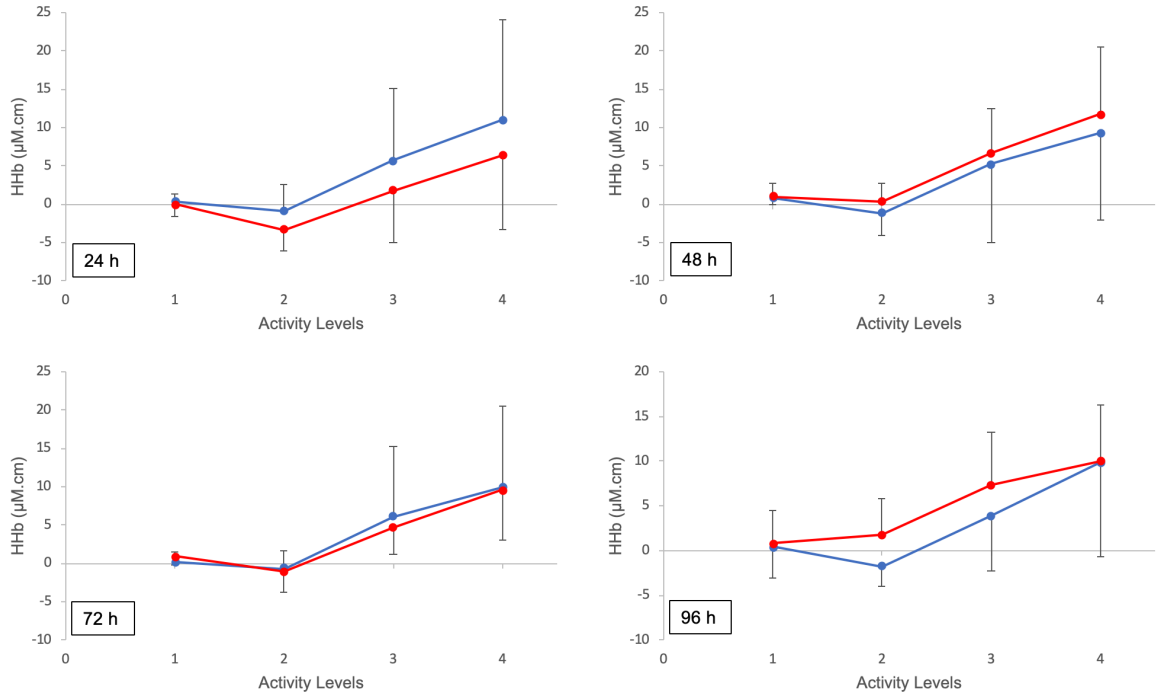

Figure 6.10: The HHb values throughout the course of the 96 h time period. With blue being representative of pre BD and red representative of post BD 4 activity levels. The activity levels equate to the following 1 = resting baseline, 2 = unloaded 15W cycling, 3 = moderate intensity and 4 = heavy intensity.

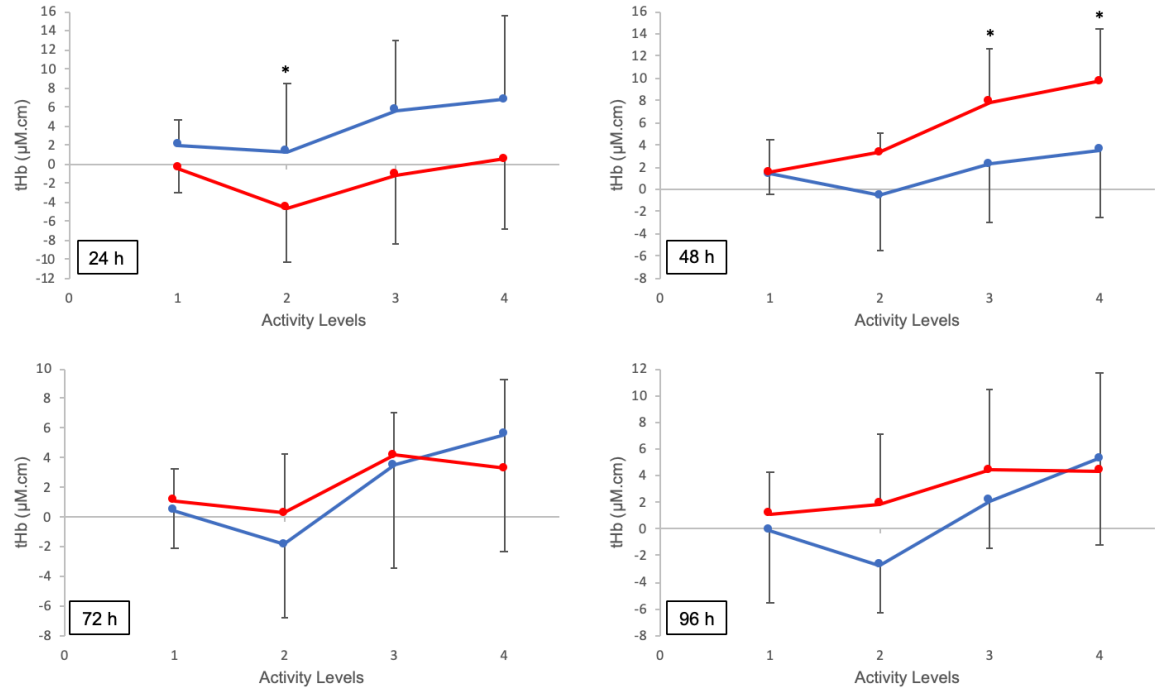

Figure 6.11: The tHb values throughout the course of the 96 h time period. With blue being representative of pre BD and red representative of post BD 4 activity levels. The activity levels equate to the following 1 = resting baseline, 2 = unloaded 15W cycling, 3 = moderate intensity and 4 = heavy intensity. The asterisks represent P < 0.05.

6.3.6 Hydration Analysis

Specific gravity of urine ranged from 1.002 to 1.031 U_{sg} with the mean and SD values being 1.016 ± 0.001 U_{sg} for pre BD, 1.018 ± 0.007 U_{sg} for post BD and 1.017 ± 0.005 U_{sg} throughout the testing period (Table 6.7). Pairwise comparisons between pre and post BD showed no significance (P = 0.295), this was also the case when comparing various time points for example post BD, time 0 (BD day) against 24 h (P = 0.183).

Table 6.7: The mean ± SD specific gravity of urine values.

Time h	Specific gravity (U_{sg}) Wk 1(Pre BD)	Specific gravity (U_{sg}) Wk 2 (Post BD)
0	1.016 ± 0.008	1.016 ± 0.008
24	1.016 ± 0.008	1.014 ± 0.007
48	1.018 ± 0.008	1.016 ± 0.009
72	1.016 ± 0.008	1.016 ± 0.009
96	1.016 ± 0.008	1.015 ± 0.009
Mean ± SD	1.016 ± 0.001	1.018 ± 0.007

6.3.7 Blood Pressure and SpO_2 Responses

Pairwise comparisons for BP between pre and post BD showed no significance in either systolic or diastolic measurements (P = 0.767 and 0.274 respectively). All other ANOVA results were also non-significant (P > 0.05). All BP measurements were below hypertensive state (140/90 mmHg) with values ranging from 105/62 to 139/83 mmHg. The mean and SD were 122.7 ± 0.5 mmHg systolic BP, 77.4 ± 1.1 mmHg diastolic BP and 122.3 ± 2.3 mmHg systolic BP, 75.6 ± 3.7 mmHg diastolic BP for pre BD (week 1) and post BD (week 2) respectively (Table 7.7), with no significance in the two systolic or diastolic values (P = 0.693 and P = 0.324 respectively). All SpO_2 responses were ~98 % throughout testing weeks with the mean pre BD value (98.0 ± 0.0) and compared to the mean post BD value (98.1 ± 0.1) showing no significance (P = 0.177).

Table 6.8: The mean ± SD values for blood pressure.

Time h	Systolic BP (mmHg) Wk 1(Pre BD)	Systolic BP (mmHg) Wk 2 (Post BD)	Diastolic BP (mmHg) Wk 1(Pre BD)	Diastolic BP (mmHg) Wk 2 (Post BD)
0	123.5 ± 10.3	124.3 ± 8.3	78.6 ± 8.1	80.0 ± 7.2
24	122.6 ± 8.8	122.4 ± 6.7	77.8 ± 7.8	69.8 ± 13.0
48	122.2 ± 11.2	124.4 ± 10.6	76.8 ± 10.5	77.2 ± 6.2
72	122.6 ± 9.1	118.8 ± 8.8	75.8 ± 7.8	74.9 ± 7.2
96	122.6 ± 11.2	121.5 ± 9.9	78.1 ± 8.1	75.9 ± 6.6
Mean ± SD	122.7 ± 0.5	122.23 ± 2.3	77.4 ± 1.1	75.6 ±3.7

6.4 Discussion/Conclusions

This work was undertaken to provide additional sub-maximal data regarding BD, as shown to be required post systematic review (Johnson, Roberts and Gordon, 2019) and to further develop the work of Gordon and colleagues (2010) thus determining the effects of BD (~ 470ml) over a period of up to 96 hours on on-transient O_2 uptake kinetics in moderate and heavy domain exercise whilst reviewing metabolic and cardiorespiratory markers and Hb concentration changes in the muscle.

Table 6.9: A summary of the hypotheses results according to significance (S) or non-significance (NS).

Time post BD (h)	Kinetic Phase	Variable	S/NS Moderate Domain	S/NS Heavy Domain
24	II	tau_2	S decrease	NS
		TD_2	NS	NS
		a_2	NS	NS
	III	tau_3	NS	S increase
		TD_3	NS	S decrease
		a_3	NS	NS
48	II	tau_2	S decrease	NS
		TD_2	NS	NS
		a_2	NS	NS
	III	tau_3	NS	NS
		TD_3	NS	S increase
		a_3	NS	NS
72	II	tau_2	NS	NS
		TD_2	NS	S decrease
		a_2	NS	NS
	III	tau_3	S increase	NS
		TD_3	S decrease	NS
		a_3	NS	NS
96	II	tau_2	NS	NS
		TD_2	NS	NS
		a_2	NS	NS
	III	tau_3	S increase	NS
		TD_3	S decrease	NS
		a_3	NS	NS

The experimental hypotheses (numbers 1 - 4) state that in the moderate and heavy domain phase II and phase III on-transient O_2 uptake kinetics will show a significant change from donating a unit of blood after 24 h and up to 96 h. Thus, the moderate domain experimental hypothesis 1 can be accepted as there was a significant change in phase II regarding tau_2 (time reduction) for 24 and 48 h post BD. Experimental hypothesis 2 can be accepted as

there was a significant change in phase III regarding tau_3 (time increased) and TD_3 (time reduction) for 72 and 96 h post BD. For the heavy domain experimental hypothesis 3, it can be accepted as there was a significant change in phase II in regard to TD_2 (time decreased) at 72 h post BD. Also, experimental hypothesis 4 can be accepted as there was a significant change in phase III regarding tau_3 (time increased) 24 h post BD and TD_3 for 24 h (time reduction) and 48 h (time increased) post BD. Table 6.9 summarises these results and demonstrates that a significant reduction of Hb and by default the O_2 carrying capacity of blood does not appear to affect all variables of the O_2 on-transient kinetics.

Although not hypothesised as previous work had indicated that Hb would fall from donating a unit of whole blood, the Hb continued to decrease until 96 h post BD. The results of the Hb appeared to affect the O_2 kinetics in the following ways: The 7.3 % reduction in Hb 24 h post BD resulted in significant decrease during phase II for tau_2 (P = 0.021) in the moderate domain at 24 h compared to pre BD, although when correlated against the Hb reduction any linear relationship was absent (r = -0.03). In the heavy domain during phase III a significant increase occurred for tau_3 (P = 0.021), but no relationship with the Hb reduction was detected (r = 0.16), although a significant decrease for TD_3 (P = 0.034) revealed a weak negative relationship with the Hb reduction (r = -0.31). Although in general agreement with Gordon and colleagues (2010), who found no significant changes in any areas 24 h post BD, these particular findings are conflicting.

The reasons for this could potentially be due to different training statuses of the participants. The $\dot{V}O_{2max}$ values were 44.15 ± 8.31 $ml \cdot kg^{-1} \cdot min^{-1}$ for this study and 53.0 ± 4.1 $ml \cdot kg^{-1} \cdot min^{-1}$ for Gordon and colleagues (2010), indicating that the cohort for this experiment lacked aerobic power in comparison. Indeed, it has been known for many decades that faster kinetics are associated with higher compared to lower $\dot{V}O_{2max}$ values (Weltman and Katch, 1976). $\dot{V}O_{2max}$ reflects the upper limit of the body's aerobic functioning (Day, et al., 2003) and thus defines the maximum capability to use O_2 and is reflected by the Fick equation ($\dot{V}O_{2max} = \dot{Q} \times (a\text{-}vO_{2diff})$). Additionally, it should be acknowledged that $\dot{V}O_2$ is also a function of O_2 delivery (Hb, $\dot{Q}$, SaO_2) and mixed venous partial pressure of O_2 ($\dot{V}O_2 = DO_2 \times P\dot{V}O_2$), with convection and diffusion processes providing O_2 transport or indeed limiting O_2 uptake. Consequently, a high $\dot{V}O_{2max}$ relies on efficient O_2 delivery and utilisation (extraction) to working muscles and cardiac output (blood flow). Individuals (such as endurance athletes) with enhanced capabilities to utilise O_2 during sub-maximal steady state exercise enable O_2 supply to meet demand with rapidity, so sparing finite anaerobic contributions. High $\dot{V}O_{2max}$ services fast kinetics, as the divisions of exercise intensity such as GET and critical power occur at high fractions

of $\dot{V}O_{2max}$, (Jones and Poole, 2009) leaving oxidative phosphorylation as the prime energy provider and limiting slow component development if at a heavy intensity. Additionally, these athletes' enhanced exercise tolerance is due to economy and efficiency, hence sub-maximally sparing glycogen by increasing fat-utilisation, using less O_2 for ATP generation, reducing O_2 deficit, buffering lactate and reducing metabolite accumulation. Consequently, during phase II a superior $\dot{V}O_{2max}$ would reduce amplitude and tau (Burnley and Jones, 2007). Thus, in this example it is justifiable to consider that the participants with higher $\dot{V}O_{2max}$ would have faster O_2 kinetics, this is certainly the case in a comparison of tau_2 between Gordon, et al. (2010) and this study pre BD (27.6 ± 7.2 s v 32.9 ± 9.4 s) respectively. However, the differences post BD (28.4 ± 6.7 s v 24.9 ± 5.4 s) imply that the more aerobically fit participants exhibited slower O_2 kinetics in this instance and hence were using more anaerobic energy by contrast to the participants of this study, although not significantly different to their pre BD value.

Another reason for the disparity in results between the two sets of participants could be due to muscle fibre composition and recruitment. Correspondence with Gordon, et al. (2010) acknowledges that the participants were trained rowers, which would indicate that they were possibly not as efficient at cycling but nevertheless a homogeneous group. It is also recognised that successful rowers have high metabolic capabilities and large muscle mass composed of 70-85 % type I fibres (Steinacker, 1993). In this study group, the gathering of a broad cohort to represent the general public and thus heterogeneous participants would suggest that fibre composition was likely disparate compared to the rowers. Thus, regarding the rowers in Gordon, et al. (2010), despite the Hb reduction it would appear that their already large oxidative capacity of the type I fibres led to no changes in muscle recruitment patterns and O_2 availability was not limiting. In the case of the fibre type recruitment of this study cohort, it is likely that type I fibres were also the predominant fibre recruited as this was comparatively short duration moderate intensity exercise (Mallory, et al., 2002), but pre BD there may have been a small involvement from type II fibres to produce the power required, possibly due to poor cycling efficiency. However, post BD and having completed four previous kinetic trials, despite the Hb reduction the speeding of this phase II section (tau_2) could be consequential to more efficient cycling, but also due to a greater slow twitch fibre recruitment drive. Force output was not required to increase, but as type I fibres fatigued a "like for like" recruitment procedure enabled more O_2 extraction despite a reduced total circulation of O_2 from Hb decline.

Reflecting on the significant reduction in time of moderate tau_2 and the significant increase in time for heavy tau_3, it is the time constant (tau) that computes the degree of the $\dot{V}O_2$ increase. For every multiple of tau $\dot{V}O_2$ rises by 63 % of the difference between the prior time value and the steady state requirement (Jones and Poole, 2012). Hence, post BD less time was taken for $\dot{V}O_2$ to reach 63 % of the total phase II rise in moderate exercise implying that O_2 availability did not influence O_2 kinetics. However, more time was taken to reach 63 % of the amplitude of phase III in the heavy domain, implying that O_2 availability did influence O_2 kinetics. Although anaerobic pathways dominate in this domain it is possible that the relative contribution from aerobic sources was affected by the reduced O_2 circulating to the working muscles. It is known that tau during phase II for $\dot{V}O_2$ (moderate exercise, 35 ± 14 s; high-intensity exercise, 39 ± 4 s) and PCr (phosphocreatine) (moderate exercise, 33 ± 12 s; high-intensity exercise, 38 ± 11 s) reflect each other, that is they simultaneously increase and decrease respectively (Rossiter, et al., 2002) and until steady state approaches PCr continues to decrease while $\dot{V}O_2$ continues to increase.

According to Kushmerick (1998), the PCr reaction depends on the degree of ATPase activity (catalysing ATP to/from ADP and Pi) and creatine kinase (CK) kinetics. CK (a protein) is a rate-limiting enzyme that drives PCr kinetics with its flux altering from substrate or concentrations of Cr, PCr, ATP and ADP, graded escalations of which controls oxidative phosphorylation (Kushmerick, 1998) and additionally large increases in ADP decrease energy release in hydrolysis and can affect ATP reactions (Sahlin and Harris, 2011). Acute inhibition of CK in isolated myocytes of Xenopus laevis (African clawed frog) produced significantly faster intracellular PO_2 kinetics compared to the control (31.8 ± 5.5 vs 49.3 ± 5.7 s) during 2 minutes of isometric tetanic contractions, which was accompanied by intracellular PO_2 falling rapidly at onset. According to the authors (Kindig, et al., 2005) this suggests that CK buffers ATP-ADP ratios. Thus, in terms of tau_2, although PCr hydrolysis itself is not dependent on O_2 availability, if there is a decrement in circulating O_2 from BD, this requires anaerobic metabolism to be engaged more. Additionally, it is conceivable that PCr was used more rapidly (decreases) and with large rises in ADP inhibiting the CK forward flux (to ATP) a speeding of kinetics occurred. With PCr use increased, metabolism enabled 63 % of the rise in phase II $\dot{V}O_2$ to be reached faster. However, it should be noted that despite the speeding of tau_2 post BD the TD did not change ($P > 0.05$), which would imply that post speeding there was also a slowing of the kinetics reflecting changes in the $\dot{V}O_2$ response. This was perhaps as a result of CK promotion (opposed to inhibition) as steady state approached. Elevated ADP activates oxidative phosphorylation due to PCr decrement continuing until supply and demand of ATP is balanced and steady state PCr is reached (Conley, Kemper and Crowther, 2001).

The existence of myoglobin as an O_2 store could be another explanation as to the speeding of tau_2. In circumstances where circulating O_2 is reduced (BD), myoglobin could potentially act as a buffer as the O_2 is instantly available, augmenting intracellular O_2 transport. Indeed, during hypoxia (12 % O_2) diffusional conductance and offloading is elevated, myoglobin-facilitated O_2 flux increases and intracellular resistance reduces (Richardson, et al., 1995). Myoglobin desaturation is rapid, within 20 s of exercise onset at 50 % of maximum O_2 consumption a partial desaturation can be noted (Richardson, et al., 1995), however the time of phase I and II (24.88 ± 5.38 s in this study post BD) are also rapid and as such maybe sufficient time to promote kinetic speeding.

Oxidative phosphorylation of O_2 is needed to produce ATP (from ADP and Pi) and during moderate intensity supply would meet demand easily. However, in the heavy domain it takes longer for steady state to be achieved and anaerobic metabolism dominates (as working above GET) therefore O_2 would not be utilised as much. Post BD tau_3 significantly increased, implying a slowing in $\dot{V}O_2$ response. Having less circulating O_2 post BD has perhaps delayed steady state being achieved, perhaps due to the muscle environment being more acidic, but also possibly due to CK in its role to slow down the response of oxidative phosphorylation (Kushmerick, 1998).

The mean lactate values shown in Figure 6.6 demonstrate that post BD the values appear higher than pre BD, but not significantly so. There were only two significantly higher values and one was the resting lactate 24 h pre to post BD and the other 48 h immediately after the heavy trial. In regards to the higher resting value (1.53 ± 0.44 mM) post BD, this is possibly as a direct result of BD. Because there is less circulating O_2, the environment may be slightly more acidic to normal due to a higher number of hydrogen ions present for resting conditions. Lactate is the end product of glycolysis, formed from pyruvate (and lactate dehydrogenase) and thus lactate depends upon pyruvate metabolism. However, pyruvate dysfunction can occur from decreased cellular O_2 and also if O_2 is reduced glucose is converted to lactate (Bakker, 2003). Hence, it is possible that lack of circulating O_2 produced a higher resting lactate value. For example, in post-operative care if lactate is > 2.0 mM O_2 delivery is increased (Bakker, 2003). The higher resting lactate value 24 h post BD appears also to be reflected within the NIRS data, which displays significantly decreased (P = 0.039) O_2Hb levels at resting baseline pre to post BD. A resting lactate range is ~0.5-1.5 mM (Higgins, 2007) and therefore the value from the participants is marginally more, but this could also be as a result of a change in the daily activity prior to arriving at the laboratory. In general, lactate values during the moderate trials were less than 4 mM, which provides an indication that participants were working below GET and as

such at a moderate intensity. The lack of statistical significance regarding BD not modifying lactate is supported by several other authors (Meurrens, et al., 2016; Gordon, et al., 2013; Hill, et al., 2013; Gordon, et al., 2010; Burnley, et al., 2006).

To progress from 24 h, a further 2.3 % reduction in Hb 48 h post BD resulted in a significant reduction of tau_2 in the moderate domain (P = 0.031), which continues on the decrement theme from the previous day. Also, in the heavy trial a significant increase was observed for TD_3 (P = 0.010). When considering NIRS, very few significant differences between pre and post BD were observed. However, the tHb saw significant increases 48 h pre to post BD during moderate (P = 0.018) and heavy intensity (P = 0.038). As previously stated, the $\dot{V}O_2$ (amplitude) during phase III remained unaffected by BD and therefore it is conceivable that in order for tHb to increase O_2 extraction at the muscle increased. This may be as a result of enhanced O_2 diffusional conductance, as shown in hypoxia (Richardson, et al., 1995; Calbet, et al., 2015) by means of additional capillaries opening, which reduces diffusion distance and increases surface area for O_2 exchange (Pittman, 2011). Also, a blood flow redistribution from muscles that are less engaged is possible, with redistribution occurring from vasoconstrictor signals to areas of less need while vaso-conductance increases to other areas (Calbet, et al., 2007). Increased blood flow (mediated by vasodilation) to the active areas is triggered from a RBC signal as a result of O_2 being offloaded from Hb (Jagger, et al., 2001). The higher lactate value post BD (P = .027) immediately following the heavy trial compared to pre BD is reflective of the fact that during the heavy trial post BD achieving steady state was significantly delayed resulting in an increased pool of fast twitch fibres being recruited, which are known to be lactate producers.

At 72 h post BD another Hb reduction (2.1 %) resulted in a significant increase in the moderate domain for tau_3 (P = 0.012) and a significant decrease for TD_3 (P = 0.009), whilst in the heavy domain tau_1 (P = 0.038) and TD_2 (P = 0.038) showed significant decreases. However, lactate and NIRS showed non-significant changes similarly to the amplitude of $\dot{V}O_2$ implying that despite the decreased circulating O_2 $\dot{V}O_2$ uptake remains unaffected by BD. In the moderate domain and 96 h post BD where Hb increased by 1.5 % a significant increase was present in tau_3 (P = 0.32) and a significant decrease for TD_3 (P = 0.029), which is the same theme seen in the previous collection point. Thus, as Hb is starting to increase the homeostatic basis within the body is returning to pre BD levels and therefore recovering, which reflects no significant changes occurring within any metabolic or cardiorespiratory factors. The only exception to this appears to be within TSI in the moderate domain where a significant decrease occurred (P = 0.006) post BD. However,

the effect size and confidence intervals would imply that the change was not meaningful (Cohens *d* = 0.36 (CI, -5.64, 6.78)). A reduction in TSI is produced from a reduction in O_2Hb and a rise in HHb, indicating an increase in muscle O_2 extraction, although no significant differences were present for O_2Hb or HHb pre to post BD at this point in time, thus it is unclear as to the cause of this significant reduction. Nevertheless, as Hb was increasing within the circulation this could have potentially allowed an augmented muscle O_2 extraction.

In regards to NIRS, it is noted that a homogeneous participant pool would provide less heterogeneous results and also participants with minimal body fat and thus similar ATT would be ideal, however this would not necessarily reflect the general exercising public who regularly donate blood. It is also noted that the vastus lateralis muscle is usually the popular choice for probe placement. However, the gastrocnemius has been identified as having almost double the slow twitch muscle fibres in comparison ~50-60 % (Edgerton, Smith and Simpson, 1975; Coggan, et al., 1992), which are highly oxidative and thus seem appropriate for analysis in a BD study concerned with circulating O_2 and use of it.

Generally, these results agree with Gordon and colleagues (2010) and $\dot{V}O_2$ is unaffected by BD. Gordon and colleagues (2010) also refer to the “tipping point” concept (Poole and Jones, 2008), an intensity where aerobic metabolism moves to anaerobic metabolism limiting O_2 delivery. They suggest that the tipping point is considerably closer to maximum O_2 consumption rather than rest and the work from this study is inclined to agree. Post BD an environment that decreases circulating O_2 is created which promotes a more hypoxic tendency and thus contributes to anaerobic functioning. However, the data within this current study reflects an unaffected $\dot{V}O_2$ amplitude and yet the tHb of the NIRS increases from 48 h onwards. Therefore, although the muscle maybe hypoxically threatened, the redistribution of blood from less active tissues prevents anaerobic metabolism occurring and moving beyond the tipping point.

To conclude, this study shows that BD produces no effect on $\dot{V}O_2$ amplitude in the moderate and heavy domains at 24 h and up to 96 h, but did exhibit significant responses for time-based components. There also appears to be a significant effect on muscle tissue in terms of tHb mainly at 48 h following a more pronounced decrease in Hb. This may indicate that an enhanced O_2 extraction at the muscle occurs, which is assisted by blood flow redistribution. This is important as it reflects a change/adaption consequential to BD.

Chapter 7: 4th Experimental Chapter

Influence of a Standard UK Blood Donation (~470 ml) on Moderate and Heavy Intensity Cycle Exercise over a Period of 24-96 Hours; Part B – Cardiac and Ventilatory Outputs

7.1 Introduction

The previous chapter began by introducing the exponential nature of O_2 uptake at the onset of exercise prior to a steady state being attained (Hill, Long and Lupton.,1924). It explained that the achievement of steady state is dependent on the intensity of exercise, with oxygen supply meeting demand at moderate levels of exercise much more readily than at heavy levels (within 2-3 minutes, Jones and Poole, 2005 and ~6-8 minutes, Gordon, et al., 2010 respectively). Exploration from the previous experimental chapter of O_2 uptake, delivery and extraction has highlighted that the transit of O_2 was affected by BD and significantly so in some variables such as time constants and tHb. However, the O_2 kinetics behaviour maybe consequential to other contributing cardiac and ventilatory variables of $\dot{V}O_2$ uptake and thus, this chapter further explores the effect of BD on moderate and heavy intensity domain cycling.

The transit of O_2 from the nose/mouth to the muscle is a journey through the lungs and the heart within the pulmonary circulation to the muscle in the peripheral circulation. The passage of O_2 commences at the upper respiratory system, moving through the nasal cavity to the pharynx and larynx before traveling to the lower respiratory system of the trachea, bronchi, bronchioles and alveoli. For air to diffuse in and out of the lungs pressure gradients exist. Air moves from high to low pressure areas and efficient gas exchange is assisted by very thin alveoli walls that can have a blood-gas barrier of only 0.3 μm in places (West, 1995). As PO_2 within the alveolar and RBC entering the pulmonary capillary is 100 and 40 mm Hg respectively, O_2 will stream along this sizeable pressure gradient causing a rise in PO_2 in the red cell (West, 1995). Thus, on leaving the alveoli towards the heart and then the systemic circulation the PO_2 is 100 mm Hg and PCO_2 is 40 mm Hg. During exercise pulmonary blood flow increases causing a reduction in time that the red cell is within the capillary and thus reducing oxygenation time (West, 1995). The same pressure gradient system operates to diffuse blood gasses in and out of muscle cells across the blood-myocyte barrier (Poole and Jones, 2012). Once the journey of O_2 from the atmosphere to the cardiovascular system and then to the muscles is complete and ATP is produced CO_2 is transported back to the lungs for expiration.

The primary variables associated with the passage of O_2 ascertained through cardio pulmonary exercise testing are tidal volume (V_T), breathing frequency (BF), stroke volume (SV), heart rate (HR) (Wasserman, et al., 2005; Jones and Poole, 2005) and arteriovenous oxygen difference (a-vO_{2diff}). A change in these variables could possibly reflect the "route" that the $\dot{V}O_2$ adopted as a result of the BD as reflected in the previous chapter. Therefore, the aim of this chapter is to explain how the transport of $\dot{V}O_2$ occurred, and probe into the contributing variables of $\dot{V}O_2$ uptake by determining the effects of BD (~470 ml) over a period of up to 96 hours on respiratory function (V_T, BF, V_E), cardiac function (HR, SV, $\dot{Q}$) and O_2 extraction (a-vO_{2diff}) at the muscle in the moderate and heavy cycling domains.

Null hypotheses:

(H_{01}): The respiratory output in the moderate domain will not be altered compared to pre BD values from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_{02}): The respiratory output in the heavy domain will not be altered compared to pre BD values from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_{03}): The cardiac responses in the moderate domain will not be altered compared to pre BD values from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_{04}): The cardiac responses in the heavy domain will not be altered compared to pre BD values from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_{05}): The O_2 extraction at the muscle in the moderate domain will not be altered compared to pre BD values from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_{06}): The O_2 extraction at the muscle in the heavy domain will not be altered compared to pre BD values from donating a unit of blood after 24 hours and across the subsequent 96 hours.

Experimental hypotheses:

(H_1): The respiratory output in the moderate domain will increase compared to pre BD values from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_2): The respiratory output in the heavy domain will increase compared to pre BD values from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_3): The cardiac responses in the moderate domain will increase compared to pre BD values from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_4): The cardiac responses in the heavy domain will increase compared to pre BD values from donating a unit of blood after 24 hours and across the subsequent 96 hours.

(H_5): The O_2 extraction at the muscle in the moderate domain will increase compared to pre BD values from donating a unit of blood after 24 hours and across the subsequent 96 hours.
(H_6): The O_2 extraction at the muscle in the heavy domain will increase compared to pre BD values from donating a unit of blood after 24 hours and across the subsequent 96 hours.

7.2 Methods

7.2.1 Participants and Study Design

Refer to page 107-110, Part A, section 6.2.1 and 6.2.2.

7.2.2 Cardiac Function

Thoracic impedance cardiography (Physioflow®, PF05L1, Manatec, Petit-Ebersviller, France) was used as a non-invasive method to gain cardiac function information, including the variables HR, SV and cardiac output ($\dot{Q}$). The participants had electrodes (Skintact® FS50) placed according to the three-dimensional triangulation method on pre-prepared skin, which included being shaved and oils and skin cells desquamated accordingly. Blood pressure was taken to complete the calibration process for signal stability. Refer to page 60-62, methods chapter 4.3.1.

7.2.3 Blood Pressure and SpO_2 Responses

With the participant sat down, after ~5 minutes of relaxation after arrival at the laboratory, a resting assessment of blood pressure (BP) (Nonin, 2120, USA) prior to Hb capillary blood sampling was taken. SpO_2 (Nonin, 2120, USA) was recorded at the same time as BP (but on the right-hand side of the body). Refer to page 71, methods chapter 4.7.1.

7.2.4 Data Analysis

Cardiac and ventilatory data were time aligned due to the difference in the data acquisition of the equipment that is data gathered via breath-by-breath (MetaLyzer®) or beats (physioflow). Following time alignment of cardiac and ventilatory data, $\dot{V}O_2$ was divided by $\dot{Q}$ to gain a-vO_{2diff} values. For details on the MetaLyzer® refer to page 56-57, chapter 4.1 and page 59-60, chapter 4.3 for the physioflow. The raw cardiac and ventilatory data for each person was placed into Excel version 16.16.11 (Microsoft Excel for Mac, 2018). The

data was then sectioned into the three intensities of workload (two moderate and one heavy intensity), with each workload placed into a new tab within the workbook. Following this each 6-minute intensity trial was subsequently taken to time zero and the data then divided and averaged into ten percent sections over the time course (6 minutes of moderate or heavy cycling) of the trials. Thus, from zero every 36 seconds data was averaged over the 6 minutes of exercise (10 % = 36 s, 20 % = 72 s, 30 % = 108 s, 40 % = 144 s, 50 % = 180 s, 60 % = 216 s, 70 % = 252 s, 80 % = 288 s, 90 % = 324 s and 100 % = 360 s). The 4 minutes of baseline prior to each square-wave workload transition were excluded from the analysis, as were the 6 minutes of recovery post transition.

Additionally, for the moderate domain exercise, each percentage time point for the assorted variables was averaged in order to get one value for this intensity for ease of analysis. To establish if there were systematic differences in the moderate domains and eligibility for combining values Bland Altman plots were used to assess bias and 95 % limits of agreement between the two sets of quantitative data. With no proportional bias detected, a one sample *t*-test was used for further indication of data reliability. All ten percent time points were statistically analysed.

7.2.5 Statistical Analysis

All variables collected have the values stated as mean ± SD. Following tests for normality (Shapiro-Wilk's), two-way repeated measures ANOVA for each time point (24, 48, 72, 96 h) and percentiles of each cardio-respiratory variable (e.g. SV) split for time were undertaken to determine the main effect for time and trial. Where main or interaction effects were found or further analysis was deemed useful one-way repeated measures ANOVA (for differences in trial/week 1 (pre BD) or 2 (post BD)) were used and repeated measures *t*-tests adopted for use between time points (e.g. 10 % at pre 24 and post 24 h). Mauchly's test was used to check sphericity and Greenhouse-Geisser correction applied where necessary. Additionally, Bonferroni correction was applied to avoid Type 1 Error for pairwise (pre to post) comparisons. Effect size was also considered due to the result being independent of sample size; refer to page 115, Part A 6.2.13 for detail on Cohen's *d* and Partial eta squared.

A level of statistical significance was set at $P < 0.05$ and statistical analysis was accomplished using SPSS version 20 (SPSS, Chicago, IL).

7.3 Results

7.3.1 Oxygen Uptake

In the moderate trial, after two-way ANOVA testing, only the 20 % time point observed a significant pairwise comparison between pre and post BD trials (P = 0.039, η_p^2 = 0.33 (CI, -1.16, -0.04)). In addition, a main effect for time was also observed (P = 0.027, η_p^2 = 0.24), however, the overall interaction effect for trial by time was not significant (P = 0.45, η_p^2 = 0.08). The 30, 40, 50, 60, 70, 80 and 90 % time points also observed significant main effects for time (P = 0.001, η_p^2 = 0.38, P = 0.001, η_p^2 = 0.40, P = 0.000, η_p^2 = 0.47, P = 0.000, η_p^2 = 0.44, P = 0.001, η_p^2 = 0.40, P = 0.007, η_p^2 = 0.30 and P = 0.001, η_p^2 = 0.37).

For pre BD, the one-way ANOVA table observed significance, firstly at 30 % (P = 0.025, η_p^2 = 0.34) with a significant pairwise comparison between 24 and 72 h (P = 0.019, (CI, -1.58, -0.13)) and 24 and 96 h (P = 0.003, (CI, -1.76, -0.13)), at 40 % (P = 0.015, η_p^2 = 0.27) with a significant pairwise comparison between 24 and 96 h (P = 0.016, (CI, -1.97, -0.18)), at 50 % (P = 0.008, η_p^2 =0 .49) with a significant pairwise comparison between 24 and 72 h (P = 0.018, (CI, -1.57, -0.13)) and 24 and 96 h (P = 0.035, (CI, -2.09, -0.06)), at 60 % (P = 0.010, η_p^2 = 0.27) with a significant pairwise comparison between 24 and 72 h (P = 0.016, (CI, -1.81, -0.10)), at 70 % (P = 0.010, η_p^2 = 0.29) with a significant pairwise comparison between 24 and 72 h (P = 0.032, (CI, -1.54, -0.06)) and 24 and 96 h (P = 0.049, (CI, -1.76, -0.01)), and lastly at 90 % (P = 0.026, η_p^2 = 0.24) with a non-significant pairwise comparison between 24 and 72 h (P = 0.050, (CI, -1.79, -0.002)).

For post BD, the one-way ANOVA indicated significance at several time points. Firstly, at 30 % (P = 0.025, η_p^2 = 0.24) with a significant pairwise comparison between 24 and 96 h (P = 0.010, (CI, -1.35, -0.17)), at 40 % (P = 0.004, η_p^2 = 0.33) with a significant pairwise comparison between 24 and 96 h (P = 0.020, (CI, -1.59, -0.12)), at 50 % (P = 0.002, η_p^2 = 0.36) with a significant pairwise comparison between 24 and 96 h (P = 0.003, (CI, -1.49, -0.30)) and 48 and 96 h (P = 0.026, (CI, -0.94, -0.05)), at 60 % (P = 0.001, η_p^2 = 0.40) with a significant pairwise comparison between 24 and 72 h (P = 0.048, (CI, -1.71, -0.01)) and 24 and 96 h (P = 0.002, (CI, -1.20, -0.39)), at 70 % (P = 0.011, η_p^2 = 0.28) with a significant pairwise comparison between 24 and 96 h (P = 0.007, (CI, -1.41, -0.22)), at 80 % (P = 0.044, η_p^2 = .22) but with no significant pairwise comparisons between collection points, and lastly at 90 % (P = 0.045, η_p^2 = 0.21) with a significant pairwise comparison between 24 and 72 h (P = 0.032, (CI, -1.48, -0.06)).

T-test analysis of pre to post BD for $\dot{V}O_2$ in the moderate trial, reported non-significance ($P > 0.05$), the mean ± SD of the percentiles is reported in Table 7.1.

In the heavy trial two-way ANOVA testing observed three significant pairwise comparisons between pre and post BD trials, firstly at 60 % ($P = 0.041$, $\eta_p^2 = 0.33$ (CI, 0.06, 2.12)), also at 70% ($P = 0.031$, $\eta_p^2 = 0.36$ (CI, 0.14, 2.45)) and at 90 % ($P = 0.046$, $\eta_p^2 = 0.32$ (CI, 0.28, 2.57)). The 20, 30, 40 and 100 % time points also observed significant main effects for time ($P = 0.028$, $\eta_p^2 = 0.23$, $P = 0.028$, $\eta_p^2 = 0.23$, $P = 0.027$, $\eta_p^2 = 0.24$ and $P = 0.045$, $\eta_p^2 = 0.27$). In addition, an interaction effect for trial by time was significant for 10 % ($P = 0.41$, $\eta_p^2 = 0.22$).

The one-way ANOVA for pre BD observed significances at 30 % ($P = 0.025$, $\eta_p^2 = 0.30$) with a significant pairwise comparison between 24 and 48 h ($P = 0.013$, (CI, -2.19, -0.24)), also at 40 % ($P = 0.017$, $\eta_p^2 = 0.26$) and 60 % ($P = 0.025$, $\eta_p^2 = 0.32$), but neither of these time points had any significant pairwise comparisons between days. The one-way ANOVA for post BD observed significances at 10 % ($P = 0.040$, $\eta_p^2 = 0.27$) and 20 % ($P = 0.007$, $\eta_p^2 = 0.30$), but again neither of these time points had any significant pairwise comparisons between days. In addition, at 40 % the ANOVA reported no significance, but significance was apparent for pairwise comparisons between 24 and 96 h ($P = 0.010$, (CI, -1.52, -0.19)). Lastly, at 100 % the ANOVA reported no significance, but significance was apparent for pairwise comparisons between 24 and 96 h ($P = 0.015$, (CI, -3.49, -0.35)).

T-test analysis of pre to post BD in the heavy trial, reported several significant differences ($P < 0.05$) at various time points, the mean ± SD of the percentiles are reported in Table 7.1. At 48 h, significance is displayed at 60 % ($P = 0.021$, Cohens $d = 0.22$ (CI, -3.85, 4.26)), while at 72 h a significant response was witnessed at 50 % ($P = 0.045$, Cohens $d = .21$ (CI, -4.16, 5.00), 60 % ($P = 0.010$, Cohens $d = 0.26$ (CI, -4.19, 4.63)), 70 % ($P = 0.010$, Cohens $d = 0.32$ (CI, -4.05, 5.55)), 80 % ($P = 0.038$, Cohens $d = 0.26$ (CI, -4.04, 5.52)) and 90 % ($P = 0.021$, Cohens $d = 0.29$ (CI, -4.14, 5.65)).

Table 7.1: Mean ± SD $\dot{V}O_2$ values over the two-week testing period.

Time Point (360s split into 10 % parts)	$\dot{V}O_2$ (ml·kg^{-1}·min^{-1})							
	Pre BD Moderate Trial (Week 1)				Post BD Moderate Trial (Week 2)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Zero	10.80 ± 1.12	10.61 ± 1.53	11.11 ± 1.46	10.50 ± 1.45	10.37 ± 1.20	10.67 ± 1.53	10.94 ± 1.92	10.46 ± 1.05
10 %	15.45 ± 2.92	15.51 ± 3.00	15.49 ± 3.05	15.74 ± 3.00	15.81 ± 3.41	15.90 ± 3.07	15.83 ± 3.36	16.18 ± 2.72
20 %	23.64 ± 5.00	23.90 ± 5.15	24.22 ± 5.07	24.39 ± 5.13	24.45 ± 5.20	24.69 ± 4.92	24.38 ± 4.87	25.02 ± 4.89
30 %	26.49 ± 5.45	26.87 ± 5.31	27.34 ± 5.31	27.44 ± 4.90	26.93 ± 5.34	27.43 ± 5.40	27.21 ± 5.03	27.69 ± 5.00
40 %	27.72 ± 5.42	28.32 ± 5.40	28.51 ± 5.59	28.80 ± 5.20	27.91 ± 5.38	28.50 ± 5.24	28.32 ± 5.14	28.76 ± 5.29
50 %	28.43 ± 5.45	29.16 ± 5.38	29.28 ± 5.42	29.50 ± 5.21	28.65 ± 5.32	29.05 ± 5.27	29.15 ± 5.09	29.54 ± 5.14
60 %	28.81 ± 5.57	29.51 ± 5.11	29.77 ± 5.51	29.66 ± 5.46	28.86 ± 5.21	29.46 ± 5.27	29.72 ± 5.76	29.89 ± 5.18
70 %	29.18 ± 5.64	29.67 ± 5.22	29.97 ± 5.38	30.00 ± 5.44	29.29 ± 5.36	29.71 ± 5.41	29.67 ± 5.34	30.10 ± 5.23
80 %	29.48 ± 5.56	30.08 ± 5.36	30.37 ± 5.82	30.30 ± 5.64	29.39 ± 5.18	30.07 ± 5.62	29.84 ± 5.21	30.11 ± 5.28
90 %	29.71 ± 5.71	30.28 ± 5.13	30.61 ± 5.63	30.54 ± 5.47	29.70 ± 5.13	30.02 ± 5.26	30.01 ± 5.34	30.47 ± 5.21
100 %	30.05 ± 5.72	30.69 ± 5.32	30.55 ± 5.60	30.61 ± 5.70	29.88 ± 5.43	30.26 ± 5.55	30.28 ± 5.30	30.37 ± 5.37
	Pre BD Heavy Trial (Week 1)				Post BD Heavy Trial (Week 2)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Zero	11.34 ± 2.05	11.32 ± 1.63	12.04 ± 1.85	12.29 ± 2.82	10.97 ± 1.05	11.94 ± 2.08	11.22 ± 1.70	11.85 ± 1.76
10 %	18.74 ± 4.10	19.21 ± 4.07	19.50 ± 4.80	18.95 ± 4.05	18.42 ± 3.76	19.07 ± 3.93	18.31 ± 4.05	19.96 ± 4.27
20 %	31.12 ± 6.59	31.54 ± 5.89	31.60 ± 7.18	31.79 ± 6.02	30.96 ± 6.50	31.33 ± 6.58	30.75 ± 6.77	32.08 ± 6.36
30 %	35.16 ± 6.81	36.38 ± 6.97	36.36 ± 7.24	35.99 ± 6.96	35.43 ± 7.03	35.97 ± 7.05	35.25 ± 7.91	35.57 ± 6.77
40 %	37.35 ± 6.72	38.73 ± 7.05	38.62 ± 7.48	38.44 ± 7.10	37.30 ± 7.06	37.83 ± 7.11	37.18 ± 8.32	38.15 ± 6.79
50 %	38.79 ± 6.91	39.93 ± 7.07	40.03 ± 7.72	40.15 ± 7.32	38.69 ± 7.05	38.94 ± 7.23	38.41 ± 8.47*	39.35 ± 6.90
60 %	39.72 ± 6.77	41.28 ± 7.19	41.31 ± 7.87	41.29 ± 7.41	39.73 ± 7.31	39.87 ± 7.14*	39.40 ± 8.74*	40.26 ± 7.03
70 %	41.08 ± 6.90	42.30 ± 7.36	42.70 ± 7.73	42.18 ± 7.41	40.77 ± 7.12	40.90 ± 7.50	40.09 ± 9.25*	41.32 ± 7.13
80 %	41.79 ± 7.09	42.77 ± 7.49	43.12 ± 7.59	43.21 ± 7.20	41.54 ± 7.14	41.69 ± 7.36	41.04 ± 9.30*	42.32 ± 7.18
90 %	42.23 ± 7.08	43.71 ± 7.69	43.90 ± 7.84	44.19 ± 7.27	41.80 ± 7.36	42.57 ± 7.27	41.48 ± 9.48*	42.97 ± 6.95
100 %	42.48 ± 7.85	43.93 ± 8.07	44.44 ± 7.83	44.86 ± 7.41	41.70 ± 7.63	43.10 ± 7.40	42.58 ± 9.48	43.62 ± 7.27

** $P < 0.05$, denotes a significant difference from Pre BD to Post BD.*

7.3.2 Tidal Volume

In the moderate trial, the two-way and one-way ANOVA testing showed no significant differences ($P > 0.05$). T-test analysis of pre to post BD for V_T reported one significantly increased time point (1.36 ± 0.26 v 1.42 ± 0.26 L), which was 24 h at 10 % ($P = 0.010$, Cohens $d = -0.27$ (CI, -0.42, -0.12)). In the heavy trial, the two-way ANOVA testing showed pairwise significant differences at 80 % ($P = 0.023$, $\eta_p^2 = 0.37$ (CI, -0.19, -0.02)), 90 % ($P = 0.013$, $\eta_p^2 = 0.45$ (CI, -0.20, -0.03)) and 100 % ($P = 0.018$, $\eta_p^2 = 0.41$ (CI, -0.19, -0.02)). However, no significant differences were observed for a main effect for time or for the overall interaction effect for trial by time ($P > 0.05$). The one-way ANOVA testing showed no significant differences ($P > 0.05$). T-test analysis of pre to post BD for V_T, reported significant increases during 24 h at 80 % (2.55 ± 0.45 v 2.73 ± 0.54 L, $P = 0.019$, Cohens $d = -0.38$ (CI, -0.63, -0.08)), 48 h at 80 % (2.63 ± 0.48 v 2.72 ± 0.49 L, $P = 0.041$, Cohens $d = -0.20$ (CI, -0.47, -0.08)) and 90 % (2.60 ± 0.51 v 2.70 ± 0.50 L, $P = 0.004$, Cohens $d = -0.20$ (CI, -0.49, -0.08)), and during 96 h at 80 % (2.63 ± 0.44 v 2.73 ± 0.54 L, $P = 0.044$, Cohens $d = -0.20$ (CI, -0.45, -0.10)), 90% (2.60 ± 0.42 v 2.73 ± 0.54 L, $P = 0.031$, Cohens $d = -0.28$ (CI, -0.52, -0.02)) and 100 % (2.58 ± 0.42 v 2.71 ± 0.51 L, $P = 0.007$, Cohens $d = -0.29$ (CI, -0.52, 0.00)). The mean ± SD values of the percentiles can be seen in Figure 7.1 and 7.2.

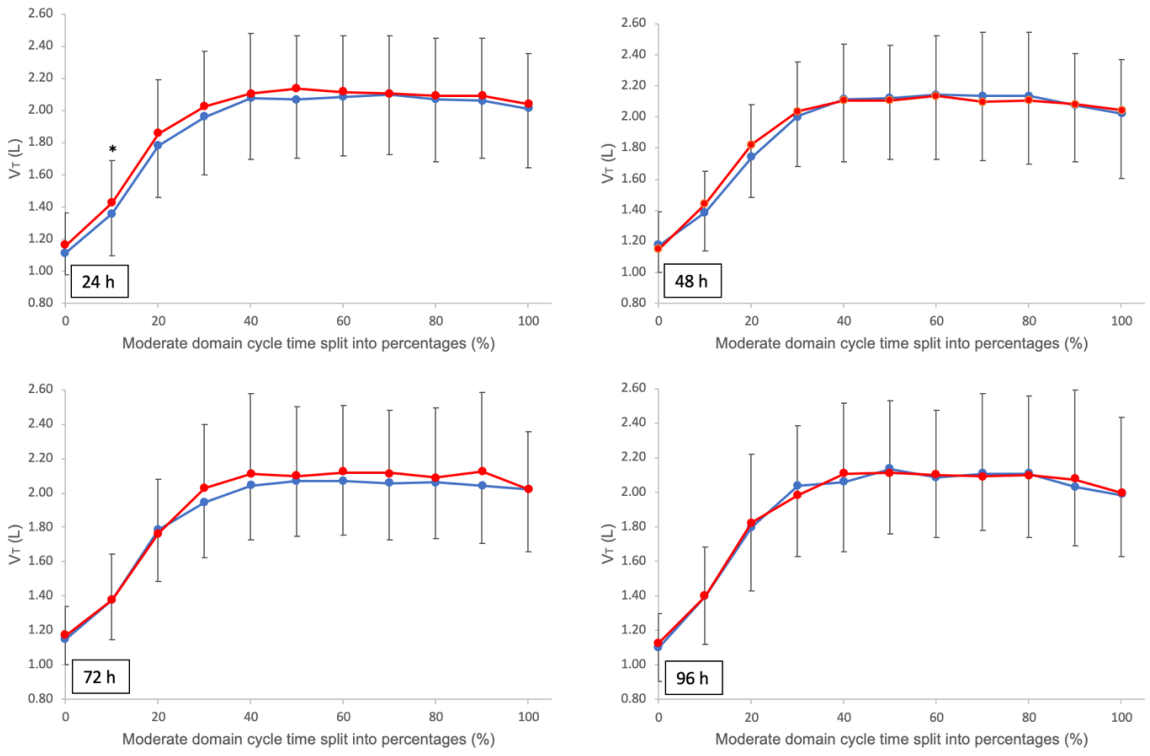

Figure 7.1: Mean pre BD (week 1 in blue) and post BD (week 2 in red) V_T during moderate intensity cycling throughout 24-96 h. The asterisk indicates significant difference (P < 0.05) pre to post BD.

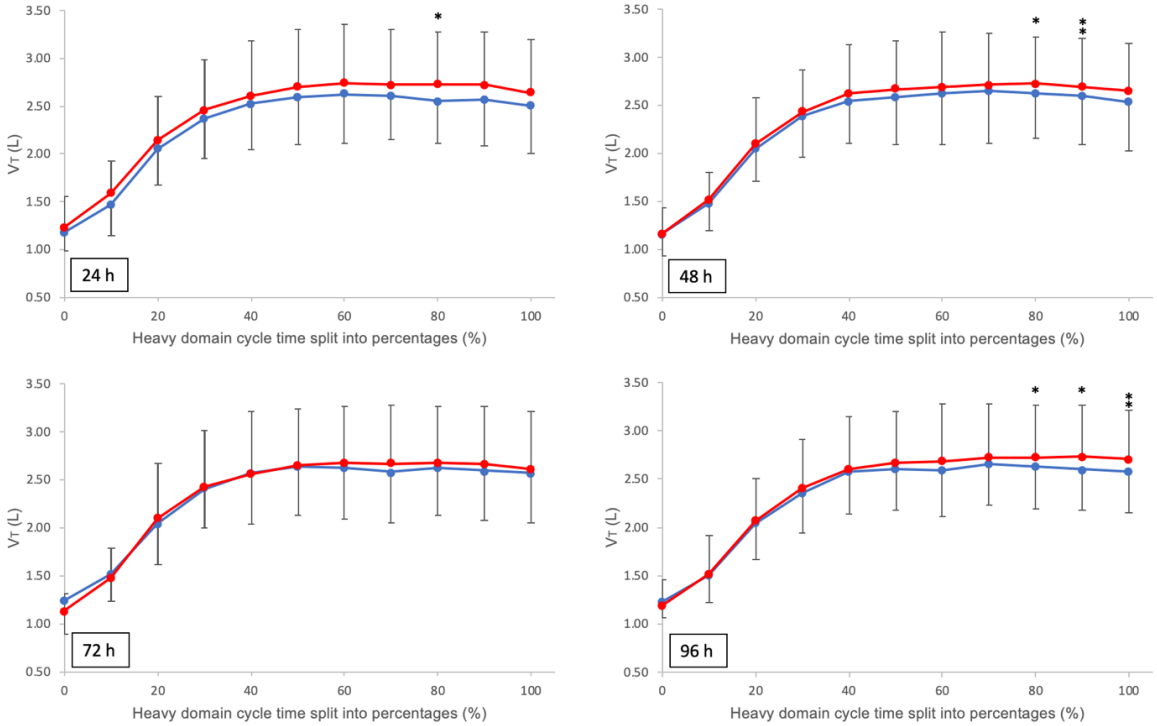

Figure 7.2: Mean pre BD (week 1 in blue) and post BD (week 2 in red) V_T during heavy intensity cycling throughout 24-96 h. The single and double asterisks indicate significant difference (P < 0.05 and P < 0.01) pre to post BD.

7.3.3 Breathing Frequency

The mean ± SD values of the percentiles for BF can be seen in Table 7.2, these values however have produced limited significant differences. The two-way ANOVA showed non-significant differences for moderate or heavy trials, while the one-way ANOVA testing demonstrated that only pre BD at 50 % for the heavy trial had a significant pairwise comparison between 24 and 48 h (P = 0.021, (CI, -4.54, -0.32)), but no significance in the one-way ANOVA. T-test analysis showed significant results for 72 h at 20 % in the moderate trial (25.79 ± 5.16 v 28.03 ± 5.79 $\cdot min^{-1}$, P = 0.002, Cohens d = -0.43 (CI, -3.35, -2.85)) and for 48 h at 10 % in the heavy trial (28.57 ± 4.38 v 27.21 ± 4.29 $\cdot min^{-1}$, P = 0.032, Cohens d = -0.33 (CI, -2.15, -2.75)).

Table 7.2: Mean ± SD BF values over the two-week testing period.

Time Point (360s split into 10 % parts)	BF(·min^{-1})							
	Pre BD Moderate Trial (Week 1)				Post BD Moderate Trial (Week 2)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Zero	22.17 ± 3.54	20.89 ± 3.01	21.75 ± 3.44	22.35 ± 3.67	21.04 ± 2.51	20.70 ± 2.01	21.18 ± 3.16	20.87 ± 3.41
10 %	24.31 ± 4.22	23.83 ± 3.65	23.67 ± 4.72	23.70 ± 4.68	23.50 ± 3.67	23.12 ± 4.00	25.04 ± 4.61	24.00 ± 4.32
20 %	25.94 ± 4.15	26.97 ± 4.75	25.79 ± 5.16	26.21 ± 5.43	26.86 ± 4.56	27.14 ± 5.33	28.03 ± 5.79**	27.32 ± 5.87
30 %	28.89 ± 5.39	28.46 ± 5.20	29.76 ± 5.89	28.76 ± 6.00	29.71 ± 5.10	29.71 ± 5.42	29.66 ± 6.33	30.59 ± 6.07
40 %	30.15 ± 6.12	29.92 ± 5.81	30.58 ± 6.30	31.52 ± 6.67	30.86 ± 5.31	31.28 ± 6.10	31.53 ± 7.12	30.93 ± 5.85
50 %	31.84 ± 6.59	31.40 ± 5.53	32.19 ± 6.42	31.39 ± 6.95	32.19 ± 6.00	32.56 ± 5.15	33.18 ± 7.44	32.78 ± 6.36
60 %	32.27 ± 6.35	32.07 ± 6.27	32.58 ± 5.89	32.57 ± 6.55	33.09 ± 6.20	32.85 ± 5.77	33.30 ± 6.67	34.05 ± 6.83
70 %	32.60 ± 6.39	32.74 ± 7.03	33.76 ± 6.63	32.82 ± 6.58	34.26 ± 6.33	33.77 ± 5.10	33.89 ± 7.27	34.80 ± 6.75
80 %	33.87 ± 7.59	33.14 ± 7.12	34.15 ± 7.05	33.86 ± 7.17	34.80 ± 6.45	34.74 ± 5.52	34.46 ± 6.91	34.84 ± 7.09
90 %	34.32 ± 6.88	34.78 ± 6.61	35.31 ± 8.38	35.64 ± 8.04	35.14 ± 6.02	34.97 ± 5.32	34.54 ± 7.17	36.39 ± 7.75
100 %	36.44 ± 7.17	36.78 ± 7.56	35.93 ± 7.58	37.18 ± 7.66	36.81 ± 6.59	36.77 ± 5.68	37.13 ± 5.98	37.61 ± 7.27
	Pre BD Heavy Trial (Week 1)				Post BD Heavy Trial (Week 2)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Zero	24.21 ± 3.49	24.13 ± 4.15	22.74 ± 5.31	23.90 ± 5.61	22.63 ± 3.51	24.26 ± 4.40	23.71 ± 3.84	24.36 ± 5.97
10 %	28.62 ± 6.56	28.57 ± 4.38	27.80 ± 5.40	27.37 ± 5.90	25.75 ± 5.87	27.21 ± 4.29*	27.56 ± 5.73	29.44 ± 7.51
20 %	32.69 ± 6.32	33.24 ± 5.55	32.59 ± 8.14	33.28 ± 7.99	31.91 ± 4.40	33.21 ± 6.73	32.34 ± 7.02	35.57 ± 7.90
30 %	37.04 ± 8.34	38.26 ± 6.03	37.01 ± 8.07	37.75 ± 7.89	37.79 ± 5.66	38.74 ± 7.29	36.90 ± 8.39	38.26 ± 7.10
40 %	39.56 ± 7.86	41.24 ± 6.78	40.13 ± 8.78	39.80 ± 8.15	40.72 ± 7.14	40.83 ± 6.93	40.03 ± 7.40	40.58 ± 7.76
50 %	41.82 ± 7.68	44.25 ± 7.50	42.21 ± 7.40	43.08 ± 9.57	41.91 ± 8.22	43.66 ± 7.34	41.94 ± 8.55	43.19 ± 7.70
60 %	43.90 ± 8.79	46.79 ± 8.41	45.40 ± 8.36	46.45 ± 9.81	43.69 ± 8.98	44.74 ± 8.91	43.76 ± 8.31	45.31 ± 7.59
70 %	47.31 ± 9.40	47.82 ± 8.49	48.86 ± 12.52	46.94 ± 10.24	46.96 ± 9.79	47.17 ± 9.17	45.93 ± 9.72	46.69 ± 9.29
80 %	50.32 ± 10.27	49.82 ± 9.46	49.44 ± 12.38	49.34 ± 11.68	48.47 ± 11.40	49.34 ± 10.04	48.08 ± 10.22	48.11 ± 9.40
90 %	51.01 ± 10.75	53.06 ± 12.14	51.93 ± 12.22	52.51 ± 12.85	50.63 ± 10.63	52.37 ± 12.18	50.34 ± 12.35	50.39 ± 10.87
100 %	53.72 ± 9.59	55.93 ± 12.97	53.82 ± 12.76	54.89 ± 13.03	51.98 ± 9.71	54.92 ± 13.04	53.80 ± 13.57	52.22 ± 10.04

** P < 0.05, ** P < 0.01, denotes a significant difference from Pre BD to Post BD.*

7.3.4 Minute Ventilation

The mean ± SD values of the percentiles for V_E can be seen in Table 7.3 and Figures 7.3 and 7.4. The two-way ANOVA for the moderate intensity revealed pairwise significance between trials at 20 % (P = 0.002, η_p^2 = 0.60 (CI, -4.73, -1.42)), 30 % (P = 0.003, η_p^2 = 0.56 (CI, -4.78, -1.24)), 40 % (P = 0.020, η_p^2 = 0.40 (CI, -3.99, -0.42)), 50 % (P = 0.012, η_p^2 = 0.45 (CI, -4.06, -0.63)), 60 % (P = 0.016, η_p^2 = 0.43 (CI, -4.62, -0.60)) and at 70 % (P = 0.012, η_p^2 = 0.45 (CI, -4.49, -0.68)). However, no significant differences were observed for a main effect for time or for the overall interaction effect for trial by time (P > 0.05). The one-way ANOVA also showed no significance (P > 0.05) in any areas.

T-test analysis of pre to post BD for V_E in the moderate trial reported several significant time points, which were at 24 h for 20 % (P = 0.025, Cohens d = -0.36 (CI, -6.04, 6.45)), 30 % (P = 0.050, Cohens d = -0.30 (CI, -7.75, 7.95)) and at 70 % there was tendency towards significance (P = 0.050, Cohens d = -.24 (CI, -9.18, 9.80)). Also at 48 h at 30 % (P = 0.019, Cohens d = -.28 (CI, -7.61, 8.37)), at 72 h for 20 % (P = 0.019, Cohens d = -0.27 (CI, -7.14, 6.87)) and 60 % (P = 0.025, Cohens d = -0.17 (CI, -9.31, 9.49)) and at 96 h at 20 % (P = 0.021, Cohens d = -0.24 (CI, -6.71, 6.97)), 30 % (P = 0.034, Cohens d = -0.20 (CI, -7.67, 8.09)), 60 % (P = 0.0004, Cohens d = -.22 (CI, -9.11, 9.22)), 70 % (P = 0.010, Cohens d = -0.18 (CI, -9.26, 9.38)) and 90 % (P = 0.024, Cohens d = -0.11 (CI, -10.06, 9.57)).

The two-way ANOVA for the heavy intensity revealed pairwise significance between trials at 20 % (P = 0.011, η_p^2 = 0.46 (CI, -3.47, -0.56)) and at 30 % (P = 0.025, η_p^2 = 0.38 (CI, -5.25, -0.42)). However, no significant differences were observed for a main effect for time or for the overall interaction effect for trial by time (P > 0.05). The one-way ANOVA testing showed two significances, firstly pre BD at 30 % revealed a pairwise significance between 24 and 48 h (P = 0.031, (CI, -7.99, -0.33)), but the ANOVA itself had no significant difference (P > 0.05) result and also post BD at 20 % the ANOVA had significance (P = 0.036, η_p^2 = 0.23), but there were no significant (P > 0.05) pairwise comparisons for this time point. T-test analysis of pre to post BD for V_E in the heavy trial reported two significance time points, which were at 24 h at 30 % (P = 0.014, Cohens d = -0.27 (CI, -12.69, 12.39)) and 96 h at 20 % (P = 0.003, Cohens d = -0.29 (CI, -10.44, 10.89)).

Table 7.3: Mean ± SD V_E values over the two-week testing period.

Time Point (360s split into 10 % parts)	V_E ($l \cdot min^{-1}$)							
	Pre BD Moderate Trial (Week 1)				Post BD Moderate Trial (Week 2)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Zero	24.38 ± 3.23	24.00 ± 3.85	24.69 ± 4.22	24.07 ± 3.98	24.04 ± 4.02	23.70 ± 3.69	24.61 ± 4.19	23.19 ± 3.82
10 %	32.14 ± 5.51	32.40 ± 6.23	32.11 ± 7.03	32.34 ± 6.53	33.03 ± 7.32	32.68 ± 6.93	33.76 ± 7.91	33.11 ± 7.65
20 %	45.76 ± 10.05	46.55 ± 10.88	45.97 ± 12.15	46.27 ± 11.44	49.56 ± 12.03*	49.10 ± 12.11	49.11 ± 12.60**	49.08 ± 12.75*
30 %	56.03 ± 13.18	56.46 ± 12.96	57.68 ± 14.43	57.41 ± 13.22	59.98 ± 14.58	60.27 ± 15.28*	59.33 ± 14.37	60.04 ± 14.65*
40 %	61.90 ± 14.65	62.50 ± 14.01	62.40 ± 15.95	63.69 ± 14.50	64.50 ± 15.40	65.12 ± 15.32	65.12 ± 15.26	64.57 ± 15.88
50 %	65.14 ± 15.11	66.11 ± 14.32	66.28 ± 16.50	65.97 ± 14.94	68.30 ± 16.30	68.08 ± 14.99	68.25 ± 16.13	68.25 ± 16.30
60 %	66.67 ± 14.80	67.69 ± 14.61	67.17 ± 16.16	67.30 ± 15.72	69.42 ± 16.76	69.34 ± 15.37	69.82 ± 17.07*	70.70 ± 16.69***
70 %	67.90 ± 15.81	68.59 ± 15.38	68.91 ± 16.09	68.75 ± 16.05	71.70 ± 17.74	70.35 ± 15.53	70.83 ± 17.91	71.59 ± 16.89*
80 %	69.34 ± 16.45	69.65 ± 15.77	69.98 ± 17.19	70.52 ± 16.83	72.24 ± 17.72	72.44 ± 15.81	70.96 ± 17.32	71.77 ± 16.91
90 %	70.14 ± 16.54	71.45 ± 15.76	71.22 ± 17.60	71.71 ± 17.59	73.14 ± 17.45	72.52 ± 16.39	71.89 ± 17.39	73.53 ± 17.12*
100 %	72.44 ± 16.36	73.42 ± 16.71	72.02 ± 17.54	73.34 ± 17.80	75.08 ± 19.27	74.17 ± 16.69	74.38 ± 17.16	73.77 ± 17.46
	Pre BD Heavy Trial (Week 1)				Post BD Heavy Trial (Week 2)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Zero	27.88 ± 3.69	27.33 ± 4.85	27.08 ± 4.09	29.20 ± 6.13	26.90 ± 3.67	27.61 ± 4.15	26.62 ± 5.25	28.37 ± 6.89
10 %	40.73 ± 8.48	41.77 ± 8.29	41.89 ± 10.41	40.68 ± 9.91	39.77 ± 9.27	41.39 ± 9.95	40.04 ± 9.54	43.63 ± 11.83
20 %	66.80 ± 16.13	67.95 ± 15.40	66.68 ± 21.27	67.63 ± 17.95	68.11 ± 16.11	69.34 ± 17.64	66.85 ± 17.42	72.82 ± 19.76**
30 %	87.11 ± 21.95	91.27 ± 20.95	89.50 ± 25.33	89.41 ± 25.47	92.81 ± 22.38*	94.57 ± 25.12	89.14 ± 24.81	92.10 ± 23.69
40 %	99.12 ± 23.50	104.47 ± 23.37	102.44 ± 26.57	102.68 ± 27.48	104.72 ± 23.25	106.56 ± 25.21	102.04± 25.62	104.57 ± 23.59
50 %	107.84 ± 25.55	113.23 ± 22.68	111.45 ± 29.01	112.30 ± 29.85	111.78 ± 24.93	116.51 ± 27.81	110.72 ± 28.92	114.34 ± 25.40
60 %	114.61 ± 28.43	121.09 ± 25.10	118.52 ± 29.55	119.94 ± 30.47	118.07 ± 26.88	118.92 ± 26.90	117.47 ± 31.77	120.62 ± 26.09
70 %	122.83 ± 31.11	125.31 ± 27.69	124.60 ± 34.03	124.31 ± 32.37	126.17 ± 29.11	127.01 ± 30.44	122.79 ± 33.63	126.06 ± 29.80
80 %	128.67 ± 35.50	130.41 ± 30.47	128.66 ± 33.92	129.03 ± 33.06	130.95 ± 32.33	134.04 ± 33.36	129.01 ± 36.30	130.24 ± 30.28
90 %	130.39 ± 33.64	135.73 ± 31.58	133.34 ± 34.40	135.53 ± 35.51	136.29 ± 32.83	140.07 ± 35.27	134.15 ± 39.38	136.19 ± 32.20
100 %	134.18 ± 35.30	139.59 ± 33.72	136.87 ± 36.32	140.80 ± 36.48	135.84 ± 31.02	143.96 ± 35.21	139.88 ± 40.29	140.31 ± 32.98

** P < 0.05, ** P < 0.01, denotes a significant difference from Pre BD to Post BD.*

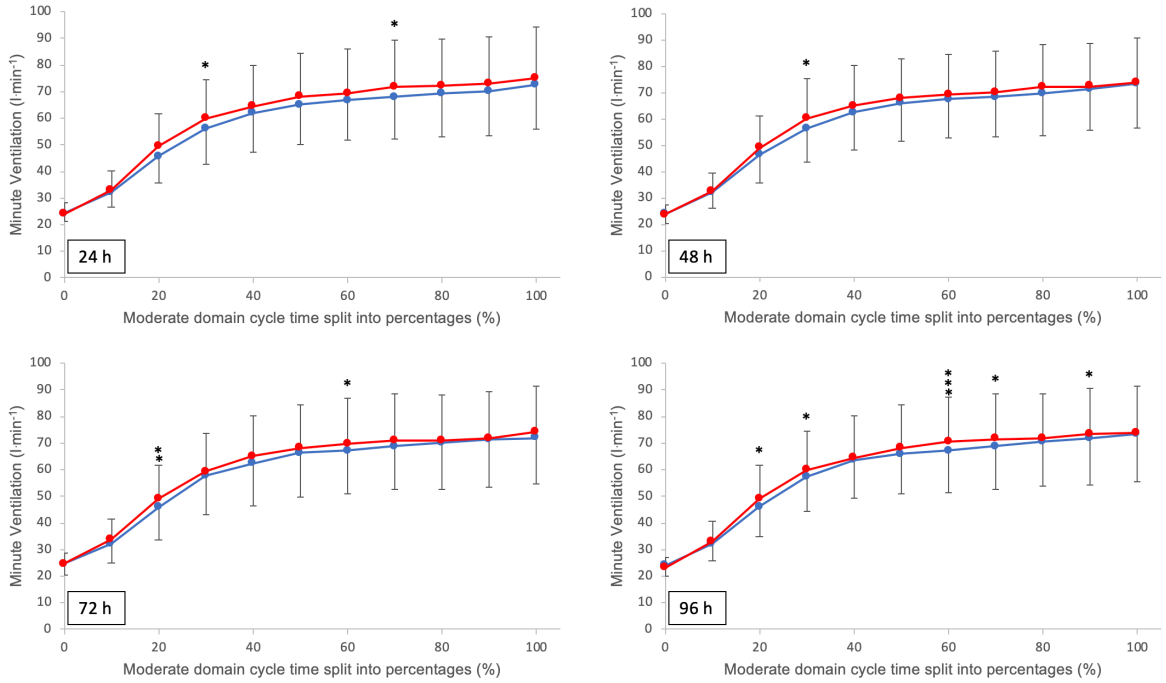

Figure 7.3: Mean pre BD (week 1 in blue) and post BD (week 2 in red) V_E during moderate intensity cycling throughout 24-96 h. The single, double and triple asterisks indicate significant difference ($P < 0.05$, $P < 0.01$ $P < 0.001$) pre to post BD.

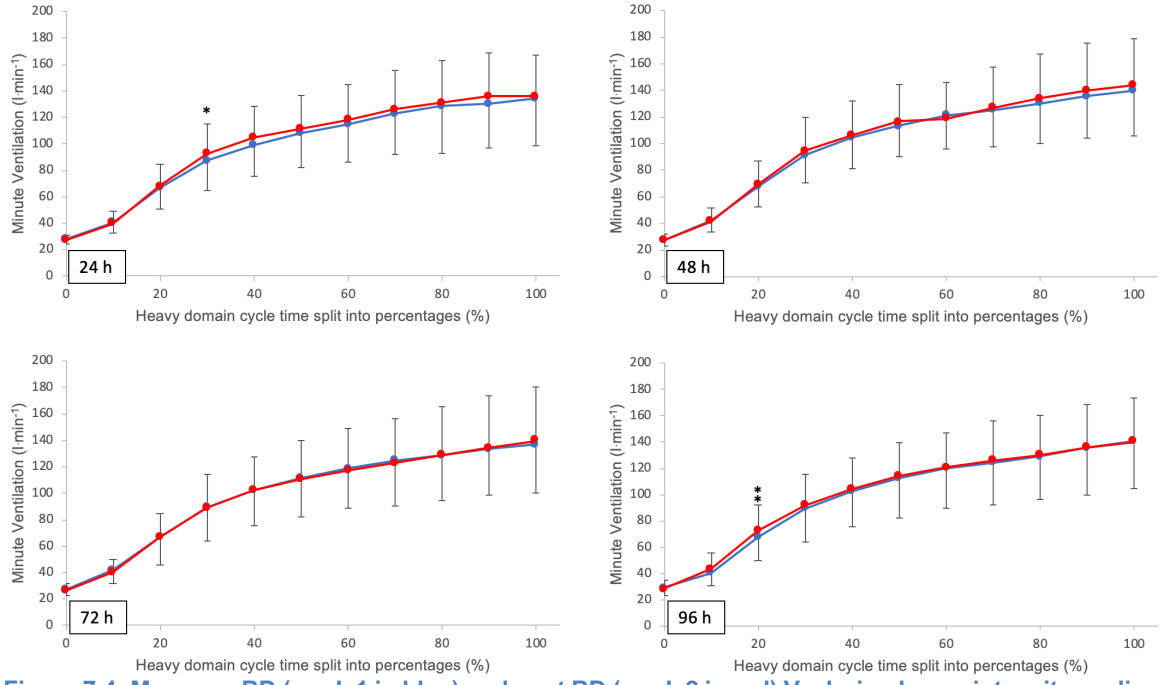

Figure 7.4: Mean pre BD (week 1 in blue) and post BD (week 2 in red) V_E during heavy intensity cycling throughout 24-96 h. The single and double asterisks indicate significant difference ($P < 0.05$ and $P < 0.01$) pre to post BD.

7.3.5 Ventilatory Equivalents

The two-way ANOVA for $V_E/\dot{V}O_2$ in the moderate intensity revealed pairwise significance between trials at 20 % (P = 0.021, η_p^2 = 0.40 (CI, -1.69, -0.17)), 30 % (P = 0.013, η_p^2 = 0.44 (CI, -1.97, -0.29)), 40 % (P = 0.025, η_p^2 = 0.39 (CI, -1.85, -0.15)), 50 % (P = 0.006, η_p^2 = 0.51 (CI, -1.75, -0.37)), 60 % (P = 0.032, η_p^2 = 0.36 (CI, -2.11, -0.12)), 70 % (P = 0.017, η_p^2 = 0.42 (CI, -2.04, -0.24)), 80 % (P = 0.022, η_p^2 = 0.39 (CI, -1.98, -0.19)), 90 % (P = 0.012, η_p^2 = 0.45 (CI, -1.72, -0.26)) and 100 % (P = 0.018, η_p^2 = 0.41 (CI, -1.71, -0.20)). However, no significant differences were observed for a main effect for time or for the overall interaction effect for trial by time (P > 0.05). Only one significance was seen regarding the one-way ANOVA, which was post BD at 10 % (P = 0.017, η_p^2 = 0.26), but there were no significant (P > 0.05) pairwise comparisons for this time point.

T-test analysis of pre to post BD for $V_E/\dot{V}O_2$ in the moderate trial reported several significant time points (Figure 7.5), which were during 24 h at 90 % (P = 0.049, Cohens d = -0.45 (CI, -2.02, 1.64)), 72 h at 20 % (P = 0.001, Cohens d = -0.67 (CI,-1.89, 0.77)), 30 % (P = 0.013, Cohens d = -0.39 (CI, -1.78, 1.12)), 40 % (P = 0.005, Cohens d = -0.57 (CI, -2.01, 1.20)), 50 % (P = 0.034, Cohens d = -0.39 (CI,-1.90, 1.41)), 60 % (P = 0.029, Cohens d = -0.47 (CI, -1.85, 1.32)), 70 % (P = 0.034, Cohens d = (CI,)), 90 % (P = 0.016, Cohens d = -0.32 (CI,-1.81, 1.49)) and 100 % (P = 0.002, Cohens d = -0.49 (CI, -2.03, 1.16)). Also, during 96 h at 60 % (P = 0.012, Cohens d = -0.41 (CI, -1.89, 1.34)), 70 % (P = 0.028, Cohens d = -0.38 (CI, -1.84, 1.48)) and 90 % (P = 0.043, Cohens d = -0.28 (CI, -2.08, 1.64)).

The two-way ANOVA for $V_E/\dot{V}O_2$ in the heavy intensity revealed pairwise significance between trials at 20 % (P = 0.004, η_p^2 = 0.55 (CI, -1.85, -0.46)), 30 % (P = 0.007, η_p^2 = 0.50 (CI, -2.53, -0.50)), 40 % (P = 0.021, η_p^2 = 0.40 (CI, -2.97, -0.30)), 50 % (P = 0.030, η_p^2 = 0.36 (CI, -3.22, -0.21)), 70 % (P = 0.028, η_p^2 = 0.37 (CI, -3.39, -0.23)), 80 % (P = 0.017, η_p^2 = 0.42 (CI, -3.13, -0.38)), 90 % (P = 0.014, η_p^2 = 0.43 (CI, -3.87, -0.53)), 100 % (P = 0.019, η_p^2 = 0.41 (CI, -3.40, -0.38)). However, no significant differences were observed for a main effect for time or for the overall interaction effect for trial by time (P > 0.05). Only one significance showed regarding one-way ANOVA's, which was with a pairwise comparison post BD between 48 and 96 h at 10 % (P = 0.017, η_p^2 = 0.26), but the ANOVA itself showed no significant difference (P > 0.05).

T-test analysis of pre to post BD for $V_E/\dot{V}O_2$ in the heavy trial demonstrated several significant time points (Figure 7.6), which were at 24 h for 50 % (*P* = 0.046, Cohens *d* = -0.43 (CI, -2.33, 2.71)) and 90 % (*P* = 0.020, Cohens *d* = -0.60 (CI, -2.59, 2.66)), 48 h at 80 % (*P* = 0.038, Cohens *d* = -0.50 (CI, -2.61, 2.63)), 72 h at 70 % (*P* = 0.043, Cohens *d* = -0.42 (CI, -2.82, 2.61)) and 100 % (*P* = 0.036, Cohens *d* = -0.54 (CI, -2.87, 2.53)) and 96 h at 20 % (*P* = 0.000, Cohens *d* = -0.50 (CI, -2.50, 1.64)) and 80 % (*P* = 0.042, Cohens *d* = -0.31 (CI, -2.86, 2.16)).

The two-way ANOVA for $V_E/\dot{V}CO_2$ in the moderate intensity revealed pairwise significance between trials at 70 % (*P* = 0.039, η_p^2 = 0.33 (CI, -1.25, -0.04)) and 80 % (*P* = 0.031, η_p^2 = 0.36 (CI, -1.15, -0.06)). However, no significant differences were observed for a main effect for time or for the overall interaction effect for trial by time (*P* > 0.05). Only one significance showed regarding one-way ANOVA, which was post BD at 10 % (*P* = 0.017, η_p^2 = 0.26), but there were no significant (*P* > 0.05) pairwise comparisons for this time point. In the heavy trial all ANOVA testing showed no significant differences.

T-test analysis of pre to post BD for $V_E/\dot{V}CO_2$ in the moderate trial reported significant time points (Figure 7.5) only during 72 h at 60 % (*P* = 0.010, Cohens *d* = -0.31 (CI, -1.78, 1.07)), 70 % (*P* = 0.006, Cohens *d* = -0.27 (CI, -1.81, 1.15)) and 100 % (*P* = 0.045, Cohens *d* = -0.29 (CI, -1.96, 0.96)). T-test analysis of pre to post BD for $V_E/\dot{V}CO_2$ in the heavy trial and all ANOVA testing showed no significant differences.

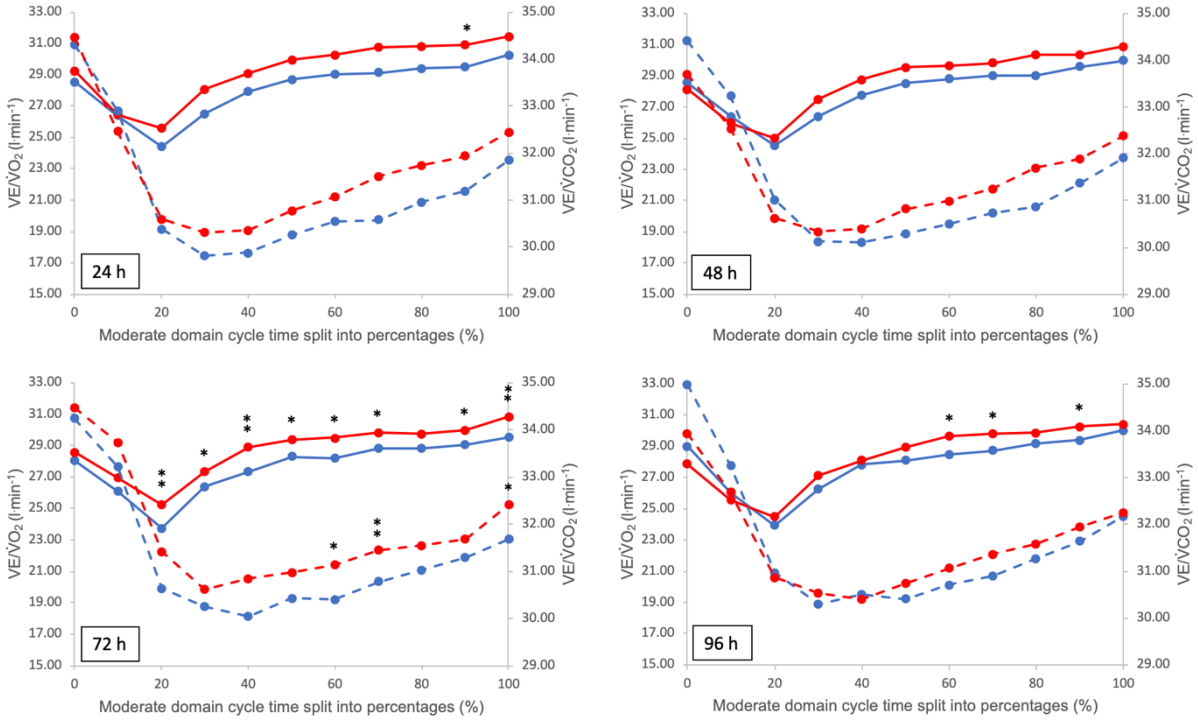

Figure 7.5: Pre BD (week 1 in blue) and post BD (week 2 in red) $V_E/\dot{V}O_2$ and $V_E/\dot{V}CO_2$ during moderate intensity cycling throughout 24-96 h. The solid line represents mean responses of $V_E/\dot{V}O_2$ and the dashed line signifies mean responses of $V_E/\dot{V}CO_2$. The single and double asterisks indicate significant difference ($P < 0.05$ and $P < 0.01$) pre to post BD. N.B. data is reported without error bars for ease of representation.

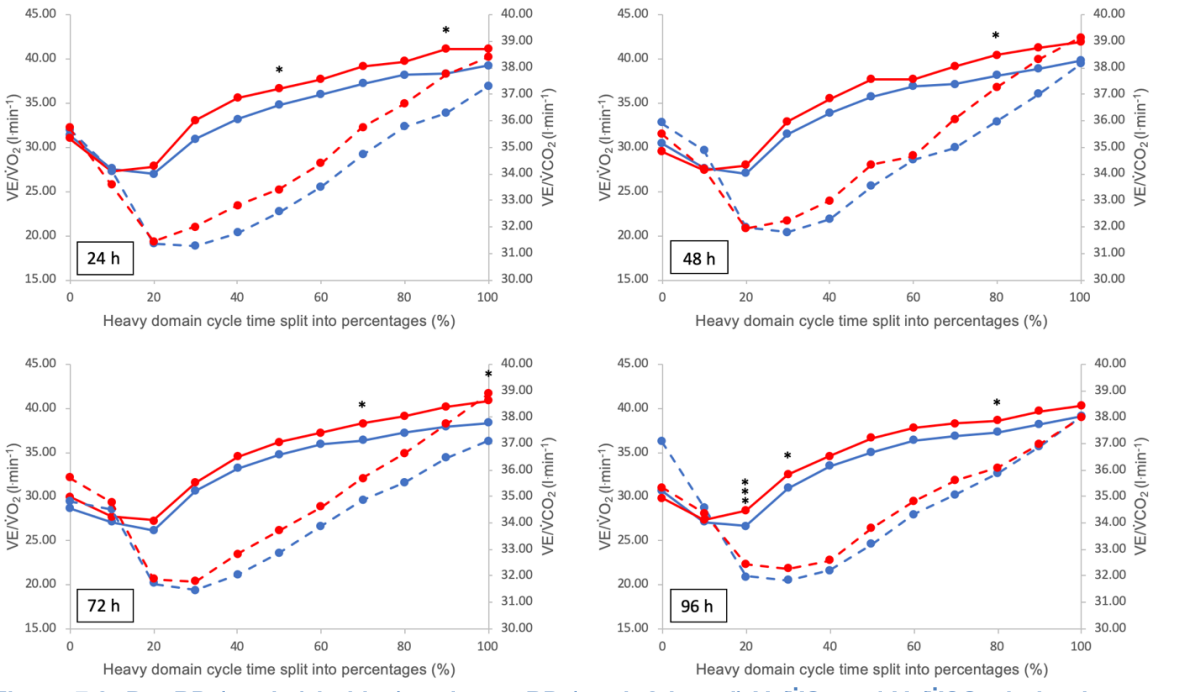

Figure 7.6: Pre BD (week 1 in blue) and post BD (week 2 in red) $V_E/\dot{V}O_2$ and $V_E/\dot{V}CO_2$ during heavy intensity cycling throughout 24-96 h. The solid line represents mean responses of $V_E/\dot{V}O_2$ and the dashed line signifies mean responses of $V_E/\dot{V}CO_2$. The single and triple asterisks indicate significant difference ($P < 0.05$ and $P < 0.001$) pre to post BD. N.B. data is reported without error bars for ease of representation.

7.3.6 Stroke Volume

Regarding SV in the moderate domain, pairwise comparisons between pre and post BD (week 1 and 2) reported significant differences between trials only at the 0 % (P = 0.034, η_p^2 = 0.35 (CI, .58, 11.89) and 10 % (P = 0.034, η_p^2 = 0.35 (CI, .56, 11.36) time point. No significant differences were observed for a main effect for time or for the overall interaction effect for trial by time (P > 0.05). No significance difference was apparent between any time points when employing one-way ANOVAs. Only one significant difference was observed when comparing pre to post BD (t-test) in the moderate trial, which was a decrease at the time point of 80 % at 24 h (146.22 ± 22.23 v 130.34 ± 28.54 ml, P = 0.030, Cohen's d = 0.64 (CI, -11.94, 15.08)).

Significance concerning the heavy trial was absent in all ANOVA results and t-tests (P > 0.05). Unlike the moderate domain detecting a data trend is not as obvious when viewing Table 7.4, with the data displaying variable tendency, it is only 24 h comparisons that have an observable reduced SV post BD.

Table 7.4: Mean ± SD of SV over the two-week testing time period.

Time Point (360s split into 10 % parts)	SV (ml)							
	Pre BD Moderate Trial (Week 1)				Post BD Moderate Trial (Week 2)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Zero	112.09 ± 19.33	109.19 ± 21.63	108.36 ± 17.65	197.64 ± 17.37	102.15 ± 21.40	108.12 ± 19.26	102.48 ± 21.59	99.60 ± 19.61
10 %	126.66 ± 20.51	122.64 ± 20.88	120.16 ± 19.82	120.81 ± 21.81	117.93 ± 23.77	122.32 ± 18.72	114.89 ± 20.92	111.32 ± 18.72
20 %	136.66 ± 23.47	130.57 ± 24.97	127.09 ± 19.45	129.41 ± 22.59	127.90 ± 25.34	130.71 ± 18.56	127.07 ± 23.46	118.11 ± 24.92
30 %	141.65 ± 22.96	134.42 ± 24.19	128.34 ± 19.72	129.64 ± 22.09	130.07 ± 27.03	134.76 ± 20.14	130.58 ± 24.34	124.52 ± 23.68
40 %	141.91 ± 22.25	136.82 ± 25.52	130.08 ± 19.57	130.41 ± 24.23	129.95 ± 27.86	134.49 ± 19.40	133.61 ± 28.15	126.32 ± 23.12
50 %	143.33 ± 26.60	137.63 ± 30.91	130.98 ± 20.84	130.17 ± 23.82	132.27 ± 26.19	133.82 ± 17.59	133.40 ± 27.97	127.51 ± 23.46
60 %	142.41 ± 26.65	133.96 ± 28.46	129.90 ± 22.03	131.27 ± 24.00	133.81 ± 29.31	134.87 ± 18.86	131.16 ± 28.15	127.42 ± 22.22
70 %	144.73 ± 27.49	135.00 ± 28.07	130.24 ± 21.98	131.06 ± 23.48	130.81 ± 27.34	132.82 ± 19.33	132.06 ± 27.51	126.75 ± 23.60
80 %	146.22 ± 22.23	134.83 ± 29.93	133.18 ± 22.47	128.82 ± 24.26	130.34 ± 28.54*	132.81 ± 20.32	132.33 ± 25.42	126.83 ± 22.36
90 %	142.61 ± 23.26	134.09 ± 30.06	130.89 ± 21.85	129.43 ± 23.62	131.88 ± 26.60	133.59 ± 17.70	131.90 ± 27.27	125.09 ± 22.70
100 %	146.79 ± 26.21	134.81 ± 28.77	131.13 ± 24.56	128.81 ± 23.04	132.31 ± 25.00	131.28 ± 14.66	131.63 ± 27.03	126.07 ± 22.75
	Pre BD Heavy Trial (Week 1)				Post BD Heavy Trial (Week 2)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Zero	112.09 ± 17.92	107.47 ± 23.66	109.59 ± 20.24	107.63 ± 21.74	106.83 ± 19.25	112.38 ± 12.33	109.59 ± 22.96	101.14 ± 17.92
10 %	133.78 ± 21.39	127.57 ± 22.07	125.74 ± 20.96	122.66 ± 23.68	121.35 ± 21.34	128.14 ± 18.27	123.81 ± 23.79	118.49 ± 25.69
20 %	141.01 ± 21.20	137.10 ± 23.78	130.11 ± 22.54	132.34 ± 25.37	129.90 ± 20.28	136.34 16.99	133.64 ± 22.87	126.26 ± 25.87
30 %	144.49 ± 27.56	139.89 ± 35.77	134.98 ± 25.15	136.23 ± 25.34	131.71 ± 32.97	139.71 ± 18.28	134.40 ± 28.79	132.40 ± 19.53
40 %	145.86 ± 27.56	141.41 ± 35.77	134.83 ± 25.15	134.40 ± 25.34	134.56 ± 32.97	135.04 ± 18.28	136.65 ± 28.79	126.37 ± 19.53
50 %	140.18 ± 23.11	137.74 ± 29.24	136.51 ± 21.04	131.18 ± 27.92	135.40 ± 32.15	132.94 ± 17.48	136.45 ± 19.15	129.14 ± 14.46
60 %	138.30 ± 23.19	134.83 ± 27.05	132.33 ± 23.63	128.88 ± 29.93	132.90 ± 33.52	137.00 ± 14.27	130.68 ± 25.43	134.75 ± 22.70
70 %	140.28 ± 29.06	133.19 ± 26.88	129.14 ± 23.23	135.67 ± 30.89	131.75 ± 29.93	134.63 ± 16.12	131.38 ± 21.86	127.82 ± 19.47
80 %	137.87 ± 23.67	137.12 ± 28.86	135.68 ± 23.18	135.49 ± 29.77	132.13 ± 26.95	142.11 ± 34.39	129.27 ± 18.63	131.91 ± 27.48
90 %	135.29 ± 23.98	134.01 ± 21.02	140.25 ± 26.98	132.57 ± 33.36	134.11 ± 28.19	128.67± 22.03	128.38 ± 18.64	126.46 ± 20.62
100 %	140.70 ± 30.21	129.43 ± 30.21	132.93 ± 35.35	125.68 ± 26.90	129.90 ± 31.35	126.11 ± 17.83	126.80 ± 15.15	129.96 ± 21.22

** P < 0.05 denotes a significant difference from Pre BD to Post BD.*

7.3.7 Heart Rate

Resting HR for each time point (24-96 h) pre BD was 65.3 ± 7.2, 65.7 ± 7.3, 66.3 ± 9.7 and 64.2 ± 11.6 b·min^{-1}. Post BD the values were 66.0 ± 7.7, 67.8 ± 7.3, 66.8 ± 8.4 and 67.7 ± 7.2 b·min^{-1}, there were no significant differences between values.

In the moderate domain, a significant difference was apparent from two-way ANOVAs in pairwise comparisons between pre and post BD (week 1 and 2) at 50 % (P = 0.045, η_p^2 = 0.32 (CI, -6.79, -.100)), 70 % (P = 0.050, η_p^2 = 0.31 (CI, -7.05, 9.11)), and 90 % (P = 0.043, η_p^2 =0 .32 (CI, -7.19, -.129)). No significant differences were observed for a main effect for time or for the overall interaction effect for trial by time (P > 0.05) and significance was absent (P > 0.05) between any time points when regarding one-way ANOVAs. Regarding HR in the moderate domain, significant (P < 0.05) increases were observed from *t*-tests when comparing pre to post BD for 72 h at 20 % (126.7 ± 10.3 v 129.8 ± 8.1 b·min^{-1}, P = 0.047, Cohen's d = -0.34 (CI, -6.17, 3.76)), 30 % (133.7 ± 10.4 v 137.1 ± 8.1 b·min^{-1}, P = 0.039, Cohen's d = -0.38 (CI, -6.24, 4.18)), 50 % (138.9 ± 11.3 v 143.0 ± 9.2 b·min^{-1}, P = 0.034, Cohen's d = -0.41 (CI, -6.80, 4.80)), 60 % (140.4 ± 11.6 v 144.4 ± 9.1 b·min^{-1}, P = 0.035, Cohen's d = -0.40 (CI, -6.96, 4.74)), 90 % (143.9 ± 12.6 v 147.6 ± 10.2 b·min^{-1}, P = 0.037, Cohen's d = -0.34 (CI, -7.45, 5.42)), and 100 % (144.3 ± 13.0 v 148.1 ± 10.1 b·min^{-1}, P = 0.033, Cohen's d = -0.34 (CI, -7.69, 5.38)). HR although not always significant had a trend to increase post BD during moderate intensity exercise (Figure 7.7).

In the heavy domain, the two-way ANOVA pairwise comparisons between pre and post BD (week 1 and 2) showed no significance (P > 0.05). However, significant differences were observed for a main effect for time at 30, 40, 60, 70 and 80 % (P = 0.023, η_p^2 = 0.34, P = 0.001, η_p^2 = 0.38, P = 0.000, η_p^2 = 0.43, P = 0.000, η_p^2 = 0.42 and P = 0.000, η_p^2 = 0.49). There was no overall interaction effect for trial by time (P > 0.05). When employing one-way ANOVAs, significance for within subjects' effects were apparent for pre BD at 60 % (P = 0.040, η_p^2 = 0.27), 70 % (P = 0.005, η_p^2 = 0.32) and 80 % (P = 0.017, η_p^2 = 0.26), indicating a possible significant difference between days, however the pairwise comparison between days showed this was not evident. At the 80 % mark for post BD, there was significance for within subject effects (P = 0.011, η_p^2 = 0.28) and there was significance between the pairwise comparison time points of 48 and 96 h (P = 0.011, η_p^2 = 0.28 (CI, .25, 5.63)). Significant (P < 0.05) increases were observed in *t*-tests (Figure 7.8) over the 20 % (147.1 ± 11.4 v 151.2 ± 9.9 b·min^{-1}, P = 0.015, Cohen's d = -0.40 (CI, -6.83, 5.20)) and 100 % (174.8 ± 10.6 v 177.5 ± 9.7 b·min^{-1}, P = 0.044, Cohen's d = -0.28 (CI, -6.29, 5.22)) marks at 72 h.

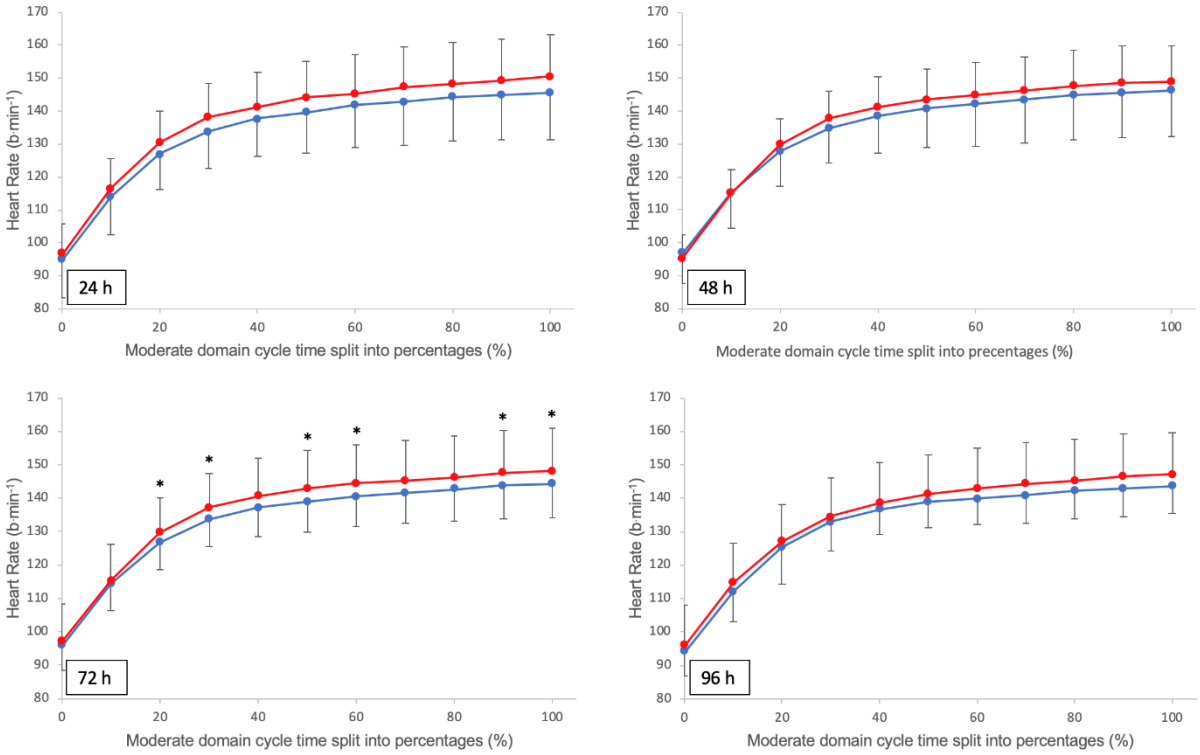

Figure 7.7: Pre BD (week 1) and post BD (week 2) HR during moderate intensity cycling throughout 24-96 h. The asterisks shown in the graph representing 72 h indicate significant difference (P < 0.05) pre to post BD.

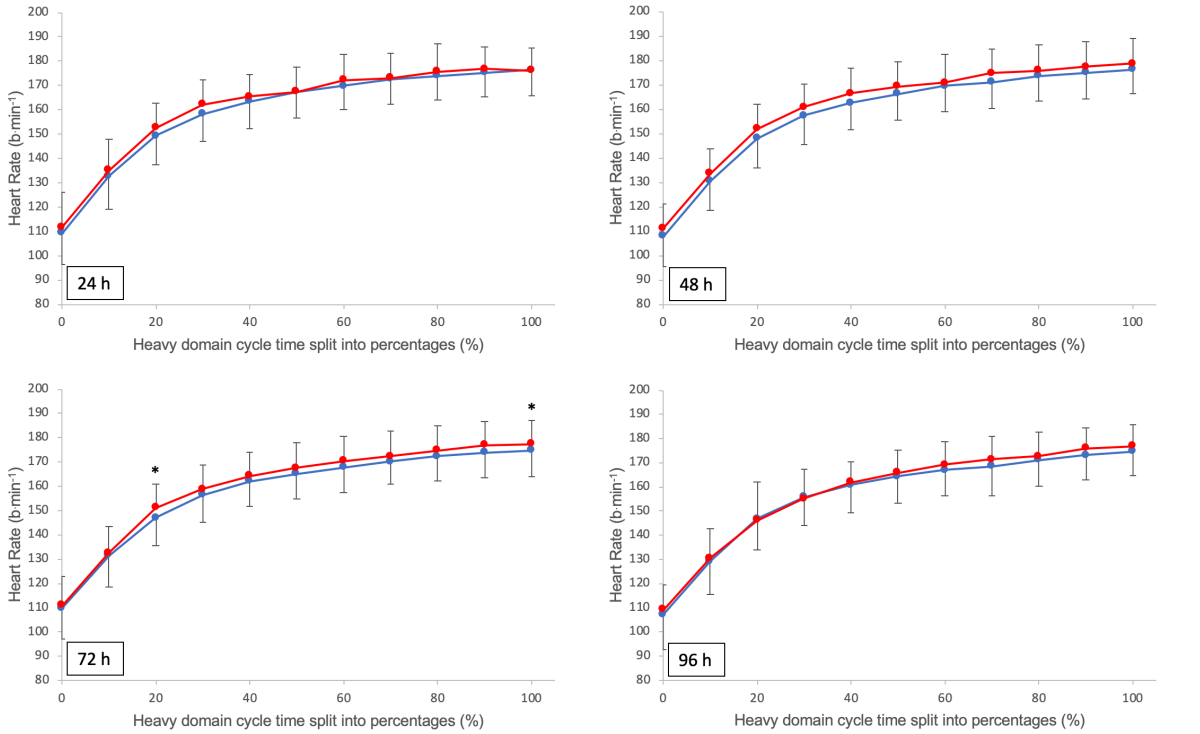

Figure 7.8: Pre BD (week 1) and post BD (week 2) HR during heavy intensity cycling throughout 24-96 h. The asterisks shown in the graph representing 72 h indicate significant difference (P < 0.05) pre to post BD.

7.3.8 Cardiac Output

The two-way ANOVA for $\dot{Q}$ in the moderate trial indicated no significantly different (P > 0.05) pairwise comparisons between pre and post BD. However, significant differences were observed for a main effect for time at 20, 30, 40, 80, 90 and 100 % (P = 0.025, η_p^2 = 0.24, P = 0.034, η_p^2 = 0.23, P = 0.039, η_p^2 = 0.22, P = 0.041, η_p^2 = 0.22, P = 0.025, η_p^2 = 0.24 and P = 0.002, η_p^2 = 0.35).There was no overall interaction effect for trial by time (P > 0.05). When regarding one-way ANOVAs, significance for within subjects' effects was apparent for pre BD only and at 100 % (P = 0.048, η_p^2 = 0.24). The two-way ANOVA and pairwise comparisons in the heavy trial indicated no significant interactions (P > 0.05), as was the case regarding a main effect for time or for the overall interaction effect for trial by time (P > 0.05). Additionally, significance was absent (P > 0.05) between any time points when regarding one-way ANOVAs. T-test analysis of pre to post BD for $\dot{Q}$ showed no significances in the moderate or heavy trials. The mean and SD results of the percentiles are displayed in Table 7.5.

Table 7.5: Mean ± SD of $\dot{Q}$ over the two-week testing time period.

Time Point (360s split into 10 % parts)	$\dot{Q}$ ($l \cdot min^{-1}$)							
	Pre BD Moderate Trial (Week 1)				Post BD Moderate Trial (Week 2)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Zero	10.59 ± 1.81	10.56 ± 2.11	10.32 ± 1.50	10.14 ± 2.09	9.88 ± 2.26	10.33 ± 1.90	9.95 ± 2.11	9.55 ± 1.83
10 %	14.34 ± 1.67	14.09 ± 2.16	13.72 ± 2.21	13.56 ± 2.72	13.75 ± 2.69	14.08 ± 2.12	13.27 ± 2.35	12.79 ± 2.71
20 %	17.19 ± 2.08	16.61 ± 2.87	16.06 ± 2.34	16.13 ± 2.74	16.62 ± 3.05	16.94 ± 2.41	16.32 ± 2.74	15.07 ± 3.52
30 %	18.80 ± 2.30	18.02 ± 2.92	17.12 ± 2.63	17.20 ± 3.06	17.91 ± 3.46	18.55 ± 2.93	17.86 ± 3.22	16.79 ± 3.52
40 %	19.40 ± 2.48	18.90 ± 3.34	17.83 ± 2.94	17.74 ± 3.31	18.26 ± 3.57	18.98 ± 2.93	18.72 ± 3.83	17.53 ± 3.36
50 %	19.92 ± 3.31	19.28 ± 4.00	18.20 ± 3.22	17.99 ± 3.31	18.96 ± 3.51	19.19 ± 2.78	19.06 ± 4.03	18.03 ± 3.57
60 %	20.07 ± 3.23	18.98 ± 3.92	18.24 ± 3.35	18.30 ± 3.48	19.35 ± 4.14	19.51 ± 3.08	18.93 ± 4.15	18.22 ± 3.47
70 %	20.56 ± 3.43	19.30 ± 3.92	18.44 ± 3.34	18.41 ± 3.47	19.19 ± 3.90	19.40 ± 3.27	19.16 ± 4.13	18.31 ± 3.67
80 %	20.96 ± 2.71	19.46 ± 4.22	19.00 ± 3.49	18.27 ± 3.61	19.22 ± 3.93	19.62 ± 3.51	19.29 ± 3.78	18.46 ± 3.52
90 %	20.57 ± 3.00	19.48 ± 4.28	18.81 ± 3.29	18.44 ± 3.48	19.59 ± 3.72	19.86 ± 3.20	19.46 ± 4.10	18.37 ± 3.60
100 %	21.23 ± 3.23	19.64 ± 4.00	18.93 ± 3.78	18.47 ± 3.54	19.84 ± 3.70	19.54 ± 2.59	19.48 ± 4.00	18.57 ± 3.66
	Pre BD Heavy Trial (Week 1)				Post BD Heavy Trial (Week 2)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Zero	12.27 ± 2.28	11.59 ± 2.61	11.92 ± 1.76	11.49 ± 2.45	11.90 ± 2.41	12.50 ± 1.74	12.15 ± 2.67	11.02 ± 1.85
10 %	17.69 ± 2.46	16.61 ± 2.48	16.51 ± 2.77	15.89 ± 3.43	16.43 ± 3.04	17.19 ± 2.82	16.22 ± 3.25	15.54 ± 3.68
20 %	20.91 ± 2.40	20.22 ± 3.25	19.19 ± 3.89	19.35 ± 3.77	19.81 ± 3.22	20.71 ± 2.86	20.07 ± 3.57	18.59 ± 4.70
30 %	22.77 ± 3.01	21.93 ± 4.39	21.08 ± 3.99	21.16 ± 4.09	21.35 ± 5.00	22.48 ± 3.48	21.40 ± 5.01	20.64 ± 4.82
40 %	23.72 ± 3.82	22.93 ± 5.69	21.86 ± 4.41	21.59 ± 4.26	22.27 ± 5.58	22.48 ± 3.33	22.41 ± 5.02	20.53 ± 3.72
50 %	23.36 ± 3.44	22.91 ± 5.02	22.57 ± 3.89	21.54 ± 4.77	22.49 ± 5.98	22.50 ± 3.29	22.92 ± 3.80	21.46 ± 3.14
60 %	23.44 ± 3.65	22.86 ± 4.78	22.20 ± 4.22	21.49 ± 5.12	22.82 ± 5.99	23.35 ± 3.17	22.35 ± 4.87	22.89 ± 4.50
70 %	24.13 ± 4.42	22.80 ± 4.65	22.04 ± 4.37	22.78 ± 5.33	22.40 ± 5.87	23.62 ± 3.33	22.72 ± 4.42	21.96 ± 4.07
80 %	23.93 ± 3.70	23.76 ± 4.89	23.41 ± 4.32	23.09 ± 5.08	23.24 ± 5.11	24.81 ± 5.16	22.74 ± 3.94	22.77 ± 4.70
90 %	23.63 ± 3.69	23.40 ± 3.70	24.27 ± 4.56	22.84 ± 5.34	23.74 ± 5.30	22.72 ± 3.85	22.81 ± 3.81	22.32 ± 4.20
100 %	24.64 ± 4.50	22.83 ± 5.37	23.28 ± 6.34	21.92 ± 4.73	22.93 ± 5.88	22.59 ± 3.56	22.59 ± 3.10	23.02 ± 4.17

7.3.9 Arteriovenous Oxygen Difference

The mean and SD values of the percentiles for a-vO_{2diff} can be seen in Table 7.6. In the moderate trial, pairwise comparisons between trials in the two-way ANOVA testing showed no significant differences. However, significant differences were observed for a main effect for time from 10 % through to 100 % (P = 0.012, η_p^2 = 0.28, P = 0.005, η_p^2 =0 .32, P = 0.004, η_p^2 = 0.33, P = 0.004, η_p^2 = 0.33, P = 0.005, η_p^2 = 0.32, P = 0.010, η_p^2 = 0.29, P = 0.005, η_p^2 = 0.32, P = 0.015, η_p^2 = 0.27, P = 0.004, η_p^2 = 0.33 and P = 0.001, η_p^2 = 0.39). There was no overall interaction effect for trial by time (P > 0.05).

One-way ANOVA in the moderate tests pre BD showed significance at 70 % (P = 0.017, η_p^2 = 0.26), with a significant pairwise comparison between 24 and 72 h (P = 0.044, (CI, 0.03, 2.44)). Also, at 80 % (P = 0.028, η_p^2 = 0.24), 90 % (P = 0.048, η_p^2 = 0.21) and 100 % (P = 0.014, η_p^2 = 0.27), but none of the other percentile bins displayed any significant pairwise comparisons between time points. One-way ANOVA in the moderate trials post BD showed significance only at 20% (P = 0.040, η_p^2 = 0.22), but with no significant pairwise comparisons between time points.

In the heavy trial, pairwise comparisons between trials in the two-way ANOVA testing showed no significant differences. However, two significant differences were observed for a main effect for time 0 % and 10 % (P = 0.007, η_p^2 = 0.30 and P = 0.017, η_p^2 = 0.26) There was no overall interaction effect for trial by time (P > 0.05). The one-way ANOVA testing for the heavy intensity had only one significant outcome, which was at 10 % post BD (P = 0.049, η_p^2 = 0.21), but with no significant pairwise comparisons between time points. No significant differences in *t*-test analysis for moderate or heavy intensity trials was apparent.

Table 7.6: Mean ± SD a-vO_{2diff} values over the two-week testing period.

Time Point (360s split into 10 % parts)	a-vO_{2diff} (ml·100ml^{-1})							
	Pre BD Moderate Trial (Week 1)				Post BD Moderate Trial (Week 2)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Zero	8.37 ± 1.90	8.18 ± 1.44	8.67 ± 1.56	8.52 ± 1.76	8.68 ± 1.79	8.39 ± 1.68	9.11 ± 2.60	8.86 ± 1.18
10 %	8.78 ± 2.52	8.93 ± 2.23	9.16 ± 2.24	9.35 ± 1.61	9.37 ± 2.26	9.10 ± 2.15	9.75 ± 2.80	10.33 ± 2.20
20 %	11.19 ± 3.34	11.75 ± 3.34	12.18 ± 3.13	12.11 ± 2.39	11.90 ± 2.64	11.75 ± 3.04	12.19 ± 3.45	13.74 ± 3.57
30 %	11.49 ± 3.36	12.16 ± 3.26	12.91 ± 3.16	12.83 ± 2.32	12.19 ± 2.67	11.93 ± 2.88	12.46 ± 3.33	13.51 ± 3.31
40 %	11.68 ± 3.42	12.31 ± 3.42	13.00 ± 3.33	13.10 ± 2.63	12.39 ± 2.55	12.14 ± 2.92	12.44 ± 3.29	13.41 ± 3.32
50 %	11.77 ± 3.53	12.49 ± 3.22	13.14 ± 3.30	13.21 ± 2.48	12.19 ± 2.31	12.24 ± 3.06	12.63 ± 3.55	13.42 ± 3.31
60 %	11.83 ± 3.57	12.89 ± 3.35	13.31 ± 3.26	13.10 ± 2.74	12.25 ± 2.84	12.24 ± 3.16	13.02 ± 3.88	13.37 ± 3.16
70 %	11.68 ± 3.47	12.72 ± 3.43	13.28 ± 3.20	13.17 ± 2.69	12.43 ± 2.63	12.40 ± 3.00	12.81 ± 3.67	13.48 ± 3.26
80 %	11.44 ± 3.03	12.88 ± 3.76	13.03 ± 3.17	13.40 ± 2.69	12.45 ± 2.62	12.48 ± 3.20	12.73 ± 3.51	13.29 ± 2.92
90 %	11.83 ± 3.34	12.91 ± 3.50	13.26 ± 3.11	13.37 ± 2.62	12.31 ± 2.47	12.28 ± 3.12	12.79 ± 3.77	13.57 ± 3.15
100 %	11.57 ± 3.10	12.85 ± 3.30	13.21 ± 3.13	13.33 ± 2.39	12.21 ± 2.56	12.50 ± 3.11	12.85 ± 3.72	13.40 ± 3.30
	Pre BD Heavy Trial (Week 1)				Post BD Heavy Trial (Week 2)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Zero	7.63 ± 2.26	8.01 ± 1.62	8.11 ± 1.48	8.55 ± 1.71	7.57 ± 1.79	7.72 ± 1.86	7.69 ± 2.30	8.70 ± 1.83
10 %	8.67 ± 2.84	9.35 ± 2.41	9.55 ± 2.77	9.60 ± 1.89	9.11 ± 2.24	9.07 ± 2.78	9.36 ± 3.21	10.64 ± 3.09
20 %	12.06 ± 3.55	12.69 ± 3.23	13.48 ± 3.86	13.23 ± 2.73	12.52 ± 2.58	12.15 ± 3.20	12.45 ± 3.46	14.52 ± 4.48
30 %	12.51 ± 3.36	13.56 ± 3.37	13.95 ± 3.28	13.70 ± 2.83	13.47 ± 2.71	12.88 ± 3.16	13.64 ± 4.21	14.33 ± 4.13
40 %	12.88 ± 3.70	14.04± 3.83	14.33 ± 3.27	14.40 ± 3.23	13.62 ± 2.48	13.52 ± 3.05	13.66 ± 4.19	15.16 ± 3.70
50 %	13.60 ± 3.94	14.36 ± 3.73	14.35 ± 3.40	15.16 ± 3.31	14.15 ± 2.90	13.92 ± 3.21	13.63 ± 4.01	14.78 ± 3.31
60 %	13.93 ± 4.19	14.68 ± 3.07	15.02 ± 3.37	15.72 ± 3.16	14.28 ± 2.97	13.74 ± 3.12	14.49 ± 4.10	14.37 ± 3.76
70 %	13.96 ± 3.81	15.15 ± 3.63	15.72 ± 3.65	15.12 ± 3.00	14.87 ± 2.77	13.98 ± 3.53	14.37 ± 4.14	15.35 ± 3.83
80 %	14.26 ± 3.96	14.67 ± 3.37	14.86 ± 3.13	15.25 ± 2.93	14.48 ± 2.73	13.89 ± 3.80	14.80 ± 4.71	15.50 ± 4.81
90 %	14.58 ± 3.85	15.18 ± 3.72	14.60 ± 3.19	16.12 ± 4.22	14.40 ± 3.53	15.34 ± 4.12	14.96 ± 4.91	15.86 ± 4.60
100 %	14.16 ± 4.12	15.82 ± 3.49	16.07 ± 5.27	16.94 ± 4.61	14.86 ± 3.24	15.47 ± 3.68	15.21 ± 4.13	15.57 ± 4.37

7.3.10 Blood Pressure and SpO_2 Responses

For BP and SpO_2 responses refer to page 129, section 6.3.7.

7.3.11 Haematological (Hb and Hct) Responses

For haematological responses refer to page 118-120, section 6.3.2.

7.4 Discussion/Conclusions

This work was undertaken to provide additional data regarding the effects of BD on sub-maximal exercise and was designed to follow the previous work within chapter 6 (O_2 kinetics). Therefore, the aim of this study was to explain the causal effect of the movement of O_2 and consequently to probe into the contributing variables of $\dot{V}O_2$ by determining the effects of BD (~470 ml) over a period of 96 hours on respiratory and cardiac function and O_2 extraction at the muscle. With the experimental hypotheses (numbers 1 - 6) stating that respiratory function, cardiac function and O_2 extraction will increase in the moderate and heavy domain from donating a unit of blood after 24 hours and across the subsequent 96 hours.

The majority of significant increases across all variables can be seen amongst the HR data 72 h post BD, which is the time period associated with the lowest Hb value post BD, consequential to haemodilution (Kanstrup and Ekblom, 1984). Although significances can be viewed for HR at 72 h, it is clear from the graphical information (Figures 7.7 and 7.8) that there was a trend for all HR data to increase post BD. Average percentage increases for HR across the moderate domain were 2.8 ± 0.5 % at 24 h, 1.4 ± 1.2 % at 48 h, 2.3 ± 0.6 % at 72 h and 2.0 ± 0.5 % at 96 h. During the heavy domain average increases were 1.2 ± 0.9 % at 24 h, 1.9 ± 0.7 % at 48 h, 1.5 ± 0.5 % at 72 h and 0.9 ± 0.8 % at 96 h. Consequently, experimental hypothesis 3 can be accepted as a significant increase occurred in the moderate domain post BD for HR during 72 h at the 20, 30, 50, 60, 90 and 100 % time bins and experimental hypothesis 4 can be accepted as a significant increase occurred in the heavy domain post BD for HR during 72 h at 20 and 100 %.

An elevated HR occurs from an acute 15 % total blood volume (TBV/BV) loss, which is the upper limit for BD set by the NHS (JPAC, 2013), equating to ~750 ml for a healthy 70 kg (~180 cm) blood donor. It should be also noted that it is possible to lose 15.5 % of TBV in a standard BD (50 kg, 150 cm female). Thus, the elevated HRs viewed in these data sets are possibly as a direct result of TBV lost, as well as less circulating O_2 availability from the decreased Hb value. With less O_2 carried per beat of the heart, HR increases to

compensate. A 10-15 % TBV decrease causes arterial baroreceptors to respond and a decreased vagal tone and increased sympathetic tone occurs heightening systemic vascular resistance and raising HR (Foëx, 1999). Additionally, Kreimeier (2000) reports that with less circulating BV, preload will decrease along with $\dot{Q}$ and HR increases to compensate. This potential response is likely to have occurred within these participants as BV will have reduced and it can be seen in the tables and graphs that HR increased and $\dot{Q}$ decreased particularly in the first 24 h. However, BV returns to normal within 48-72 h (Klein and Anstee, 2007), due to the advancing plasma volume increase (Calbert, et al., 2006) from transcapillary refill (interstitial to intravascular compartment fluid movement). Therefore, the most pronounced effect for the increased HR at 72 h is most likely predominately associated with reduced Hb as opposed to TBV.

Although, general significance is absent for SV with the exception of 80 % at 24 h in the moderate domain, it can be seen from Table 7.6 that SV shows a trend to reduce post BD. Average percentage decreases for SV during all of the moderate domain were 8.2 ± 1.5 % at 24 h, 1.0 ± 1.2 % at 48 h, 0.1 ± 2.6 % at 72 h and 4.2 ± 2.6 % at 96 h. During the heavy domain average decreases were 5.9 ± 2.7 % at 24 h, 0.4 ± 3.0 % at 48 h, 1.4 ± 3.3 % at 72 h and 2.7 ± 3.6 % at 96 h. A 15 % blood loss can result in a decreased SV (Karam, et al., 1994), which could result in hypovolemia (decreased intravascular volume), a condition which can also result from dehydration (Kreimeier, 2000). Hypovolemia will lower venous return thus decreasing SV. Although BV will not return to normal until ~72 h post BD, by 24 h the BV would likely have begun to rise due to transcapillary refill and as the participants within this study were not dehydrated, as seen from the hydration analysis no significance is present. In regards to the current data set, the average BV loss across the participants was 10 % (12 % for females and 9 % for males).

V_T increased generally post BD, but significantly so in the heavy domain, therefore experimental hypothesis 2 can be accepted for V_T during 24 h at 80 %, 48 h at 80 % and 90 %, and 96 h at 80, 90 and 100 %, while at 72 h an increase similarly occurred, but significance was absent. In addition, in the moderate domain experimental hypothesis 1 can be accepted, as there was a significant increase in V_T 24 h post BD at 10 % and a significant increase for BF 72 h post BD at 20 %. Elevations in BF and breathing depth increase V_T, in order to inspire more O_2 and expel more CO_2. Average percentage increases of $\dot{V}CO_2$ in the moderate domain were 2.80 ± 2.43 % at 24 h, 2.12 ± 2.28 % at 48 h 1.53 ± 1.71 % at 72 h and 2.73 ± 2.03 % at 96 h. In the heavy domain they were 0.76 ± 2.09 at 24 h, 0.30 ± 1.73 at 48 h, -2.39 ± 1.53 at 72 h and 1.83 ± 3.12 at 96 h. As a decreased circulating O_2 was present, it may be the case that the need to take on more

O_2 and thus expel more CO_2 generated from an increased metabolism caused the V_T increase compared to pre BD levels, although in this instance BF did not produce a significant concomitant increase too. Respiratory rate (BF) is generally deemed to be normal following <15 % blood loss, but may develop abnormal rapid breathing with a 15 – 30 % loss (Nolan and Pullinger, 2014). Based on this information, the lack of statistical significance in BF is justified, as based on the participants within this study the largest blood loss encountered was ~13 %. There was however increased V_E post BD, which was significantly different mainly in the moderate domain across varying percentages across all testing periods. V_E is a composite value derived from BF and V_T, as such any changes even subtle ones may have been sufficient enough to produce the statistical significance observed. Possible reasons for the significant increases in V_T could be due to an increased level of CO_2 and decreased levels of O_2. It has been demonstrated during CO_2 rebreathing that V_E increases from the reduction in O_2 and increased CO_2 (Skow, et al., 2015). Confirmation that CO_2 had likely increased comes from the lactate values that were amplified post BD. The maintenance of blood gases are detected via chemoreceptors, with the peripheral sensors sensitive to increases in PCO_2 and decreases in arterial PO_2 (Skow, et al., 2015).

Ventilatory equivalents ($V_E/\dot{V}O_2$ and $V_E/\dot{V}CO_2$) are measures of ventilatory efficiency/performance (Tharion and Subramani, 2011). Post BD $V_E/\dot{V}O_2$ and $V_E/\dot{V}CO_2$ can be seen to increase in both intensities across the whole test duration with almost no exceptions and indeed significantly so in many cases when regarding $V_E/\dot{V}O_2$ (Figures 7.5 and 7.6), which was predominantly so at 72 h when Hb was at its lowest value. $V_E/\dot{V}O_2$ increased due to V_E increasing and $\dot{V}O_2$ decreasing. The increased V_E is to eliminate CO_2 produced from increased metabolism and is supported by the lactate values (chapter 6.3.4), which increased pre to post BD (which was ~5 % at 72 h following moderate domain exercise). The amount of air ventilated to exhale 1 L/min of CO_2 is reflected by $V_E/\dot{V}CO_2$ and what can be observed between pre to post BD is average percentage increases of 1.48 ± 1.20 % at 24 h, 0.64 ± 1.66 % at 48 h, 1.85 ± 0.64 % 72 h and 0.12 ± 1.39 % at 96 h during the moderate domain and 2.00 ± 1.66 % at 24 h, 1.46 ± 1.90 % at 48 h, 2.37 ± 1.25 % at 72 h and 0.39 ± 1.87 % at 96 h during the heavy domain. These values and Figures 7.5 and 7.6 provide an observation that pre to post BD the $V_E/\dot{V}CO_2$ increased because of augmented peripheral chemoreceptor sensitivity to the (decreased) O_2 a system that aids to deter tissue hypoxia (Ponikowski, et al., 2001). Furthermore, at 96 h less CO_2 was produced per L of O_2, which coincides with Hb beginning to restore and O_2 carrying capacity increasing.

Although decreases can be observed in $\dot{V}O_2$ these are not significant decrements in either intensity domains, except for 72 h during heavy, when Hb was at the lowest values. In agreement with the previous chapter (6, Part A), it is plausible that myoglobin in its role as an O_2 store within muscle is assisting in maintaining the required amount of $\dot{V}O_2$ needed for the exercise. This O_2 storing role of myoglobin is supported by diving mammals such as penguins where breath holding (hypoxic diving) increases myoglobin concentrations (Ponganis, et al., 2010). Similarly, in human free divers it is suggested that myoglobin-mediated O_2 diffusion is probably essential particularly in long dives to maintain muscle oxygenation (Kjeld, et al., 2018). Although initially there is a rapid desaturation of myoglobin within 20 s of exercise commencement (at ~50 % or ~60 % of $\dot{V}O_{2max}$ in normoxia or hypoxia respectively), the levels remain constant up to maximum and during rest rapid re-saturation occurs (Richardson, et al., 1995). Therefore, under the submaximal exercise domains and when the O_2 delivery is insufficient following BD, the myoglobin becomes an O_2 reserve. Secondly, it could also be that in addition to the use of myoglobin and particularly when moving into heavy exercise when the myoglobin store is possibly waning, the next compensatory mechanism is to increase the respiratory dynamics. $\dot{V}CO_2$ generally increased post BD, the values of which are not shown here as they were not significant, but have been confirmed via lactate increases (chapter 6). This resulted in an increase of the breathing mechanisms in order to increase alveolar O_2 tension and decrease PCO_2. This two-fold process of myoglobin use and increased respiratory dynamics, appears to assist $\dot{V}O_2$ in maintaining a similar trajectory for most testing periods pre to post BD.

BD was non-significant in regards to O_2 extraction at the muscle, thus despite the reduced Hb concentration and therefore lower circulating O_2, the a-vO_{2diff} remained unchanged. Therefore, experimental hypothesis 5 and 6 have to be rejected as no significant increases occurred in the moderate or heavy domains pre to post BD with regard to O_2 extraction at the muscle (a-vO_{2diff}). This unchanging situation is possibly reflected by the fact that $\dot{Q}$ was largely unaffected. Additionally, the O_2 bound to myoglobin is possibly responsible for the lack of change during the moderate intensity in a-vO_{2diff}. As the waning myoglobin store has no restorative opportunity during heavy intensity exercise (additional to having previously assisted in the moderate intensity trials), the a-vO_{2diff} still remains unchanged possibly due to a redistribution of blood, as indicated by the tHb increases (particularly at 48 h) obtained from NIRS in chapter 6. Furthermore, during the heavy domain participants were close to maximum a-vO_{2diff}. For example, a-$vO_{2diffmax}$ at $\dot{V}O_{2max}$ was 15.12 ± 3.06 $ml \cdot 100ml^{-1}$ and at 24 h pre and post BD a-$vO_{2diffmax}$ during the last 36 s of the heavy trial was 14.46 ± 4.18 $ml \cdot 100ml^{-1}$ and 15.09 ± 3.29 $ml \cdot 100ml^{-1}$ respectively. Maximum a-vO_{2diff}

is reached at ~64 % of $\dot{V}O_{2max}$ according to Leprêtre, Koralsztein and Billat (2004) or 60 - 65 % according to Grassi, et al (1999). Therefore, it is possible that a change in extraction pre to post BD would not be evident via a-vO_{2diff} because the participants were working at ~80 % of $\dot{V}O_{2max}$, which is higher than 65 % during the heavy domain.

To conclude, the period that appears the most affected by BD is at 72 h and the reduced circulating O_2 most certainly plays a role in changes in variables such as the HR increasing. Although in many variables only subtle and not always significant changes were apparent, it is prudent to consider that modifications have been made via BD to the movement of O_2. While the body appears to adjust to maintain homeostasis, there is little doubt within this study that Hb levels continue to decrease up to 72 h post BD. Therefore, although the cautionary period of the day after BD exists, in terms of performance levels in sports, maybe an explanation of how cardiac and respiratory mechanism may alter for several days after BD should be made available to the donor.

Chapter 8: 5th Experimental Chapter

Influence of a Standard UK Blood Donation (~470 ml) on Multiple Sprint Capability over a Period of 24-96 Hours

8.1 Introduction

Multiple sprints will produce varying acute physiological responses according to the influence of variables such as the duration of exercise/rest, exercise intensity, number of efforts/sets performed, time between efforts/sets, recovery intensity between efforts/sets and ergometer choice (Buchheit and Laursen (2013a). Performing multiple sprints is perhaps better known as high intensity interval training (HIIT) by the general public or sprint interval training (SIT). Exercising at high intensity for a short period of time such as 20 or 30 seconds and then resting for a similar time span to recover is a representative principle for HIIT, but an array of protocols exists that manipulate the prior mentioned variables and intensities (% $\dot{V}O_{2max}$), with the exercise lasting ~5-40 minutes (Buchheit and Laursen., 2013b).

An integration of metabolic processes is in use during multiple sprints and the energy system dominating will depend on the duration of the sprint. For example, a sprint of up to 10 seconds will cause ATP-PCr to dominate, if the time is ~15-30 seconds then anaerobic glycolysis will predominate. Although aerobic contributions appear initially redundant in comparison to anaerobic systems, as more sprints are performed, a greater reliance on oxidative metabolism occurs (as muscle O_2 demand increases with each repetition). Thus, more of a contribution from this system is made to the overall energy production, particularly if recovery is inadequate, as PCr resynthesis and inorganic phosphate removal are O_2 dependent processes (Glaister, 2005). It is also a condition of multiple sprint exercise that there is insufficient O_2 for the muscles metabolic demand (a type of cellular hypoxia) (Gordon, et al., 2020). Evidence suggests that in 2 x 30 s sprints 34 ± 2 % and 49 ± 2 % of the energy during the first and second sprint respectively is derived from aerobic metabolism (Bogdanis, et al., 1996). However, the contribution of the oxidative system will vary immensely according to variables such as time. A review article (Gastin, 2001) reported the aerobic contribution in high intensity exercise to vary from as low as 3 % up to 98 %. Additionally, a decline in power output is observed in 10 x 6 s sprints (by ~27 % for the mean power and ~33 % for the peak power) and anaerobic ATP decline by ~65 % from the first to last sprint (Gaitanos, et al., 1993), which can be attributable to aerobic metabolism influence and hindrance of glycolytic rate (Nevill, et al., 1996).

If O_2 is limited due to hypoxia (compared to that of normoxia), there is an increased peripheral fatigue and the recovery of PCr concentration (%) is reduced (Glaister, 2005). Chapter 6 identified that when circulating O_2 is limited the phase II kinetics (tau_2) altered, which was suggested to be attributed to PCr's rapid decrease and the slowing of the creatine kinase (CK) reaction. Given that PCr resynthesis is O_2 dependent, it is feasible to assume that in a repeated sprint scenario where Hb has been compromised and therefore circulating O_2 then sprint performance may be influenced. With research into this type of exercise accompanied by BD appearing neglected, this study aims to determine the effects of BD over a period of up to 96 hours on power output during four repeated 15 s sprints in a moderately trained population.

Null hypotheses:
(H_{01}): Peak power output (PPO) will not be altered compared to pre BD values from donating a unit of blood after 24 hours and up to 96 hours.
(H_{02}): Peak power output per kg (PPO/kg) will not be altered compared to pre BD values from donating a unit of blood after 24 hours and up to 96 hours.
(H_{03}): Mean power output (MPO) will not be altered compared to pre BD values from donating a unit of blood after 24 hours and up to 96 hours.
(H_{04}): Fatigue index (FI) will not be altered compared to pre BD values from donating a unit of blood after 24 hours and up to 96 hours.

Experimental hypotheses:
(H_1): Peak power output (PPO) will decrease compared to pre BD values from donating a unit of blood after 24 hours and up to 96 hours.
(H_2): Peak power output per kg (PPO/kg) will decrease compared to pre BD values from donating a unit of blood after 24 hours and up to 96 hours.
(H_3): Mean power output (MPO) will decrease compared to pre BD values from donating a unit of blood after 24 hours and up to 96 hours.
(H_4): Fatigue index (FI) will decrease compared to pre BD values from donating a unit of blood after 24 hours and up to 96 hours.

8.2 Methods

8.2.1 Participants and Study Design

Testing was conducted in the morning over a two-week period with a rest week between testing phases. Week one (W1) was pre-BD and week two (W2) was post BD, Figure 8.1 depicts the activity according to days. Monday on W1 established haematological levels and $\dot{V}O_{2max}$, subsequent visits during W1 were for Sprint Interval Testing (SIT). SIT comprised 4 x 15 s sprints at a resistance of 0.07 $W \cdot kg^{-1}$ of body mass with 90 s of unloaded pedalling prior to each effort. W2 was identical except the participant donated blood on Monday post haematological testing only. The study design is an experimental before and after self-controlled design, that is the participants acted as their own controls following exposure to BD. The data for this experimental chapter was collected following completion of two moderate and one heavy intensity exercise trials (chapter 6 and 7) and involved the same participants. Therefore, sample size, participant details (body mass etc.), inclusion criteria and study design are detailed on pages 107-110, chapter 6 (section 6.2.1 and 6.2.2).

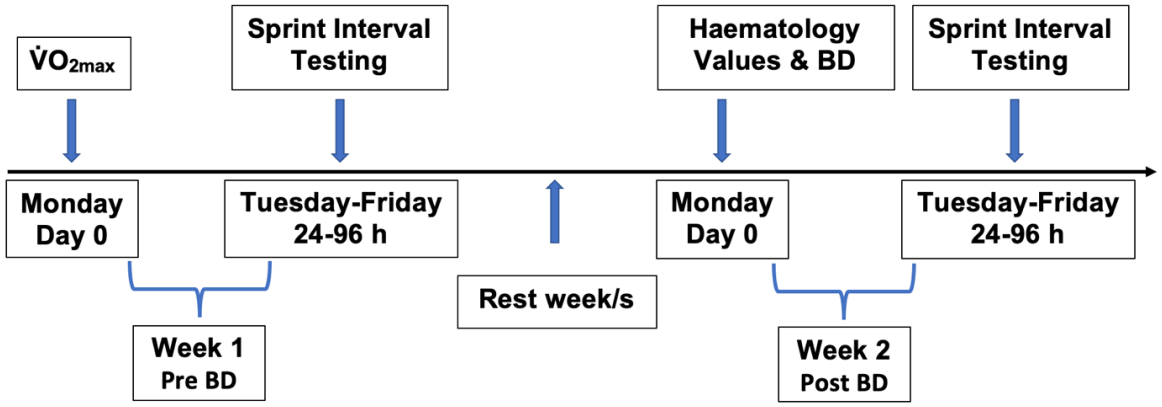

Figure 8.1: Timeline of domain testing.

8.2.2 Haematological Responses

Using pre-calibrated equipment (EKF Hemo Control, EKF diagnostic, Germany), capillary blood (~10 µl) from a finger was examined for haemoglobin and haematocrit levels prior to all fitness testing procedures. Additionally, each participant made a standard blood donation (~470 ml) at a National Blood Transfusion Service within a few hours of haematological levels being established on the Monday (Day 0) of the second week of testing. Refer to page 65, methods chapter 4.4.1 for detail on procedure.

8.2.3 Blood Lactate Responses

Blood lactate (BLa) was assessed (Biosen C-Line, EKF diagnostic, Germany) via capillary blood from the fingertip prior to commencement of SIT to gain a resting value, although it should be noted that this testing occurred following ~40 minutes of sub-maximal exercise. Additionally, it was taken immediately post each sprint. Refer to page 67, methods chapter 4.5.1 for detail on procedure.

8.2.4 Hydration Analysis

Refer to page 68, methods chapter 4.6.1 for detail on procedure.

8.2.5 Data Analysis

All sprint data (derived from the software of the Lode) was gathered for each person and placed into Excel version 16.16.11 (Microsoft Excel for Mac, 2018). Fatigue index (FI) was calculated accordingly (peak power output – minimum power output)/PPO x 100 (Naharudin and Yusof, 2013).

8.2.6 Statistical Analysis

All variables collected have the values stated as mean ± SD. Following tests for normality (Shapiro-Wilk's) a combination of repeated measures *t*-tests, one-way repeated measures ANOVA (for differences in pre to post BD) and two-way repeated measures ANOVA to compare within and between trials. Mauchly's test was used to check sphericity and Greenhouse-Geisser correction applied where necessary. Additionally, Bonferroni correction was applied to avoid Type 1 Error for pairwise (pre to post) comparisons. Effect size was also considered; refer to page 115, section 6.2.13 in chapter 6 for detail on Cohen's *d* and Partial eta squared.

A level of statistical significance was set at $P < 0.05$ and statistical analysis was accomplished using SPSS version 20 (SPSS, Chicago, IL).

8.3 Results

8.3.1 Peak Power Output

Two-way ANOVA revealed no significant differences between the whole of week 1 and 2 trials or any trial days e.g. pre to post BD 24 h. However, a main effect for time was observed for the whole experimental period (P = 0.001, η_p^2 = 0.35), at 24 h (P = 0.001, η_p^2 = 0.39), 48 h (P = 0.013, η_p^2 = 0.35), 72 h (P = 0.006, η_p^2 = 0.31) and 96 h (P = 0.002, η_p^2 = 0.37). The overall interaction effect for trial by time was not significant except at 48 h (P = 0.031, η_p^2 = 0.27). One-way ANOVA showed significant differences only post BD for the following: 24 h (P = 0.002, η_p^2 = 0.47) with a significant pairwise comparison between sprints 1 and 4 (P = 0.028, (CI, 14.28, 298.39)) and sprints 2 and 4 (P = 0.005, (CI, 30.46, 170.71)), 48 h (P = 0.001, η_p^2 = 0.51) with a significant pairwise comparison between sprints 1 and 4 (P = 0.010, (CI, 32.56, 261.94)) and sprints 2 and 4 (P = 0.013, (CI, 22.35, 204.98)), 72 h (P = 0.005, η_p^2 = 0.32), but with no significant pairwise comparison between sprints and 96 h (P = 0.009, η_p^2 = 0.29) with a significant pairwise comparison between sprints 1 and 3 (P = 0.028, (CI, 7.67, 160.33)) and sprints 1 and 4 (P = 0.014, (CI, 14.62, 146.21)). Figure 8.2 visually displays the decremental nature of wattage following each sprint. The percentage decrease of PPO between sprint 1 to 4 were 7.87 % pre BD and 16.03 % post BD at 24 h, 5.17 % pre BD and 14.99 % post BD at 48 h, 2.82 % pre BD and 8.39 % post BD at 72 h, and 3.75 % pre BD and 8.13 % post BD at 96 h.

The absolute PPO mean ± SD values are displayed in Table 8.1, pre to post BD from *t*-test analysis showed only two significant different values, which concerned sprint 1 during 48 h (P = 0.020, Cohens d = -0.40 (CI, -126.91, 113.36) and 72 h (P = 0.002, Cohens d = -0.38 (CI, -116.20, 117.59).

Table 8.1: Mean ± SD peak power outputs pre to post BD over four 15 second sprints across the four days (24-96 hours) of sprint testing.

h	PPO (W)							
	Sprint 1		Sprint 2		Sprint 3		Sprint 4	
	Pre BD	Post BD	Pre BD	Post BD	Pre BD	Post BD	Pre BD	Post BD
24	938.08 ± 215.66	970.00 ± 208.72	904.25 ± 216.94	915.08 ± 198.96	876.67 ± 233.60	849.25 ± 171.81	864.25 ± 223.27	814.50 ± 167.64
48	900.42 ± 223.60	982.08 ± 201.07*	886.83 ± 238.18	948.50 ± 176.02	855.75 ± 199.38	876.83 ± 169.44	853.83 ± 190.12	834.83 ± 159.89
72	915.33 ± 204.70	990.75 ± 208.50**	948.58 ± 248.64	970.58 ± 238.44	911.08 ± 192.95	906.00 ± 172.50	889.50 ± 202.71	907.58 ± 202.17
96	950.33 ± 196.08	989.42 ± 199.23	922.17 ± 200.64	960.75 ± 224.62	894. 92 ± 191.27	905.42 ± 224.62	914.67 ± 195.67	909.00 ± 212.92

** P < 0.05, ** P < 0.01, denotes a significant difference from Pre BD to Post BD.*

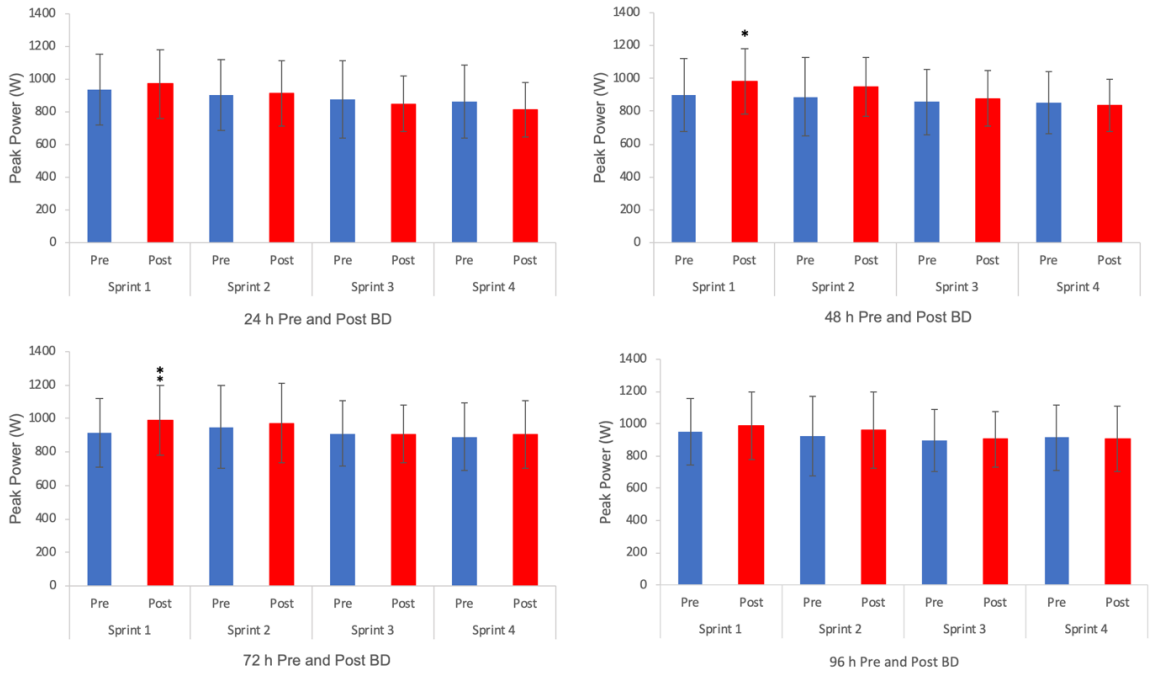

Figure 8.2: The mean PPO for sprints 1 to 4 for 24 to 96 h. With * denoting P < 0.05 pre to post BD and ** denoting P < 0.01 pre to post BD.

8.3.2 Peak Power Output/kg (PPO/kg)

Two-way ANOVA revealed no significant differences between the trials. However, a main effect for time was observed for the whole experimental period (P = 0.000, η_p^2 = 0.43), at 24 h (P = 0.000, η_p^2 = 0.43), 48 h (P = 0.009, η_p^2 = 0.38), 72 h (P = 0.013, η_p^2 = 0.27) and 96 h (P = 0.001, η_p^2 = 0.39). The overall interaction effect for trial by time was non-significant except at 48 h (P = 0.013, η_p^2 = 0.28). One-way ANOVA demonstrated significant differences only post BD for the following: 24 h (P = 0.001, η_p^2 = 0.51) with a significant pairwise comparison between sprints 1 and 3 (P = 0.037, (CI, 0.07, 2.80)), 1 and 4 (P = 0.016, (CI, 0.32, 3.42)), 2 and 3 (P = 0.049, (CI, 0.00, 1.57)), and lastly sprints 2 and 4 (P = 0.002, (CI, 0.44, 1.99)), 48 h (P = 0.000, η_p^2 = 0.53) with a significant pairwise comparison between sprints 1 and 4 (P = 0.007, (CI, 0.48, 3.18)), 2 and 3 (P = 0.047, (CI, 0.01, 1.76)), and lastly sprints 2 and 4 (P = 0.011, (CI, 0.30, 2.53)), 72 h (P = 0.006, η_p^2 = 0.31) but with no significant pairwise comparison between sprints and 96 h (P = 0.005, η_p^2 = 0.32) with a significant pairwise comparison between sprints 1 and 3 (P = 0.025, (CI, 0.11, 1.91)) and 1 and 4 (P = 0.015, (CI, 0.18, 1.95)).

The absolute PPO/kg mean ± SD values are displayed in Table 8.2, pre to post BD from *t*-test analysis showed only two significant different values, which concerned sprint 1 during

48 h (P = 0.023, Cohens d = -0.58 (CI, -1.79, 0.30) and 72 h (P = 0.001, Cohens d = -0.55 (CI, -1.56, 0.48) (Figure 8.2).

Table 8.2: Mean ± SD peak power per kilogram outputs pre to post BD over four 15 second sprints across the four days (24-96 hours) of sprint testing.

h	PPO/kg (W·kg^{-1})							
	Sprint 1		Sprint 2		Sprint 3		Sprint 4	
	Pre BD	Post BD	Pre BD	Post BD	Pre BD	Post BD	Pre BD	Post BD
24	11.66 ± 1.52	12.06 ± 1.07	11.18 ± 1.60	11.41 ± 1.52	10.82 ± 1.77	10.63 ± 1.44	10.68 ± 1.61	10.19 ± 1.42
48	11.22 ± 2.14	12.26 ± 1.56*	10.95 ± 1.92	11.85 ± 1.35	10.63 ± 1.62	10.97 ± 1.35	10.57 ± 1.20	10.43 ± 1.36
72	11.45 ± 1.79	12.39 ± 1.81**	11.81 ± 2.13	11.91 ± 1.94	11.37 ± 1.46	11.32 ± 1.36	11.08 ± 1.51	11.29 ± 1.59
96	11.88 ± 1.69	12.33 ± 1.40	11.50 ± 1.58	11.96 ± 1.87	11.15 ± 1.49	11.33 ± 1.44	11.38 ± 1.26	11.27 ± 1.60

** P < 0.05, ** P < 0.01, denotes a significant difference from Pre BD to Post BD.*

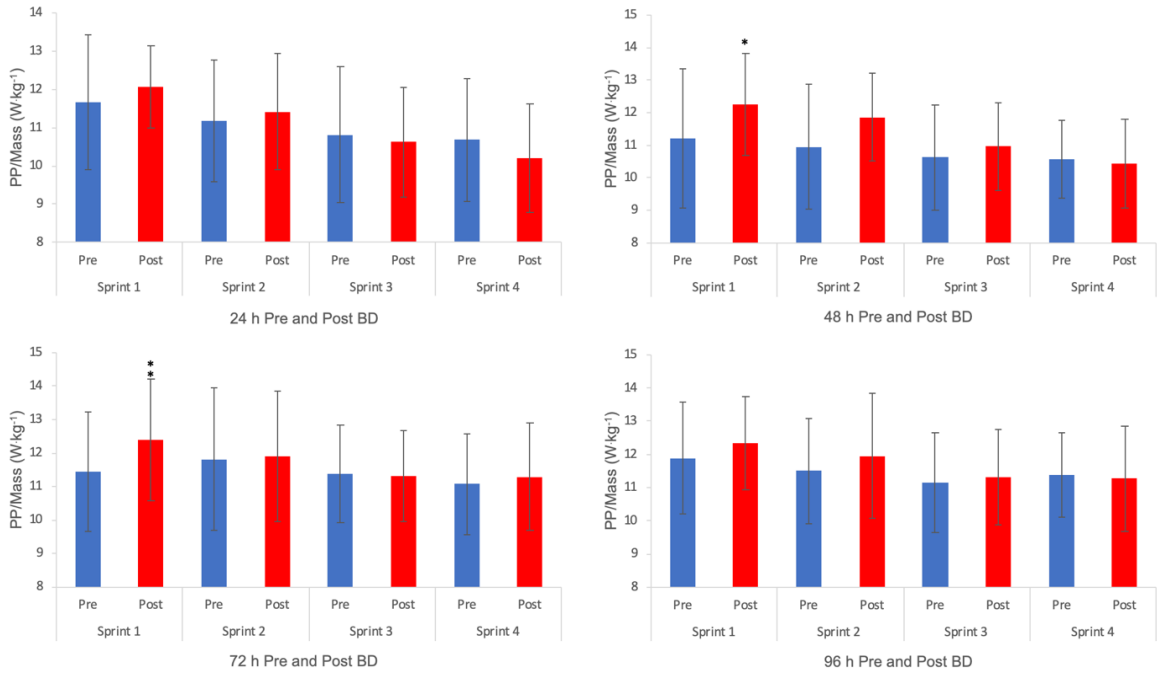

Figure 8.3: The PPO/kg (mass) for sprints 1 to 4 for 24 to 96 h. With * denoting P < 0.05 pre to post BD and ** denoting P < 0.01 pre to post BD.

8.3.3 Mean Power Output (MPO)

Two-way ANOVA revealed no significant differences across pre and post BD periods, however it is noteworthy that at 96 h pairwise comparisons between trials were significant (P = 0.001, η_p^2 = 0.63 (CI, -39.34, -12.94)). A main effect for time was observed for the whole experimental period (P = 0.000, η_p^2 = 0.75), 24 h (P = 0.000, η_p^2 = 0.79), 48 h (P = 0.000, η_p^2 = 0.67), 72 h (P = 0.000, η_p^2 = 0.80) and 96 h (P = 0.000, η_p^2 = 0.80). The overall

interaction effect for trial by time was non-significant. One-way ANOVA showed significance pre BD for 24 h (P = 0.000, η_p^2 = 0.67), 48 h (P = 0.003, η_p^2 = 0.47), 72 h (P = 0.000, η_p^2 = 0.67) and 96 h (P = 0.000, η_p^2 = 0.76). For post BD, significance was observed for 24 h (P = 0.000, η_p^2 = 0.76), 48 h (P = 0.000, η_p^2 = 0.66), 72 h (P = 0.000, η_p^2 = 0.65) and 96 h (P = 0.000, η_p^2 = 0.73). Significant pairwise comparisons are shown in Table 8.4. Figure 8.4 visually displays the decremental nature of wattage following each sprint. Pre and post BD the percentage decrease of MPO between sprint 1 to 4 were 17.94 % pre BD and 17.52 % post BD at 24 h, 12.58 % pre BD and 13.19 % post BD at 48 h, 13.18 % pre BD and 13.81 % post BD at 72 h, and 15.15 % pre BD and 13.01 % post BD at 96 h.

The MPO values (mean ± SD) are displayed in Table 8.3, pre to post BD from *t*-test analysis showed three significantly different values, which concerned sprint 1 (P = 0.016, Cohens d = -0.16 (CI, -81.07, 80.72), 2 (P = 0.009, Cohens d = -0.21 (CI, -76.89, 81.96) and 4 (P = 0.003, Cohens d = -0.26 (CI, -76.62, 76.51) during 96 h (Figure 8.4).

Table 8.3: Mean ± SD mean power outputs pre to post BD over four 15 second sprints across the four days (24-96 hours) of sprint testing.

h	Mean Power (W)							
	Sprint 1		Sprint 2		Sprint 3		Sprint 4	
	Pre BD	Post BD	Pre BD	Post BD	Pre BD	Post BD	Pre BD	Post BD
24	719.33 ± 146.52	729.83 ± 144.29	676.83 ± 135.22	660.83 ± 145.27	627.58 ± 155.38	619.92 ± 117.81	590.25 ± 137.09	601.92 ± 133.41
48	704.17 ± 157.94	717.83 ± 150.20	658.75 ± 146.15	679.25 ± 133.21	633.08 ± 135.67	632.00 ± 143.12	615.58 ± 130.77	608.92 ± 135.05
72	712.83 ± 144.11	728.92 ± 163.05	694.25 ± 159.24	677.42 ± 143.75	645.92 ± 149.90	646.33 ± 130.38	618.83 ± 141.25	628.25 ± 130.31
96	721.67 ± 143.00	743.17 ± 142.94*	675.67 ± 135.54	703.50 ± 145.23**	641.67 ± 148.88	662.75 ± 124.68	612.33 ± 134.96	646.50 ± 135.70**

** P < 0.05, ** P < 0.01, denotes a significant difference from Pre BD to Post BD.*

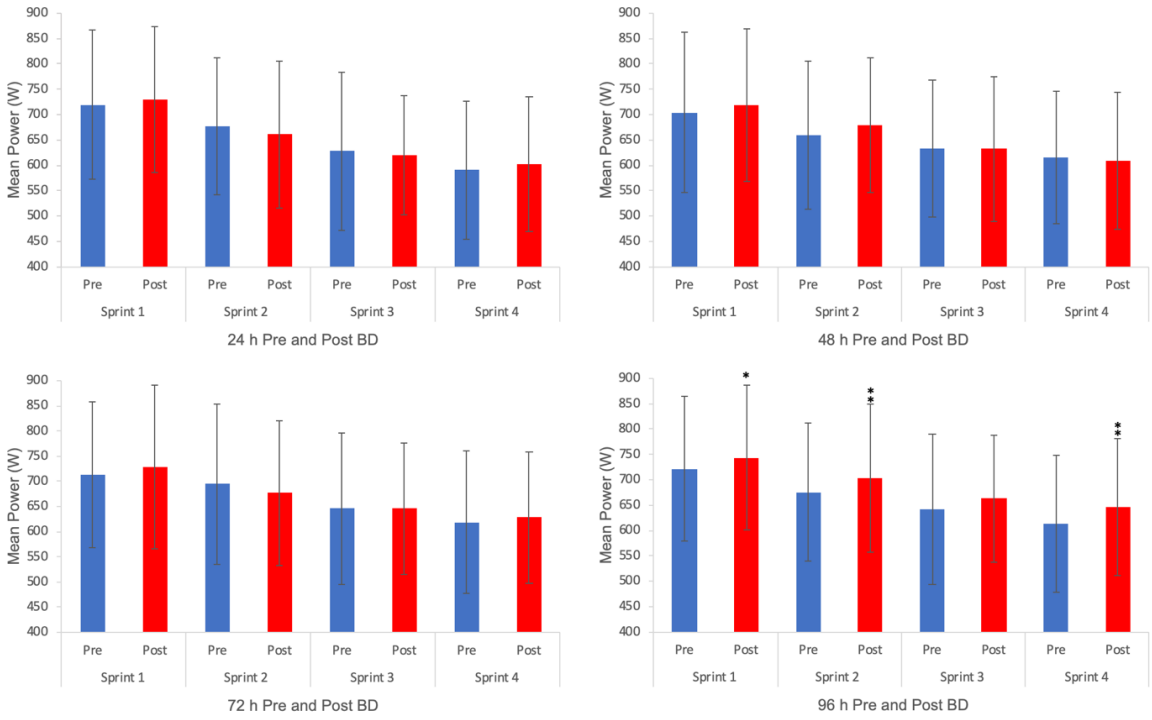

Figure 8.4: The MPO for sprints 1 to 4 for 24 to 96 h. With * denoting P < 0.05 pre to post BD and ** denoting P < 0.01 pre to post BD.

Table 8.4: Significant MPO results for pairwise comparisons following one-way ANOVA tests.

Pre/Post BD and Hour	Sprint Number Comparisons	Significance Level (P =)	95% Confidence Interval for Difference	
			Lower Bound	Upper Bound
Pre 24 h	1 – 2	0.033	2.89	82.11
	1 – 3	0.004	29.95	153.55
	1 – 4	0.001	51.75	206.41
	2 – 3	0.035	2.83	95.67
	2 – 4	0.001	34.70	138.47
Pre 48 h	1 – 3	0.027	7.01	135.16
	1 – 4	0.028	8.42	168.75
Pre 72 h	1 – 3	0.004	20.54	113.30
	1 – 4	0.001	39.76	148.24
	2 – 3	0.000	25.44	71.23
	2 – 4	0.000	38.19	112.64
Pre 96 h	1 – 2	0.010	10.10	81.70
	1 – 3	0.002	28.85	131.15
	1 – 4	0.000	61.55	157.12
	2 – 3	0.011	7.35	60.65
	2 – 4	0.000	43.17	83.50
Post 24 h	1 – 2	0.002	24.67	113.33
	1 – 3	0.000	58.88	160.96
	1 – 4	0.000	66.75	189.09
	2 – 3	0.018	6.21	75.62
	2 – 4	0.001	27.16	90.67
Post 48 h	1 – 3	0.006	23.95	147.72
	1 – 4	0.002	42.05	175.78
	2 – 3	0.001	18.95	75.55
	2 – 4	0.002	27.77	112.90
Post 72 h	1 – 2	0.018	7.78	95.22
	1 – 3	0.009	18.97	146.20
	1 – 4	0.001	44.73	156.60
	2 – 4	0.001	19.69	78.65
Post 96 h	1 – 2	0.007	10.12	69.21
	1 – 3	0.000	45.71	115.12
	1 – 4	0.000	51.03	142.30
	2 – 3	0.017	6.58	74.92
	2 – 4	0.003	19.06	94.94

8.3.4 Fatigue Index

Two-way ANOVA showed significantly different pairwise comparisons between pre and post BD (P = 0.036, η_p^2 = 0.34 (CI, -6.87, -0.29)). Additionally, there were significantly different pairwise comparisons at 24 h (P = 0.039, η_p^2 = 0.33 (CI, -14.51, -0.44)) and 48 h (P = 0.020, η_p^2 = 0.40 (CI, -15.70, -1.68)). A main effect for time was observed for the whole experimental period (P = 0.000, η_p^2 = 0.48), at 24 h (P = 0.000, η_p^2 = 0.43), 48 h (P = 0.001, η_p^2 = 0.37), 72 h (P = 0.000, η_p^2 = 0.60) and 96 h (P = 0.000, η_p^2 = 0.55). The overall interaction effect for trial by time was not significant except at 24 h (P = 0.019, η_p^2 = 0.26). One-way ANOVA showed significant differences pre BD for 24 h (P = 0.000, η_p^2

= 0.43) with significant pairwise comparisons between sprints 1 and 4 (P = 0.023, (CI, -29.75, -1.93)) and sprints 2 and 4 (P = 0.023, (CI, -24.27, -0.25)), for 48 h (P = 0.004, η_p^2 = 0.33) between sprints 1 and 4 (P = 0.023, (CI, -26.67, -1.71)), for 72 h (P = 0.000, η_p^2 = 0.55) between sprints 1 and 3 (P = 0.003, (CI, -20.94, -4.50)), sprints 1 and 4 (P = 0.001, (CI, -26.56, -7.23)) and sprints 2 and 4 (P = 0.028, (CI, -14.96, -0.71)) and for 96 h (P = 0.003, η_p^2 = 0.46) between sprints 1 and 2 (P = 0.005, (CI, -13.49, -2.32)), sprints 1 and 3 (P = 0.005, (CI, -23.67, -4.21)) and sprints 1 and 4 (P = 0.009, (CI, -33.08, -4.30)). One-way ANOVA showed significant differences post BD for 24 h (P = 0.008, η_p^2 = 0.30) but with no pairwise comparisons, for 72 h (P = 0.003, η_p^2 = 0.34) with a significant pairwise comparison between sprints 1 and 4 (P = 0.013, (CI, -18.78, -2.01)) and for 96 h (P = 0.001, η_p^2 = 0.37) between sprints 1 and 2 (P = 0.024, (CI, -15.67, -0.95)), sprints 1 and 3 (P = 0.022, (CI, -16.79, -1.12)) and sprints 1 and 4 (P = 0.020, (CI, -19.07, -1.47)).

The Fatigue Index values (mean ± SD) are displayed in Table 8.5, pre to post BD from *t*-test analysis showed four significantly different values concerning 24 h, sprint 1 (P = 0.012, Cohens d = -0.58 (CI, -8.15, 6.15) and 2 (P = 0.023, Cohens d = -0.99 (CI, -9.89, 9.29) and 48 h, sprint 1 (P = 0.034, Cohens d = -0.92 (CI, -7.14, 6.24) and 2 (P = 0.023, Cohens d = -0.66 (CI, -10.65, 8.02) (Figure 8.5).

Table 8.5: Mean ± SD Fatigue Index pre to post BD over four 15 second sprints across the four days (24-96 hours) of sprint testing.

H	Fatigue Index (%)							
	Sprint 1		Sprint 2		Sprint 3		Sprint 4	
	Pre BD	Post BD	Pre BD	Post BD	Pre BD	Post BD	Pre BD	Post BD
24	38.69 ± 13.38	45.72 ± 11.90*	42.28 ± 15.72	58.44 ± 18.18*	48.51 ± 14.14	54.37 ± 13.99	54.54 ± 15.30	55.40 ± 13.43
48	39.20 ± 11.00	49.66 ± 12.66*	46.89 ± 17.66	57.28 ± 15.33*	49.42 ± 15.05	58.13 ± 17.65	53.38 ± 15.05	58.57 ± 22.30
72	43.69 ± 12.65	49.64 ± 18.13	52.74 ± 14.30	54.76 ± 17.40	56.40 ± 15.23	57.22 ± 15.84	60.58 ± 17.63	60.04 ± 17.63
96	44.15 ± 11.80	46.99 ± 7.84	52.06 ± 13.12	55.30 ± 13.83	58.09 ± 17.17	55.95 ± 11.88	62.84 ± 20.54	57.26 ± 12.72

** P < 0.05 denotes a significant difference from Pre BD to Post BD.*

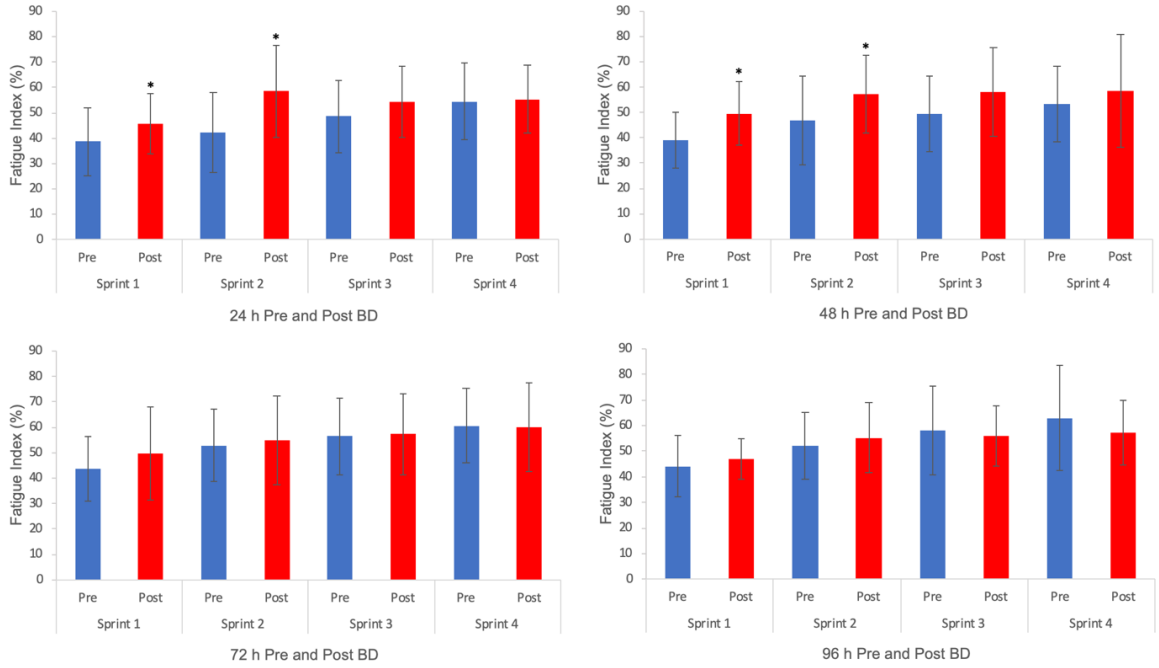

Figure 8.5: The mean FI for sprints 1 to 4 for 24-96 h. With * denoting P < 0.05 pre to post BD.

8.3.5 Blood Lactate Responses

Table 8.6 summarises the lactate responses pre and post BD and the mean lactate profile is shown in Figure 8.6. It should be noted that the resting lactate is not a "true" resting value due to the SIT testing occurring post ~40 minutes of sub-maximal exercise with insufficient time to gain a value of ~1 mM.

Two-way ANOVA testing between the pre and post BD trials showed non-significant difference in pairwise comparisons (P = 0.118). However, a main effect for time was observed (P = 0.000, η_p^2 = 0.91) and the overall interaction effect for trial by time was also significant (P = 0.047, η_p^2 = 0.18). There was a significant pairwise comparisons only at 48 h (P = 0.036, η_p^2 = 0.33 (CI, -1.94, -0.08)), but a main effect for time was observed at 24 h (P = 0.000, η_p^2 = 0.91), 48 h (P = 0.000, η_p^2 = 0.92), 72 h (P = 0.000, η_p^2 = 0.92) and 96 h (P = 0.000, η_p^2 = 0.93). In addition, at 72 h the overall interaction effect for trial by time was significant (P = 0.015, η_p^2 = 0.33). In regards to one-way ANOVA testing in all the pre and post BD days, the within-subject analysis displayed significance (P = 0.000 with η_p^2 ranging from 0.87 to 0.93) with pairwise comparisons between resting lactate and after the first sprint being non-significant (P > 0.05) on all days except for the first 24 h post BD (P = 0.004 (CI, -1.06, -0.19)) and 96 h post BD (P = 0.037 (CI, -0.91, -0.02)). The pairwise comparisons between all other lactate collections were significant (values ranging from P = 0.000 to P = 0.042).

The mean values pre to post BD showed no significance in *t*-tests, however significance was observed when comparing 48 h, sprint 4 pre to post BD (P = 0.044, Cohens d = -0.72 (CI, -2.11, 0.42)), 72 h, sprint 2 (P = 0.042, Cohens d = -0.61 (CI, -1.71, 0.48)) and 72 h, sprint 3 (P = 0.036, Cohens d = -0.71 (CI, -1.89, 0.48)).

Two-way ANOVA testing for mean lactate delta Δ values between the whole experimental period pre and post BD showed non-significant difference (P = 0.118) in pairwise comparisons, although a main effect for time was observed (P = 0.000, η_p^2 = 0.67). There was non-significant pairwise comparisons between trials at 24, 48, 72 or 96 h, but a main effect for time was observed at 24 h (P = 0.000, η_p^2 = 0.77), 48 h (P = 0.000, η_p^2 = 0.62), 72 h (P = 0.000, η_p^2 = 0.63) and 96 h (P = 0.000, η_p^2 = 0.67). In regards to one-way ANOVA testing in all the pre and post BD days, the within-subject analysis displayed significance (of between P = 0.000 and 0.006, with η_p^2 ranging from 0.40 to 0.60). Pairwise comparisons (Table 8.8) pre BD and post BD between Δ post sprint 1 – rest and Δ post sprint 2 – post sprint 1 were all significant (values ranging from P = 0.000 to P = 0.009) except 72 h pre BD. Pairwise comparisons pre BD and post BD between Δ post sprint 2 – post sprint 1 and Δ post sprint 3 – post sprint 2 showed one significant value at 24 h post BD (P = 0.036 (CI, 0.08, 2.80)). Pairwise comparisons pre BD and post BD between Δ post sprint 3 – post sprint 2 and Δ post sprint 4 – post sprint 3 showed no significant values.

The mean lactate delta Δ values (Table 8.7) pre to post BD showed only one significant *t*-test, which was 72 h Δ post sprint 2-1 (P = .013, Cohens d = -1.02 (CI, -1.69, -0.30).

Table 8.6: Mean ± SD lactate values over the two-week period of SIT.

Time h	Wk 1 - Pre BD Lactate (mM)				
	Resting	Post Sprint 1	Post Sprint 2	Post Sprint 3	Post Sprint 4
24	6.10 ± 2.24	6.31 ± 2.42	8.98 ± 2.25	11.22 ± 2.46	12.77 ± 2.67
48	5.78 ± 2.47	6.25 ± 2.54	8.82 ± 2.32	10.33 ± 2.46	12.04 ± 2.46
72	5.57 ± 2.32	6.28 ± 2.39	8.40 ± 1.94	10.65 ± 2.09	12.26 ± 2.36
96	5.66 ± 2.66	6.06 ± 2.48	8.77 ± 1.98	11.03 ± 1.83	13.01 ± 1.72
Mean ± SD	5.78 ± 0.23	6.23 ± 0.11	8.74 ± 0.25	10.81 ± 0.40	12.52 ± 0.45
Time h	**Wk 2 - Post BD Lactate (mM)**				
	Resting	**Post Sprint 1**	**Post Sprint 2**	**Post Sprint 3**	**Post Sprint 4**
24	6.11 ± 2.59	6.74 ± 2.35	10.09 ± 1.88	11.99 ± 1.81	13.21 ± 1.67
48	6.06 ± 2.55	6.76 ± 2.82	9.93 ± 2.23	11.94 ± 1.97	13.58 ± 1.00*
72	5.71 ± 2.35	6.21 ± 2.29	9.54 ± 1.92*	12.06 ± 2.10*	13.62 ± 2.04
96	5.61 ± 2.02	6.07 ± 2.12	8.76 ± 1.55	11.23 ± 1.40	12.88 ± 1.88
Mean ± SD	5.88 ± 0.25	6.45 ± 0.36	9.58 ± 0.59	11.81 ± 0.39	13.32 ± 0.35

** P < 0.05 denotes a significant difference from Pre BD to Post BD.*

Table 8.7: Mean ± SD Δ lactate values over the two-week period of SIT. PS = Post Sprint

Time h	Wk 1 - Pre BD Δ Lactate (mM)			
	PS1 – Rest	PS2 – PS1	PS3 – PS2	PS4 – PS3
24	0.21 ± 0.81	2.68 ± 1.50	2.24 ± 0.69	1.56 ± 0.88
48	0.48 ± 0.62	2.57 ± 1.49	1.50 ± 0.79	1.71 ± 0.96
72	0.71 ± 0.82	2.13 ± 1.18	2.25 ± 0.87	1.61 ± 0.70
96	0.40 ± 0.62	2.71 ± 1.28	2.25 ± 0.94	1.98 ± 0.84
Mean ± SD	0.45 ± 0.21	2.52 ± 0.27	2.06 ± 0.37	1.71 ± 0.19
Time h	**Wk 2 - Post BD Δ Lactate (mM)**			
	PS1 – Rest	**PS2 – PS1**	**PS3 – PS2**	**PS4 – PS3**
24	0.62 ± 0.44	3.35 ± 0.95	1.91 ± 1.12	1.22 ± 1.18
48	0.70 ± 0.72	3.17 ± 1.68	2.01 ± 0.86	1.64 ± 1.13
72	0.49 ± 0.78	3.33 ± 1.27*	2.53 ± 1.34	1.56 ± 0.75
96	0.47 ± 0.44	2.69 ± 1.09	2.47 ± 1.20	1.66 ± 0.93
Mean ± SD	0.57 ± 0.11	3.13 ± 0.31	2.23 ± 0.31	1.52 ± 0.20

** P < 0.05 denotes a significant difference from Pre BD to Post BD.*

Table 8.8: Significant Δ lactate values for pairwise comparisons following one-way ANOVA tests over the two-week period of SIT. PS = Post Sprint

Pre/Post BD and Hour	Δ Lactate Comparisons	Significance Level (*P* =)	95% Confidence Interval for Difference	
			Lower Bound	Upper Bound
Pre 24 h	PS1-Rest v PS2-PS1	0.004	-4.16	-0.78
	PS1-Rest v PS3-PS2	0.001	-3.13	-0.92
	PS1-Rest v PS4-PS3	0.009	-2.38	-0.31
Pre 48 h	PS1-Rest v PS2-PS1	0.010	-4.72	-0.46
	PS1-Rest v PS3-PS2	0.008	-1.80	-0.25
	PS1-Rest v PS4-PS3	0.023	-2.32	-0.15
Pre 72 h	PS1-Rest v PS3-PS2	0.001	-2.45	-0.63
Pre 96 h	PS1-Rest v PS2-PS1	0.003	-3.82	-0.81
	PS1-Rest v PS3-PS2	0.001	-2.84	-0.87
	PS1-Rest v PS4-PS3	0.000	-2.41	-0.76
Post 24 h	PS1-Rest v PS2-PS1	0.010	-4.72	-0.46
	PS1-Rest v PS3-PS2	0.008	-1.80	-0.25
	PS2-PS1 v PS3-PS2	0.036	0.08	2.80
	PS2-PS1 v PS4-PS3	0.000	1.10	3.15
Post 48 h	PS1-Rest v PS2-PS1	0.009	-4.37	-0.57
	PS1-Rest v PS3-PS2	0.010	-2.33	-0.30
Post 72 h	PS1-Rest v PS2-PS1	0.001	-4.35	-1.32
	PS1-Rest v PS3-PS2	0.002	-3.30	-0.77
	PS2-PS1 v PS3-PS2	0.001	-1.66	-0.47
	PS2-PS1 v PS4-PS3	0.013	0.35	3.19
Post 96 h	PS1-Rest v PS2-PS1	0.001	-3.44	-1.01
	PS1-Rest v PS3-PS2	0.003	-3.31	-0.69
	PS2-PS1 v PS3-PS2	0.024	-2.25	-0.14
	PS2-PS1 v PS4-PS3	0.018	0.16	1.91

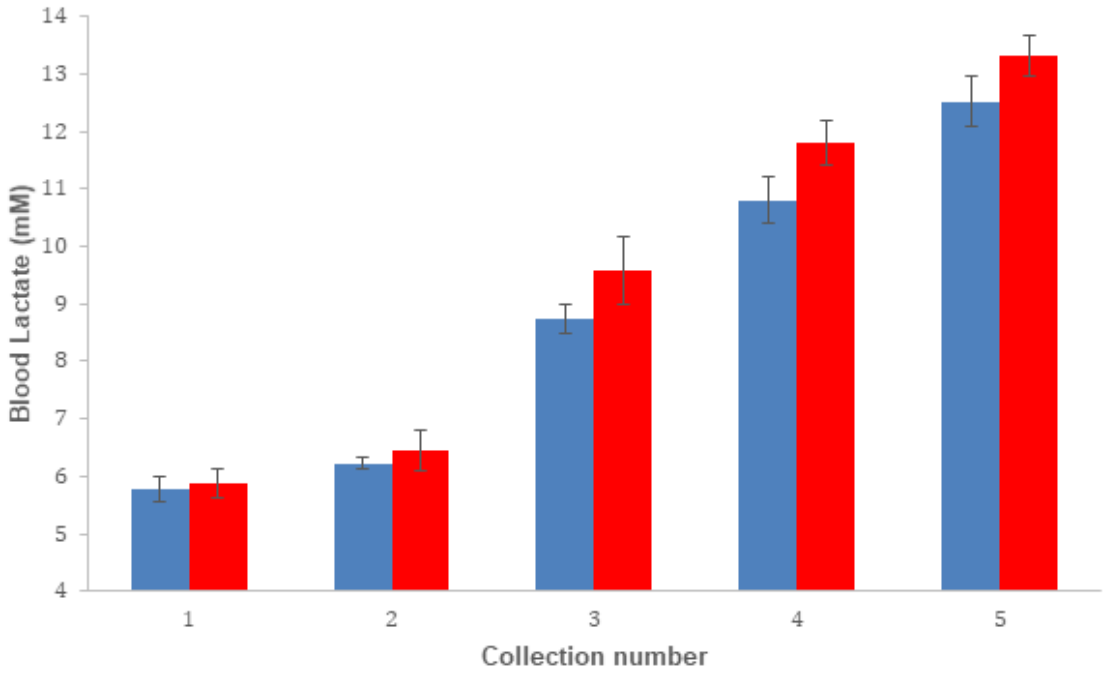

Figure 8.6: The mean lactate profile over the five collection points (of resting and post each sprint), with the blue data being representative of week one (pre BD) and red representative of week two (post BD).

8.3.6 Haematological (Hb and Hct) Responses

Figure 8.7 and Table 8.9 summarise the haematological responses pre and post BD, with two-way ANOVA pairwise comparisons for Hb between week 1 and 2 reporting significance (P = 0.000, η_p^2 = 0.83, (CI, 0.71, 1.31) between trials. Significant differences were also observed for a main effect for time (P = 0.000, η_p^2 = 0.70 and) and for the overall interaction effect for trial by time (P = 0.000, η_p^2 = 0.58). Significant differences were absent in the one-way ANOVA pre BD (week1), but significance was present for post BD (week 2) (P = 0.000, η_p^2 = 0.77). Pairwise comparisons of each day to Hb levels at 0 h (BD day) showed significance (P = 0.000). BD resulted in a 7.3 % Hb decrease from time 0 to 24 h, also a decrement from 24 to 48 h of 2.3 %, a further reduction of 2.1 % from 48 to 72 h and between 72 and 96 h a 1.5% increase occurred. This was similar for Hct when moving through the days as previously described for Hb, with the decrements of 7.2, 2.3, 2.6 % and an increase of 1.9 % when compared to baseline. T-test analysis was significant post BD at 24, 48, 72 and 96 h (P = 0.000).

Table 8.9: Mean ± SD Hb and Hct values over the two-week testing period.

Time h	Hb ($g \cdot dL^{-1}$) Wk 1(Pre BD)	Hb ($g \cdot dL^{-1}$) Wk 2 (Post BD)	Hct (%) Wk 1(Pre BD)	Hct (%) Wk 2(Post BD)
0	14.71 ± 0.73	14.58 ± 0.69	43.25 ± 2.18	42.83 ± 2.04
24	14.56 ± 0.58	13.52 ± 0.95***	42.92 ± 1.78	39.75 ± 2.93***
48	14.43 ± 0.69	13.20 ± 0.64***	42.33 ± 2.02	38.83 ± 1.85***
72	14.37 ± 0.73	12.92 ± 0.87***	42.08 ± 2.11	37.83 ± 2.48***
96	14.32 ± 0.61	13.12 ± 0.92***	41.92 ± 1.78	38.58 ± 2.64***
Mean ± SD	14.48 ± 0.16	13.47 ± 0.66***	42.50 ± 0.57	39.57 ± 1.95***

****P < 0.001 denotes a significant difference*

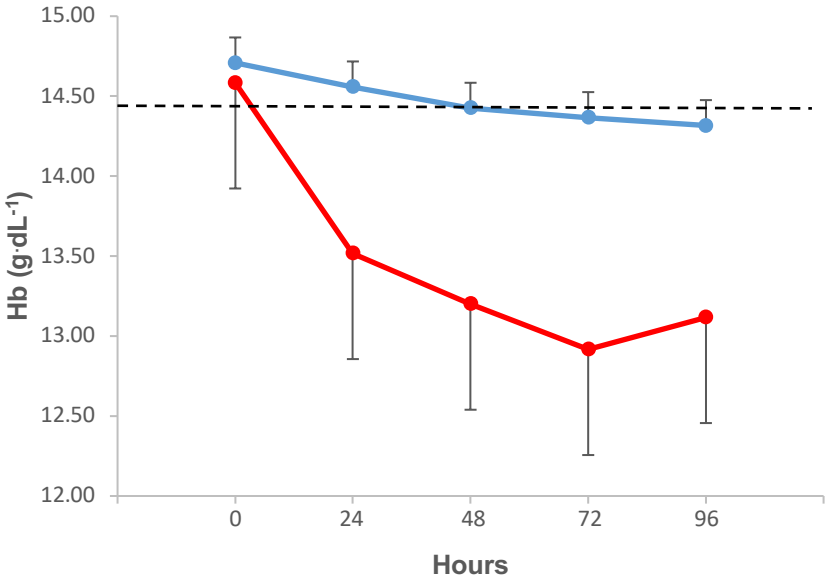

Figure 8.7: Mean pre and post BD Hb values over the two-week testing period. The blue Hb responses are the mean pre BD (week 1) values and the dotted black line represents the mean value for that week. Mean Hb values for post BD (week 2) is shown in red. The zero on the x-axis represents day 0 (the Hb value obtained prior to BD).

8.4 Discussion/Conclusion

The purpose of this study was to examine the effect of acute BD over a 96 h period on repeated sprint capability. In terms of PPO post BD a higher wattage compared to pre BD was generated in all but a few cases and significantly so during 48 and 72 h on the first sprint. The time taken to get to PPO was relatively short (~3 s) and the importance of PCr in the first 10 s assisted in the power output (Hirvonen et al., 1987; Gastin, 2001). It is possible that due to the limited O_2 as Hb was reduced at 24 h, 48 h and 72 h post BD, that the significant differences were because more PCr was utilised in the initial sprint and aerobic metabolism contributed more in the successive sprints. Mean power also displayed a tendency to have a higher value post BD, with significance in all sprints except

number 3 at 96 h. The fatigue index was higher post BD in most instances, with the first two sprints during 24 and 48 h showing significance. Although not reported in the results the power decline in most instances was more pronounced post BD. As a direct pairwise comparison pre to post BD, a significant difference was only observed at 48 h (P = 0.022) and *t*-tests for 24 h and 48 h in sprint 1 demonstrated significance too (P = 0.023 and P = 0.023 respectively).

The reduction in power over the time course of each sprint is a pattern followed by both pre and post BD conditions, similar to that expressed during multiple sprint work in healthy individuals (Smith and Billaut, 2012). This potentially indicates that PCr degrades and restoration time is insufficient, thus promoting an increased reliance on anaerobic glycolysis in producing ATP across the repeated sprints, an increase of lactate from glycogen degradation will occur, and as the final sprints ensue oxidative metabolism will be supporting to maintain the power (Gaitanos, et al., 1993). However, as BD changes the body's internal environment in terms of less circulating O_2, it would be conceivable that performance of multiple sprints post BD may be impaired. Although that does not appear to be the case here, thus in terms of PPO, PPO/kg, MPO and FI, the experimental hypotheses of these variables decreasing post BD in comparison to pre BD has to be rejected because generally the post BD results are higher. Nevertheless, Hb decreased significantly over the course of 96 hours and the reduced supply of circulating O_2 appears to have influenced power outputs, but not in the direction of the stated hypotheses.

As both PPO and MPO increased post BD and in some cases significantly, this could be a consequence of recruiting higher order threshold motor units (impacting neuromuscular function) a result of the O_2 decrement. Furthermore, as blood lactate concentration increased (although not significantly) post BD. This is a function of both lactate influx into slow twitch oxidative fibres and crucially lactate efflux from fast twitch fibres further strengthening the argument (Gladden, 2000). The fast twitch fibres used in sprinting have limited O_2 delivery and instead rely more on glycolytic metabolism to produce ATP. However, in comparison to slow twitch fibres, these fast twitch fibres have a larger fractional O_2 extraction per unit of work due to larger diffusion gradients. This is coupled with an increased mean (blood) transit time, which gives more prominent muscle O_2 diffusion (McDonough, et al., 2005), suggesting that these fibres are potentially assisted by and benefitted from increased blood perfusion in repeated sprints under hypoxic conditions (Faiss, et al., 2013).

In terms of metabolic data, the blood lactate results increased as multiple sprints ensued. The Δ values are important to consider, as in this study the participants had previously taken part in exercise prior to the sprints. The pattern displayed by the Δ results is that the increase becomes less as more sprints were performed indicating that the contribution from oxidative metabolism had increased. For example, in pre and post BD PS1-rest showed little change, but the change was more dramatic between PS2-PS1, resulting in significant values between PS1-rest and PS2-PS1 ranging from $P = 0.000$ to $P = 0.009$. This escalation was intensified post BD, an indication that the PCr had not recovered sufficiently resulting in an increased reliance on substrate level glycolysis leading to an increased lactate efflux. The amplification of the post BD values possibly reflects the reduced O_2 availability at the cell, resulting in a greater glycolytic contribution. Between PS3-PS2, the change in blood lactate value reduced, this was also the case for PS4-PS3, but the Δ values were less post BD, potentially reflecting increased cellular fatigue compared to pre BD and reduced glycolytic contribution. For the average MPO the percentage difference for sprints 3 to 2 pre and post BD was 6 % and in sprints 4 to 3 it was 5 % pre BD and 3 % post BD, the power decline pre and post BD particularly regarding the last two sprints assisted in corroboration of the previous statement of a more aerobic contribution. Blood lactate increased generally throughout, possibly due to the deficit of PCr in continuing on sprinting beyond 10 s to 15 s.

PPO was higher and the power decline was greater post BD. The power decline increased per sprint because although fast twitch fibres were recruited, they reached peak force rapidly and have been shown to have a fourfold PPO to that of slow twitch fibres, but also fatigue more rapidly (Komi, 2010). Hence the peak power cannot be sustained over this particular experimental fixed time period and so the use of slow twitch fibres was promoted and escalated as sprints proceeded, as these fibres can produce the tension/force required with less fatigue over a longer time period (Komi, 2010). However, the power decline was generally exaggerated per sprint post BD possibly due to the O_2 diffusion gradient reducing across the blood-myocyte barrier, which enhanced the need for increased muscle O_2 diffusion to meet O_2 demand in fast twitch fibres (McDonough, et al 2005). As a result of having less circulating O_2, the extracellular and intracellular diffusion gradient to the slow twitch fibre may have reduced, thus power decline increased from less perfusion, as exemplified by Jung, et al. (1999). The authors stated that O_2 partial pressure within tissue was limited by O_2 supply and depletion of O_2, and when physical activity increased, energy turnover does too, along with O_2 demand to working muscles. Similarly, BD increased energy turnover too, as removing blood volume increased O_2 cost in the later section of sprinting, which made sprints harder to perform due to decreased O_2 available.

It is also worth considering that the slow twitch fibres in particular were already fatigued from the previous ~40 minutes of exercise encountered during three bouts of constant load exercise (chapter 6, 7). Additionally, post BD all effects such as increased power decline were heightened due to issues such as an increase in lactate caused from a lack of circulating O_2 (Bakker, 2003) due to the Hb reduction and increases in both cellular acidity and CO_2, resulting in the O_2 disassociation curve exhibiting a rightward shift to promote release of O_2 to tissues, thus decreasing O_2 diffusion. Myoglobin (found in all fibres except type 2B), unlike Hb does not show cooperativity (due to only one polypeptide sub unit) and hence the curve is not sigmoidal, but hyperbolic (Ordway and Garry, 2004) and does not significantly move in response to changes in cellular conditions. It also binds O_2 more tightly than Hb and thus is a good store for O_2 until needed, especially when partial pressure is very low (~2 mmHg) in intense exercise (Richardson, et al., 1995). In previous chapters, the importance of myoglobin was considered, with research showing the benefits of its role in buffering and augmenting intracellular O_2 transport by increasing O_2 flux, particularly in times of need such as during hypoxia (Richardson, et al., 1995). A rapid desaturation of myoglobin occurs within 20 s of exercise commencement (at ~50 % or ~60 % of $\dot{V}O_{2max}$ in normoxia or hypoxia respectively) and during rest, rapid re-saturation occurs (Richardson, et al., 1995). Based on this it could be speculated that at supra maximal intensity levels such as in repeated sprinting, desaturation could occur possibly within ~5 s, but nevertheless be present to assist in the repeated sprints.

With no apparent previous research into this area (BD and multiple sprints), it is difficult to make any direct comparisons. Although not alike, information from hypoxic studies is perhaps useful. According to Billaut and Buchheit (2013), repeated sprint performance (10 x 10 s) in hypoxia maybe reduced due to muscle reoxygenation capacity being diminished during rest periods and in prolonged sprints, muscle O_2 availability is important (to restore PCr), hence performances are impacted. Furthermore, Smith and Billaut, 2012 reported that muscle deoxygenation increases with O_2 availability reduced during an initial sprint and is then constant, suggesting muscle O_2 extraction will not constrain repeated sprints and is supported from a 12.5 % increase of deoxygenation from the vastus lateralis muscle in hypoxia compared to normoxia (Billaut and Buchheit, 2013). The results from this current study appear to agree with Smith and Billaut (2012) that there is no constraint as a result of muscle O_2 extraction on repeated sprints as both MPO and PPO have increased pre to post BD. Legrand, et al. (2005) indicated that the reduced O_2 availability causes a greater muscle O_2 extraction, furthermore, in both Billaut and Buchheit (2013) and Smith and Billaut (2012) hypoxia reduced the total work output. In addition, the initial output from the first sprint was reduced, which is contrasting to this study, as the outputs increased,

although judgements between two different methods of O_2 reduction is perhaps not comparable, i.e. BD and gas concentration changes. There are differences; contrastingly, Hb is reduced with BD, but increases of Hb occur from hypoxic exposure, which would increase O_2 carrying capacity (Windsor and Rodway, 2007).

Power could have increased simply because participants got better at sprinting, possibly by more co-ordinated muscular work patterns. None of the participants were sprint cyclists as testing was aimed at a general public cohort, thus a degree of technical improvement maybe causal to improving power. Another theory for power outputs increasing post BD is possibly due to the anticipation of knowing what to expect from pre BD week and this may have enhanced muscle recruitment and thus power output. Muscle recruitment in terms of co-ordination and quantity of muscle mass is controlled via the CNS in high-intensity exercise (Billaut, 2011). Thus, there is a possibility that the CNS upregulated muscle activity, however, if there was an upregulation, it would be converse to findings from electromyography assessments that show (as a result of hypoxia) depressed O_2 delivery to tissues (brain de-oxygenation) reduces central neural drive (Amman and Kayser, 2009).

To conclude, the reduced supply of circulating O_2 as a result of Hb reduction from BD did appear to have influenced and indeed increased power outputs. As the sprints were relatively short, PCr dictated in the first 10 s and a lack of oxidative phosphorylation was initially present, which may have primarily compromised any O_2 tissue exchange. However, as power had generally increased it appears that in combination with a potentially greater O_2 extraction or unconstrained muscle O_2 extraction and type two fibre recruitment drive, BD had significantly enhanced sprint ability in many cases. It should also be noted that there was the potential that the results may have been blunted due to the previous ~40 minutes of exercise (chapter 6 and 7) (and is hence a possible limitation to this study), although the pattern of response in pre BD was still reflective of repeated sprinting if previous exercise had not have been present.

Chapter 9: Thesis Discussion

This thesis set out to provide an awareness of BD on the delivery and utilisation of O_2 during exercise across a five day intervention period and to provide insight into the effects that BD has both sub-maximally and supra-maximally, so that the results may aid individuals to make more informed decisions on whether to donate or not. Figure 9.1 indicates key findings from the experimental chapters.

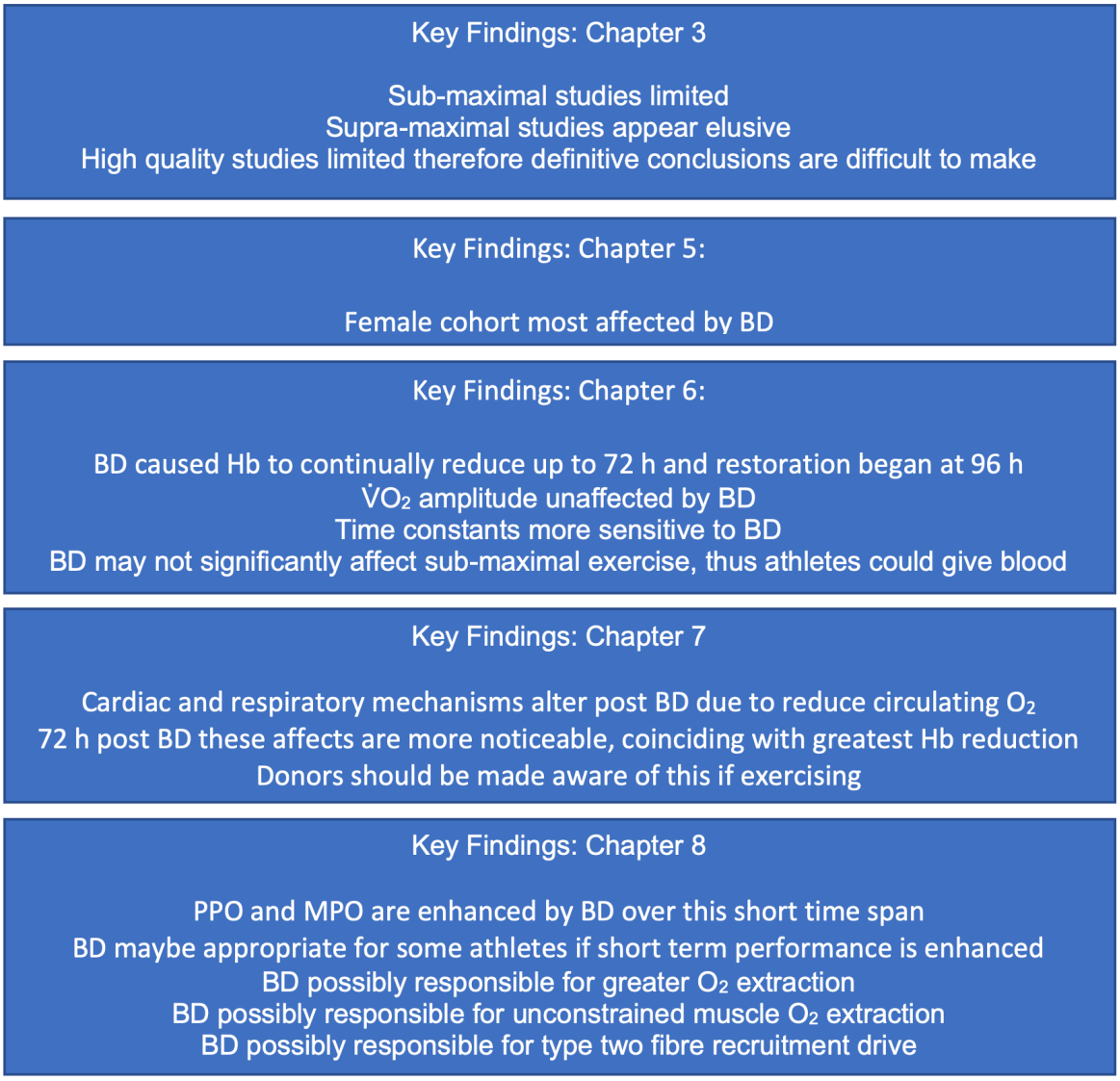

Figure 9.1: Key findings from the experimental chapters.

The systematic review and meta-analysis established that with only eight studies eligible for inclusion in the review, there were to date not enough high-quality experiments to draw any definitive conclusions regarding BD and cardiorespiratory outcomes. Additionally, it steered future chapters, as it revealed that many studies were over short time frames (24-48 h) and with one day of post BD testing, involving predominately $\dot{V}O_{2max}$ investigations and sub-maximally data was very limited (only 2 studies). Thus, it was showing the need for sub-maximal experimental work post BD and indicating that the chapters examining O_2 uptake kinetics and cardiorespiratory responses were justified. Furthermore, no investigative studies on supra-maximal intensities and BD could be identified, thus vindicating experimentation in this area. It was also established that a cohort to reflect the blood donating general public should be recruited and not just young athletic participants.

Chapter 5 examined if cardiorespiratory fitness and anthropometrics had an impact on haematological responses post BD and served to establish who was most affected by BD, which could potentially influence recruitment of participants in further chapters. Significance and strong correlations were observed, particularly when contemplating each individuals Hb loss in a 470 ml BD. However, to fully understand to a greater depth parameters such as cardiorespiratory responses, GET, O_2 pulse, HR_{max} and $\dot{V}O_2$ sub-maximally, they needed to be observed before and after BD. This therefore demonstrated the need for further work on the cardiorespiratory responses. Additionally, it was established that the female cohort was most affected regarding the Hb decreases, therefore justifying the inclusion of them in future work. However, if males had significant results in future testing, it would be anticipated that females would likely produce significant responses too and thus if recruitment of females was problematic it should not be dwelled upon.

The O_2 uptake kinetics chapter (6) aimed to provide additional sub-maximal data that was deemed to be lacking from the analysis of the systematic review, and recruited male and female participants that ranged from 22 to 61 years. One of the significant findings from this chapter was that Hb continued to decrease until 96 h post BD, which suggests that there is less circulating O_2 for several days post BD. However, despite this being the case other than the time constant and time delay ($tau_{2/3}$ and $TD_{2/3}$), the O_2 kinetics and thus the $\dot{V}O_2$ was deemed to be unaffected by BD. However, tHb from NIRS did show significant increases particularly at 48 h post BD; postulated to be due to enhanced O_2 extraction at the muscle from blood flow redistribution, which reflects an adaptation consequential to BD. This particular chapter concluded that 6 minutes of moderate or heavy intensity cycling

appeared to be unaffected by BD and thus a blood donor could donate blood and not have their $\dot{V}O_2$ uptake kinetics and by extension, sub-maximal exercise significantly affected. Chapter 7 was used to explore the cardiac and ventilatory variables that contribute to O_2 uptake and further investigate the effects of BD on moderate and heavy intensity domain cycling. It was seen that all variables increased post BD, but the majority of significant increases was seen in the HR data 72 h pre to post BD, which was the day of the lowest Hb value too. The 72 h period seemed to be most affected by BD and although as previously mentioned O_2 uptake kinetics appeared generally unaffected by this, cardiac and respiratory mechanisms had altered and thus, an awareness of these changes due to a lack of circulating O_2 should be accessible to the donating public who exercise.

The final experimental chapter examined repeated sprint capability, and was included as the systematic review found no evidence of supra-maximal intensity studies researched in conjunction with BD. The rejection of all the experimental hypotheses as BD was thought to impair repeated sprint ability was postulated to be because of the seemingly greater O_2 extraction or unconstrained muscle O_2 extraction and type two fibre recruitment drive that enhanced the power outputs. The implications of this in terms of performance is potentially beneficial with regards to power generation over a short time period, however many formats of repeated sprints exist, thus this is a small “snap shot” that could result from BD.

The commonality that is linking each experimental chapter is that there was a Hb reduction, which resulted in decreased circulating O_2. Moving from rest to normal exercising steady state conditions (pre BD), O_2 kinetics alter (e.g. phase II is longer in duration than phase I); blood flow is redistributed, as when circulation increases from the exercise the transit time of a RBC within capillaries decreases and therefore the offloading time of O_2 from Hb decreases (Calbet, et al., 2007). However, post BD Hb levels reduced (mean Hb pre BD 14.48 ± 0.16 $g{\cdot}dL^{-1}$ to post BD 13.47 ± 0.66 $g{\cdot}dL^{-1}$), resulting in deceased O_2 carrying capacity, thus, when exercising under steady state conditions the decreased transit and oxygenation time is now also accompanied with less O_2 bound to Hb; hence less oxygenation. This decreased PO_2 (O_2 gradient) results in a slower O_2 exchange across the blood myocyte barrier altering O_2 kinetics. The reduced PO_2 affects the amount of O_2 extracted per heartbeat and the mean transit time of the RBC into a cell is decreased as the blood flows faster spending less time in contact with the capillary walls (Figure 9.2). The cardiac tissue and exercising muscles therefore exert more energy, which can be seen from the changes in the kinetics data (tau^2 speeding tau3 slowing) (Figure 9.2). Thus, cardiac function is affected by the reduced circulating O_2, particularly because the heart is an aerobic organ and the exercising HR increases on each post BD day in comparison to

pre BD, but the greatest significance can be viewed at 72 h post BD (when Hb decreased the most). It should be appreciated that during exercise, cardiac output (blood flow) is directed mainly to the exercising muscles, for example with a 20 L/min output, 0.5 L/min goes to the heart and 18 L/min goes to the muscle (Joyner and Casey, 2015), but the volume of 0.5 L/min going to the heart following BD will contain less Hb. The resultant increase of HR seen in the cardiac output data suggests the PCO_2 increased as reflected in V_E along with its composites BF and V_T (Skow, et al., 2015), as the excess CO_2 needs to be expelled from the cell (Figure 9.2). It is possible that this sequence of events all result from a decreased circulating O_2, and are likely derived from changes that have occurred at a muscular level. This is because there appears to be no evidence suggesting that respiration is significantly affected at rest following BD. In chapters 6 and 7 the role of myoglobin was discussed as a source of O_2 storage in the muscle in times of need, such as in hypoxia and BD conditions, thus maintaining the required amount of O_2 needed for exercise (Figure 9.2). Its assistance maybe more evident in moderate exercise, as myoglobin has a finite store as it desaturates rapidly (Richardson, et al., 1995) and although it is found in both slow and fast twitch fibres it is more associated with slow twitch muscle fibres (Ordway and Garry, 2004), thus the heavy intensity exercise is supported more via respiratory increases and fast twitch muscle fibre recruitment (Figure 9.2).

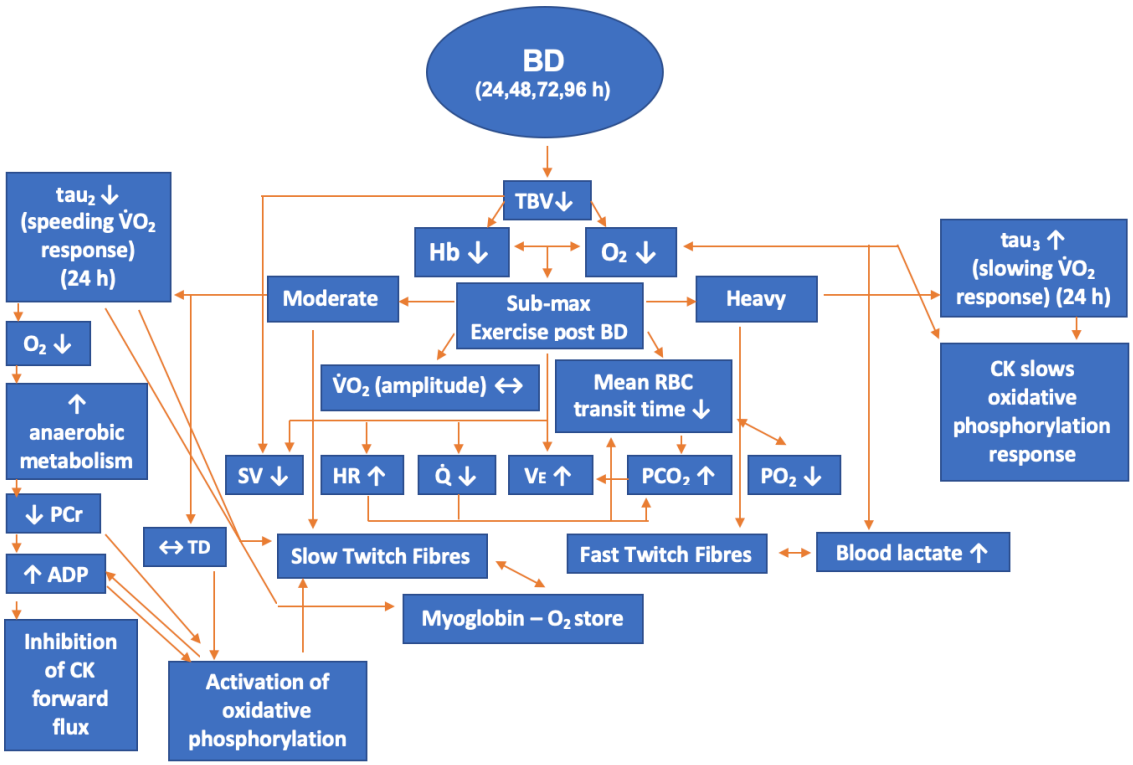

Figure 9.2: Physiological interactions of BD on sub-maximal exercise.

During heavy exercise the $\dot{V}O_2$ slow component is derived from a gradual loss of muscle proficiency (Krustrup, et al., 2009) and increased fast twitch fibre recruitment (Figure 9.2) to maintain power production (Cannon, et al., 2011). It is postulated that the fast twitch recruitment is additionally increased when there is less circulating O_2 (from BD), something reflected during the heavy trial post BD as steady state was significantly delayed, resulting in an increased pool of fast twitch fibres being recruited. This was confirmed via higher post BD lactate scores (pre BD = 10.56 ± 2.08 mM, post BD = 11.39 ± 2.28 mM; $P = 0.027$). Crucially these fibres are also used during supra-maximal exercise. Chapter 8 (multiple sprint capability) displayed increased (mean and peak) power outputs post BD, hypothesised to be due to increased recruitment of the fast twitch fibres, as a result of less circulating O_2 and further supported from blood lactate increases (e.g. post sprint 4 pre BD 12.52 ± 0.45 mM v post BD 13.32 ± 0.35 mM) (Figure 9.3). The power decline (across the four sprints) also increased post BD, potentially due to fast twitch fibres reaching peak force rapidly but also fatiguing rapidly in comparison to pre BD. Similarly, to the work related to O_2 uptake kinetics and cardio-ventilatory variables, the power decline per sprint post BD is amplified from O_2 diffusion gradient issues. Specifically, the gradient reducing across the blood-myocyte barrier; augmenting the requirement for amplified muscle O_2 diffusion to meet O_2 demand in fast twitch fibres (McDonough, et al., 2005) (Figure 9.3). Additionally, the decreased PO_2 and mean transit times (blood) also applies regarding supra-maximal power outputs. The decreased O_2 would also have a direct effect on the resynthesis of PCr during the recovery from the multiple sprints. It is suggested that PCr recovers at a slower rate post BD as its resynthesis is an O_2 dependent processes (Glaister, 2005), but there is less circulating O_2, (which also limits O_2 partial pressure within tissue Jung, et al. (1999)) and muscle O_2 requirement escalates with sprint repetitions; higher lactate scores are produced, which contributes to the higher fatigue index seen post BD (Figure 9.3).

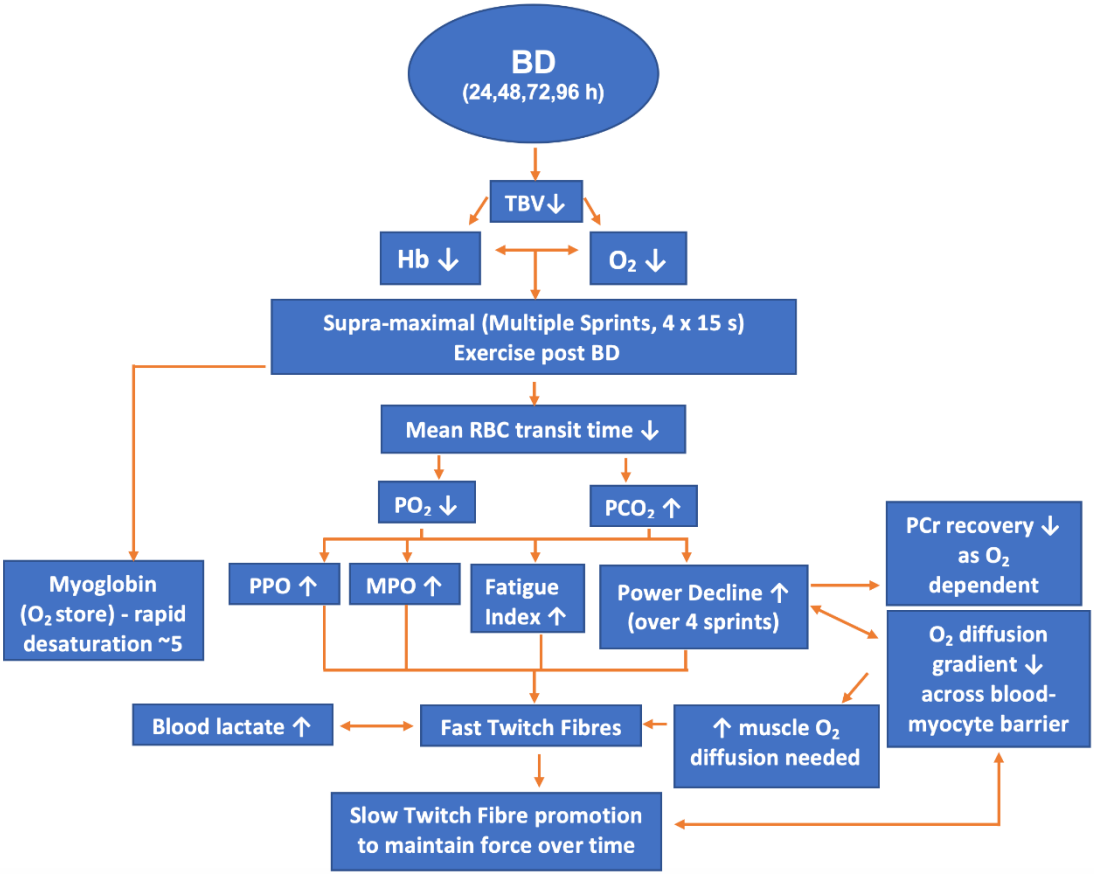

Figure 9.3: Physiological interactions of BD on supra-maximal exercise.

Although not qualitatively investigated in this study, it was interesting to note that throughout the days of testing in the post BD week, participants feelings of well-being appeared to reflect the Hb pattern. The participants were not aware of their Hb values, but when asked how they felt post testing the responses such as “that was harder than yesterday or it felt easier today” seemed to match the Hb increases or decreases.

When exercise testing females, it may be worth considering the menstrual cycle, due to issues such as reduced strength/power in the second half of a menstruation cycle (Gordon, et al., 2013). However, it is not necessarily so straight forward, for example, if a female participant uses contraception where menstruation is absent or does not have regular cycles, then there is an absence of knowledge as to when the menstrual phase occurs. In terms of this study, a female participant ~5 days prior to BD week and throughout testing happened to have what she deemed to be heavy menstruation. Like all participants, her Hb levels reduced post BD, and were prior to BD 13.7 and then 12.4, 11.8, 11.2 & 11.4 $g{\cdot}dL^{-1}$. According to World Health Organisation (WHO, 2011b) non-pregnant females should have a minimum Hb of 12.0 $g{\cdot}dL^{-1}$ to avoid being under the threshold for anaemia. According to these values, she was under the threshold for three testing days. Blood loss

from menstruation can range from ~30 ml to more than 80 ml (Hallberg, et al., 1966) and it is impossible to know in this case how much blood was lost consequential to menstruation, but it could be that the Hb values were exacerbated due to menstruation being coupled with BD. Although her blood loss from BD was 11 % and it would have equated to 13 % with an additional 80 ml lost from menstruation, these values do not exceed the 15 % maximum volume loss tolerance level for BD.

Chapter 10: Limitations and Recommendations

Limitations

Limitations identified within this thesis mainly concern participants. Firstly, the final three experimental chapters sample sizes were under powered (12) in comparison to the power calculation recommendations (14). Female participant numbers were reduced compared to males. A more even age range spread may have been more representative of the general public in that the majority of participants were in their twenties and there was no representation of people in their fifties. In regards to data gathered, there was a lack of NIRS participant data due to equipment failure. Finally, with regards to the supra-maximal research, only one kind of HIIT approach was used, but different elements of time, activity, intensity etc. exist. Additionally, it was performed at the end of ~40 minutes of submaximal intensity training.

Recommendations

An obstacle to overcome within this field is the recruitment of participants willing to not only exercise to a set of conditions, but who also need to donate blood. On recruiting participants, even if participants willingly attend to donate, the donation does not always happen, due to many issues, such as low Hb levels, the phlebotomist not accessing veins correctly, medical reasons and poor blood flow leading to incomplete donations. It would therefore be beneficial and recommended to work alongside the NHSBT, as they have access to regular existing donors.

Working with the NHSBT is also a recommendation as it may assist with ‘blinded’ research. It is advantageous as this type of research aids to eliminate bias. This study did not use this approach and it appears most BD studies do not either, as discovered from the systematic review where only one study adopted a sham bleed (Meurrens, et al., 2016). This is potentially due to ethical implications, logistical difficulties or the sense that it may be an inappropriate invasive procedure if there is no outcome (BD).

The NHSBT were aware of the work being undertaken in this thesis because although the study was ethically approved via ARU the blood donations were performed at an approved NHSBT centre and therefore permission was granted by them to allow this. One of the stipulations they stated for them to agree to this was that there should be 24 h between exercise and BD. However, this thesis and other investigators (Mora-Rodriguez, et al.,

2012) within this field have found no scientific evidence that states donors should not exercise prior to 24 h after BD. As such, a recommendation that exercise and BD should/could be investigated within this time period too seems acceptable.

In regards to additional research, there are still unanswered questions, such as what happens in excess of 6 minutes of moderate or heavy intensity exercise? Six minutes was used in this study as it built on the work from Gordon and colleagues (2010) and additionally, it is deemed sufficient time to get to steady state. However, people generally exercise recreationally for longer than 6 minutes and also do not necessarily stay at a steady state, for example when running up an incline or changing the type of activity within an exercise class. Therefore, it would be a recommendation to use an exercise to reflect what people normally do when exercising e.g. a 20/30-minute run/bike at 70-80% of $\dot{V}O_{2max}$.

The supra-maximal research should be performed without the possible effects from previous exercise conducted on the same day and thus be researched separately and additionally with a comparison against another form of HIIT. For example, 6 – 8 bouts of 20 s sprints at 170% of $\dot{V}O_{2max}$ (supra-maximal), with 10 seconds rest between the sprints (Tabata, et al., 1997) or bouts of > 20-30 s (SIT) combined with recovery (Buchheit and Laursen, (2013b).

The role of myoglobin has been discussed in this thesis in terms of its O_2 storage capabilities and its benefits in times of need e.g. BD, thus there is the possibility that myoglobin played a role after BD. Therefore, further research is recommended with the approach that Richardson, et al. (1995) adopted, that is with the use of magnetic resonance spectroscopy (MRS) to detect myoglobin saturation and O_2 gradients. PCr has also been discussed in this thesis, for example in regards to its resynthesis being O_2 dependent. A recommendation for how BD affects PCr kinetics could be determined with MRS too. Rossiter and colleagues (2002) investigated the asymmetry of phosphocreatine concentration and O_2 uptake using MRS and therefore this approach could aid to determine how much BD impacts PCr.

Chapter 11: Conclusions

At present there appears to be little information available regarding exercise after BD within the guidance from the NHS. This thesis has endeavoured to provide further understanding into this area by using sub-maximal and supra-maximal exercise over the time period of 24 – 96 h, thus providing a novel insight into exercise post BD. The supra-maximal exercise was researched to try to appreciate what may happen in a HIIT class, because these types of classes are very popular within the leisure industry and the data regarding this exercise genre and BD appears elusive.

Although it is stated that Hb may take 6-12 weeks to return to normal (NHS, Blood and Transplant, 2018), it is not mentioned that Hb may continue to fall for several days after donation. This thesis has shown that Hb decrements can impact exercise (but not always significantly) in excess of the cautionary period of 12 - 24 h post BD, and the experimental chapters have demonstrated the effect on physiological variables and as such this information could/should be mentioned within guidance from the NHS, Blood and Transplant. Furthermore, these findings are generalisable, as a sample with a wide age-range was gathered (thus relating to the blood donating general public). If potential blood donors had a firmer understanding of how the physiological mechanisms are affected, then it could lead to an alteration in member numbers (increase or decrease). Specifically, there could be increases within the athletic population (who think BD may impact their performance), particularly if they were cognisant of certain facts such as there are no significant impacts on $\dot{V}O_2$ from BD at moderate and heavy intensities.

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Appendices

Appendix 1. Publication – The Acute Effects of Whole Blood Donation on Cardiorespiratory and Haematological Factors in Exercise: A Systematic Review

PLOS ONE

RESEARCH ARTICLE

The acute effects of whole blood donation on cardiorespiratory and haematological factors in exercise: A systematic review

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Abstract

Background

This systematic review aimed to collect the relevant historical and current literature to produce an informed analysis of the acute effects on cardiorespiratory and haematological factors following whole blood donation (~ 470 ml) during exercise. Testing the hypothesises that blood donation produces either no changes (Null) or produces significant changes (alternate) in haematology, $\dot{V}O_2$, heart rate, exercising power and time.

Methods

Four databases of medical and science orientations were searched with terms sensitive to connections regarding exercise, blood donation (400–500 ml)/haematology, $\dot{V}O_2$, heart rate, exercising power and time. The study retrieval process utilised the PRISMA approach and selection was via an adapted scoring method according to the Consensus based Standards for the selection of health Measurements Instruments (COSMIN). Systematic review focused on 24–48 hrs post donation. Details of the PRSIMA checklist can be found in the accompanying online document.

Results

Following scrutiny of 48 research papers by two independent assessors 8 experimental studies were included. Four studies showed a mean reduction for difference in $\dot{V}O_{2max}$ (- 2.4 ± 1.4 ml·kg^{-1}·min^{-1}) and a medium effect size (-0.26). No statistical significance was present at the mean meta-analysis level, also the case for heart rate, time to exhaustion and power. A mean reduction was seen in haemoglobin (- 1.05 g·dL^{-1}), haematocrit (- 3.71%) and red blood cells (- 0.44 Mio μL^{-1}), very large effect size was observed (Cohen's *d*, -0.75, -1.16 and -4.23 respectively) and statistical significance (95% CI, -2.04, -0.54; -4.59, 2.28 and -4.37, -4.10 respectively).

Conclusion

Although individual studies show that $\dot{V}O_{2max}$ Is reduced from blood donation pooled results show that $\dot{V}O_{2max}$ is indeed not significantly reduced from blood donation 24–48 hrs post donation. Additionally sub-maximally there isn't enough data to produce substantial comparatives. Furthermore, this systematic review demonstrates that there are not enough high-quality studies regarding cardiorespiratory outcomes following blood donation.

Introduction

Currently in the UK ~ 470 ml of blood is collected from donating a single unit [1] with blood transfusions distributed to treat medical conditions including cancer, anaemia and blood disorders, be used in surgery, including emergency and cardiac and for blood loss following child birth. In the UK alone 6,000 donations are needed every day and 200,000 new donors are needed each year in order to ensure that there is a sustained availability of blood [2].

A number of confounding reasons have been attributed to the progressive decline in new blood donors, including an increase in exotic holidays and tattoos [3,4] both of which can preclude the donation of blood coupled with an increasing population of people from non-white ethnic groups [5]. An additional population group who are potentially contributing to this decline are the sport enthusiasts and recreational/professional athletes, who may be concerned about the effect of BD on their performance [6]. Early concerns regarding BD affecting athletic performance were noted in the mid 1900's where Springfield College, Massachusetts ruled that no man could be a blood donor while participating in varsity sport, due to reported unfavourable effects following BD such as wrestlers collapsing after competition and endurance lost in other athletes [7]. Additionally, reduced health, in terms of decreasing energy levels and becoming prone to infections or disease, as a result of BD is a fear of the non-donor [8]. Although not specific guidance for high-performance competitive athletes, the American Red Cross [9] suggest that a marginal decrease in exercise tolerance may be noticed up to a week after BD. Additionally, where exercise is concerned, according to NHS Blood and Transplant [10] and American Red Cross [9], prudence should be executed immediately after blood donation.

There are consequences to BD such as decrements in blood/plasma volume (B/PV) and haemoglobin (Hb). While the BV returns to normal within 24–48 hours (48–72 hrs, [11]), due to the progressive PV expansion [12], red cell mass and thus Hb restoration takes much longer, which according to Klein and Anstee [11] and NHS blood and Transplant guidelines [13] can be 3–6 weeks or 6–12 weeks respectively. Indeed, a restoration rate in Hb of 36 +/- 11 days was observed following removal of 550 ml of blood [14].

The biological responses to acute BD are well documented, indeed early work from Karpovich and Millman [7] highlighted that a Hb decrement affected athletic performance, while later work from Balke and colleagues [15] proposed that from a BD of 500 ml there was a 9% decline in maximal oxygen uptake ($\dot{V}O_{2max}$) and more recently Gordon and contemporaries [16] showed a 4.65% fall in $\dot{V}O_{2max}$ from a 450 ml donation. Additionally associated markers of aerobic fitness have been shown to be altered following BD such as maximum aerobic power output (W_{max}) [17] and time to exhaustion (T_{Ex}) [18,19,20]. There is little doubt that decreasing blood volume via BD will diminish oxygen carrying capacity, as haemoglobin (Hb) concentration will have been reduced and it is the primary source for the conveyance of

oxygen. The iron containing protein Hb inside developing and mature red blood cells is important, in terms of arterial O_2 content >99% of O_2 is transported by Hb [21].

Currently the Cochrane Database regarding systematic review [22] shows no publications concerning the effects that BD has on exercise. There has however recently been a systematic review [23] concerning the effects of a standard whole blood donation on oxygen uptake and exercise capacity. The authors chose to include studies using a variety of blood volumes from 400–500 ml and across different donation time periods and exercise domains. The aim of this current systematic review is to build on the work from Van Remootel and colleagues [23] and produce an informed analysis of the acute effects of BD (~ 470 ml) on maximal oxygen uptake and associated cardio-metabolic markers (specific to haematology), within the time period of 12–24 hr post donation as it is suggested that exercise within the time period should be approached with caution [9,10]. Testing the hypothesis that blood donation produces either no changes or produces significant changes in haematology, $\dot{V}O_2$, heart rate (HR), exercising power and time.

Method

The systematic review was carried out from guidance of Systematic Reviews [24] and by using the bibliographic management software RefWorks, Proquest, USA. Exact duplicates and close duplicates (a term that allows duplicates to be discovered despite minor differences being present, for example varying combinations of author names or differing titles and publication years), were assessed and then removed for subsequent analysis.

Literature search of data base sources and search terms

From the beginning of December 2016 to the end of January 2018, Pubmed, Web of Science, SPORTDiscus and Scopus were used to obtain literature from the early 1900's to January 2018, due to the databases being medical and science orientated. Search terms were created in order to be sensitive to all possible connections regarding exercise, blood donation/haematology, $\dot{V}O_2$, HR, exercising power and time. Hit numbers (which were absent from Van Remoortel et al. [23]) were Pubmed 3243, Web of Science 426, SPORTDiscus 249 and Scopus 5713. The following terms are an example of the search strategy used in Pubmed (a full strategy is available in the online supplementary material).

"blood donation" OR "blood collection" OR "blood withdrawal" OR "blood centre" OR "Blood center" OR "blood service" OR "blood bank" OR "blood transfusion" OR "phlebotom*" OR "plasma don*" OR "blood volume" OR "blood donor*"

"sport" OR "exercise" OR "physical endurance" OR "hiking" OR "cycling" OR "athletic" OR "training" OR "working out" OR "work out" OR "strenuous activity" OR "VO2max" OR "maximal aerobic power" OR "Oxygen carrying capacity"

"men" OR "man" OR "male" OR "sportsman" OR "sportsmen" OR "woman" OR "women" OR "female" OR "sportswomen" OR "Sportswoman" OR "athlete" OR "adult" OR "healthy" OR "humans" OR "people" OR "fit"

Inclusion and exclusion criteria

Restricting the publications to English and using the Population, Interventions, Comparators, Outcomes and Study designs (PICOS) strategy enabled studies to be selected accordingly (see supplementary material). Studies that were accessible as conference abstracts were excluded, as were blood expansion studies, studies assessing muscular strength and studies involving

participants > 66 years and < 17 years in age. The age range selected was according to blood transfusion services in the UK [25].

Study retrieval process and quality assessment

The final studies selected for the systematic review were rated with an adapted scoring method [26] according to the Consensus based Standards for the selection of health Measurements Instruments (COSMIN) and the threshold for eligibility set at adequate or greater. For each sub-section of the tool a worst score counts scenario was adopted, with scores scaled from 4 (very good) to 1 (inadequate). This scoring method considers issues such as, control groups, allocation concealment, blinding, selective outcome reporting, quantity of cited publications, aims and hypothesis, appropriate variables, sampling method, size and description, validity and reliability of equipment, study design, descriptive statistics, statistics, research setting, calculations, inferences, findings and significances.

To ensure that the studies selected were of good quality, two assessors (DJ and DG) individually assessed the texts for eligibility according to the exclusion and inclusion criteria and additionally according to the previously mentioned COSMIN threshold.

Data extraction and analysis

Using the spreadsheet software package Microsoft Excel, Microsoft Corp, Washington, USA (2011) a database was formed with the haematological and cardiorespiratory/respiratory variables extracted from the evaluated studies for analysis.

The variables of Hb, haematocrit (Hct), RBC's, VO_{2max}, maximum heart rate (MaxHR), maximum aerobic power output and time to exhaustion were all considered for inclusion in the systematic review, since they were the most widely reported variables relating to haematological and cardio-vascular responses and regarded according to their mean and standard deviation pre and post donation results. The post donation values were classified according to time frames, which included immediately, 2 hrs, 24–48 hrs, 48–72 hrs, 7 days, 14 days, 21 days and 28 days. However, for the purpose of this evaluation the systematic review focused on 24–48 hrs for two reasons. Firstly, the time period had sufficient data to analyse and secondly the time period is closest to being straight after donation and suggested by the NHS [10] and American Red Cross [9] to be approached with caution regarding exercise. For an outcome to be included for systematic review there needed to be at least two studies that reported a variable of interest. Valentine, Pigott and Rothstein, [27] suggest that although two studies is not ideal it will provide a conclusion and provides an analysis strategy.

Cohens *d* and 95% confidence intervals were calculated for each study. Cohens *d* (standardized difference in means) is expressed as the difference between two (raw) means divided by the pooled standard deviation (SD) of the groups ($M1 - M_2$ / SD) [28]. Effect sizes for Cohens *d* are evaluated as, very large > 1.3, 0.8–1.3 (large), 0.5–0.8 (medium), < 0.2–0.5 (small). The mean Cohens *d* value was calculated by individual studies being weighted by the variance of the data, thus expressed as $\overline{SE} = \frac{\Sigma(ES_i w_i)}{\Sigma w_i}$ [29] or $Mean\ Cohens\ d = \frac{Sum(individual\ Cohen\ d\ from\ each\ study\ x\ inverse\ of\ variance\ from\ each\ study)}{Sum\ of\ inverse\ of\ variance}$.

Results

Following scrutiny of 48 research papers for their eligibility and reducing that number to 20 after further inspection (regarding to PICO components), 8 studies were deemed suitable for inclusion in the systematic review following the COSMIN check, with the publication dates of those included ranging from 1997–2016 as highlighted in Fig 1. Regarding assessment of

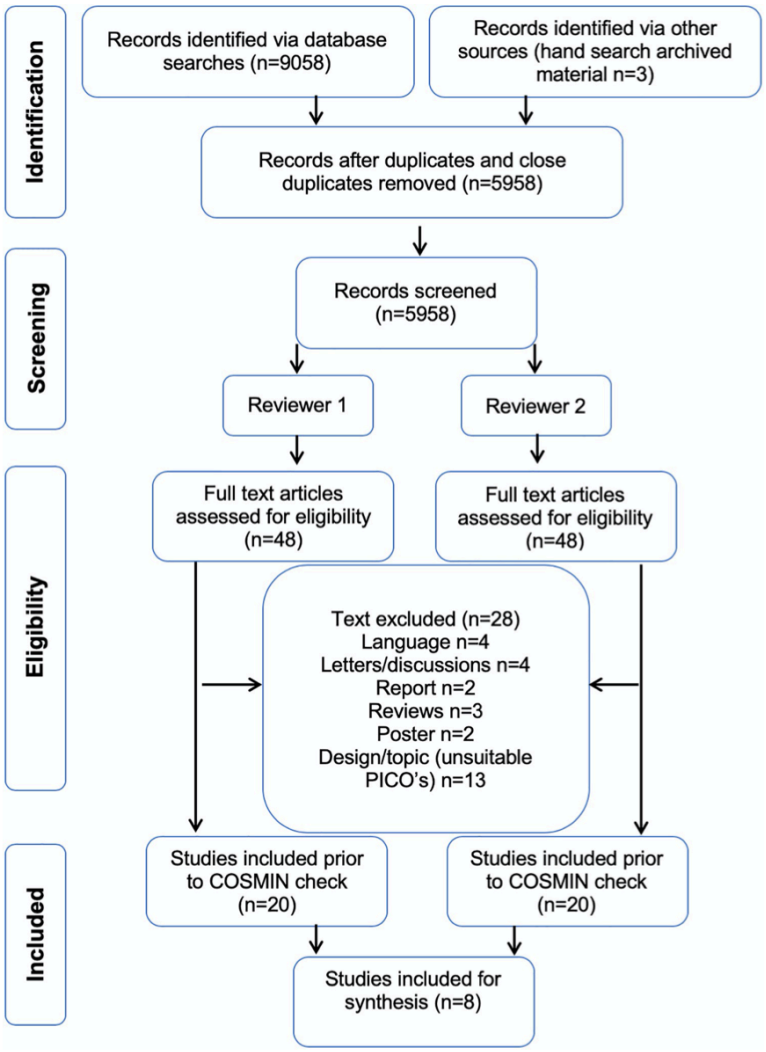

Fig 1. Flow diagram based on the PRISMA statement [30] illustrating the identification and selection process for the study retrieval.

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publication bias summarized effects (95% CI = -1.05,0.16 and I^2 = 0%) of the main variable (VO_{2max}) was assessed, and a Limits of Agreement plot showed that the two assessors were considered to be in agreement (bias = 0.12, CI = -0.25,0.55.

Overview of studies included

All 8 studies included were experimental in nature, incorporating a before and after blood donation approach, with only the Meurrens, et al. [20] study using a control (sham bleed)

group. Participant numbers ranged from 9–24 (13.5 ± 4.9) and hence appeared low, unfortunately only two studies, that of Judd, et al. [31] and Meurrens, et al. [20] provided power calculations to reflect sample size, although justification for sample size was presented in all studies except Krip, et al. [32].

The majority of the participants were male (91%) and the mean age of all who took part was 23.9 ± 1.5 with a mean height of 179.2 ± 3.4 cm and a mean mass of 76.4 ± 5.9 Kg.

Cardiorespiratory variables

Oxygen Uptake ($\dot{V}O_2$). The exercise mode for all studies was cycle ergometer, with a protocol that induced exhaustion and thus $\dot{V}O_{2max/peak}$ with the exception of Gordon, et al. [33] and Krip, et al. [32], which were submaximal studies. Table 1 shows an overview of the protocols used by all researchers.

Gordon, et al. [33] has submaximal data in the moderate (below Gas Exchange Threshold (GET), specifically 80% of the difference between rest and GET) and heavy domains (between GET and $\dot{V}O_{2max}$, which was 50% of the difference between GET and $\dot{V}O_{2max}$), but cannot be compared to any other studies due to no parallels. Similarly, with little data to compare against and no apparent parallels this is the case also for the Krip, et al. [32] study.

The participants athletic descriptions (Table 1) included physically active and healthy to well-trained athletes, resulting in $\dot{V}O_{2max}$ reported mean value before blood donation of 51.31 ± 7.10 ml·kg^{-1}·min^{-1} (Table 2) (when converting all the values (including Burnley and

Table 1. Overview of the 9 studies included. Sample size varied, as did the combination of male (M) and female (F) participants. Blood donation volumes ranged from 450–500 ml. With age, height and mass reported as mean ± SD.

Study	Sample, size (N) & sex (M/F)	Blood drawn (ml)	Age (yrs)	Height (cm)	Mass (Kg)	Exercise mode & overview of protocol
Burnley, et al., 2006	10 (M) 1 (F) Physically active & healthy	450	23 ± 6	177 ± 4.0	77.2 ± 11	Cycle: 0 W for 3 min then 80% of difference between GET and $\dot{V}O_{2peak}$ (severe intensity domain) to exhaustion.
Gordon, et al., 2010	6 (M) 4 (F) Regular sport participants	450	21 ± 2	175.2 ± 5.1	66.4 ± 2.8	Cycle: Test 1, 6 min at 80% of respiratory exchange ratio or Test 2, 50% of the difference between $\dot{V}O_{2max}$ and respiratory exchange ratio.
Gordon, et al., 2013	15 (M) Well trained athletes	450	23.3 ± 4.5	180.1 ± 6	77.4 ± 13.1	Cycle: increase ramp from 100W by 25w/min at 80rpm to exhaustion.
Hill, Vingren & Burdette., 2013	2 (F), 7 (M) Healthy uni students	450	23 ± 1 (F) 25 ± 3 (M)	170 ± 11 (F) 182 ± 2 (M)	60 ± 8 (F) 92 ± 15 (M)	Cycle: Following a 4 min warm up at 79 ± 15 W and 4 min rest, 80rpm held at 274 ± 2 W to exhaustion.
Judd, et al., 2011	2 (F) 10 (M) Moderately active	450	24.3 ± 5.2	181 ± 8	87.5 ± 15.3	Cycle: starting at 1.5 /1.0 kp (men and women respectively), ramp increase of 0.5 kp per 2min at 80rpm to exhaustion.
Krip, et al., 1997	12 (M) (6 gave blood) Endurance trained	500	24.8 ± 0.5	N.D.	73.9 ± 2.2	Cycle: 6 min at 60rpm at successive work rates to attain target HR's (maximum of 180).
Meurrens, et al., 2016 #	24 (M) (16 gave blood) Moderately trained	470	25.6 ± 0.76	180.2 ± 1.82	73.8 ± 1.96	Cycle: Starting at 70W and increasing by 30W every 2 min at 85-90rpm to exhaustion.
Ziegler, et al., 2014	20 (M) Healthy men	450	25.9 ± 1.6	185 ± 2	78.7 ± 3.3	Cycle: 6 min warm up at 100W, 35W/min to exhaustion.

N.B. N.D. denotes no data,

#denotes this study is a RCT design and as such is not included in the meta-analysis.

https://doi.org/10.1371/journal.pone.0215346.t001

Table 2. $\dot{V}O_{2max}$ values pre donation (Unbled) in all 8 studies are shown with post donation (Bled) values for the studies that collected 24–48 hrs data. Gordon, et al. [33] was a submaximal test post donation in the moderate and heavy domains involving oxygen uptake kinetics and thus the values are not reported in this table.

Study	$\dot{V}O_2$ & $\dot{V}O_{2max/peak}$ ml·kg^{-1}·min^{-1} (unless otherwise specified)	
	Unbled	Bled (24-48hrs)
Burnley, et al., 2006	3.79 ± 0.64 *♯	3.64 ± 0.61 *♯
Gordon, et al., 2010	53.0 ± 4.1	-
Gordon, et al., 2013	51.3 ± 7.6	48.4 ± 7.9 ^
Hill, Vingren & Burdette., 2013	40 ± 4.0	36.0 ± 4.0
Judd, et al., 2011	46.6 ± 7.0	44 ± 6.5
Krip, et al., 1997	64.1 ± 1.9 $	-
Meurrens, et al., 2016	56.7 ± 1.5	53.9 ± 1.4
Ziegler, et al., 2014 [34]	49.7 ± 1.7♯	-

N.B.

* denotes values in l·min^{-1},

^ denotes that this time period was 48-72hrs.

♯ denotes the Burnley [18] study post donation showing a $\dot{V}O_{2peak}$ value, as this was a test performed in the severe intensity domain to exhaustion and the Ziegler, et al. study showing $\dot{V}O_{2peak}$ values, with their criteria to exhaustion and a respiratory exchange ratio of more than 1.2.

$ denotes the Krip, et al. [32] study showing submaximal unbled end $\dot{V}O_2$ values.

https://doi.org/10.1371/journal.pone.0215346.t002

team [18] and to ml·kg^{-1}·min^{-1}). With values ranging from 40 ± 4.0 [19] to 64.1 ± 1.9 ml·kg^{-1}·min^{-1} [32].

Table 2 shows that regardless of the time frame in a bled situation there is a trend for BD to decrease $\dot{V}O_{2max}$ values from the pre donation values. With focus on the time frame of 24–48 hrs post donation, under meta-analysis examination, evidence from the four studies within this bled time period showed a mean reduction for difference in $\dot{V}O_{2max}$ (- 2.4 ± 1.4 ml·kg^{-1}·min-1), with a small effect size, -0.26 (Fig 2). No statistical significance is present at the mean meta-analysis level due to the 95% CI passing through 0 (-1.46, 0.94). Hill, Vingren & Burdette [19] had significance in the decreased of VO_{2max} with a large effect size (Cohen's *d*, -1) and confidence intervals of -1.97 to -0.03. Of the remaining studies all had small Cohen's *d* effect sizes, but no statistical significance.

Heart rate (HR). The HR pre and post donation values were only acknowledged in three studies (Table 3), with Krip, et al. [32] only presenting post donation HR results. On viewing, the values are similar across time frames. Indeed, the meta-analysis showed no statistical significance for any of the results, all the CI's passed through "0", with very small Cohens *d* effects across all research too (Fig 3).

Time to exhaustion (TEx). TEx pre and post donation evidence was recorded from five studies (Table 4), which are the same groups shown in Table 2, which related to $\dot{V}O_{2max/peak}$ results. With the exception of the Judd and contemporaries [31] study TEx shows an inclination to decrease in a Bled condition from Unbled across all time frames. For the time frame of 24–48 hr the meta-analysis (Fig 4) showed a mean reduction in TEx (- 17.40 s), with a large effect size produced (Cohen's *d*, -0.88). No statistical significance is present at the mean meta-analysis level due to the 95% CI passing through 0 (-37.29, 35.52).

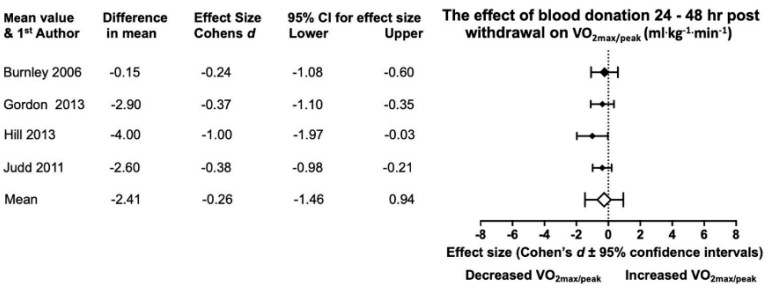

Fig 2. Statistical results from four studies of pre and 24–48 hr post BD in $\dot{V}O_{2max/peak}$. With the diamonds reflecting the effect size of the studies. The white diamond shows the overall mean effect size. All effect sizes are Cohen's *d* and 95% confidence interval, with heterogeneity of $I^2 = 0\%$.

https://doi.org/10.1371/journal.pone.0215346.g002

Table 3. Maximal HR values pre donation (Unbled) and post donation (Bled) over various time frames.

Study	HRmax (b·min^{-1})	
	UnBled	Bled (24-48hrs)
Gordon, et al., 2013	184.9 ± 10.1	185.0 ± 10 ^
Judd, et al., 2011	186 ± 12	181 ± 19
Krip, et al., 1997	N/A	187 ± 3
Meurrens, et al., 2016	187±4	187 ± 3

N.B.

^ denotes that this time period was 48-72hrs. No pre value was available for Krip, et al. [32]

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Maximum aerobic power output (W_{max}). W_{max} pre (329.7 ± 42.4) and post (329.5 ± 45.7) donation evidence could only be gathered from one study [16] and the time period for this was 48-72hrs, thus no meta-analysis could be performed.

Haematological variables

Hb. Hb pre and post donation evidence was recorded from five studies (Table 5), across these studies there is a decrement in Hb under Bled conditions, which reveals an 8.06%

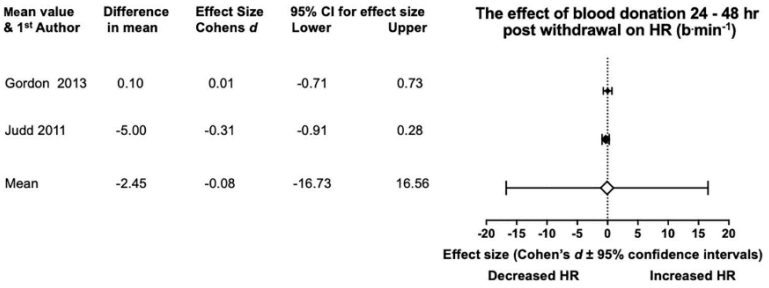

Fig 3. Statistical results from two studies of pre and 24–48 hr post BD in HRmax. With the diamonds reflecting the effect size of the studies. The white diamond shows the overall mean effect size. All effect sizes are Cohen's *d* and 95% confidence interval, with heterogeneity of $I^2 = 0\%$.

https://doi.org/10.1371/journal.pone.0215346.g003

Table 4. TEx pre and post donation (Unbled and bled) values over various time periods.

Study	TEx (s)	
	UnBled	Bled (24-48hrs)
Burnley, et al., 2006	375 ± 129	321 ± 99
Gordon, et al., 2013	611.4 ± 101.4	610.8 ± 109.8 ^
Hill, Vingren & Burdette., 2013	268 ± 16	250 ± 22
Judd, et al., 2011	586 ± 168	589 ± 189
Meurrens, et al., 2016	1014 ± 54	966 ± 60

N.B.

^ denotes that this time period was 48-72hrs.

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decrease in Hb levels. The meta-analysis (Fig 5) showed a mean reduction in Hb (- 1.05 $g \cdot dL^{-1}$) across four relevant studies, with a medium effect size produced (Cohen's *d*, -0.75). No statistical significance is present at the mean meta-analysis level due to the 95% CI passing through 0 (-2.04, 0.54). Gordon, et al. [16] showed a large effect size and statistical significance as

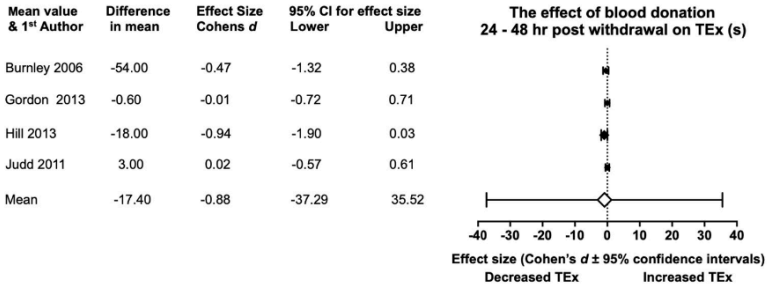

Fig 4. Statistical results from four studies of pre and 24–48 hr post BD in TEx (s). With the diamonds reflecting the effect size of the studies. The white diamond shows the overall mean effect size. All effect sizes are Cohen's *d* and 95% confidence interval, with heterogeneity of $I^2 = 11.57\%$.

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Table 5. Hb pre and post donation (Unbled and bled) values over various time frames, with the Krip, et al. [32] and Ziegler, et al. [34] studies not reporting post donation values.

Study	Hb ($g \cdot dL^{-1}$)	
	UnBled	Bled (24-48hrs)
Burnley, et al., 2006	15.4 ±0.9	14.7 ± 1.3
Gordon, et al., 2010	14.2 ± 1.5	13.1 ± 1.5
Gordon, et al., 2013	15.6 ±1.2	14.1 ± 1.6 ^
Hill, Vingren & Burdette., 2013	14.9 ± 0.8	14.0 ± 1.7
Krip, et al., 1997	15.8 ± 0.5	-
Meurrens, et al., 2016	15.6 ± 0.2	13.7 ± 0.3
Ziegler, et al., 2014	9.3 ± 0.11 *	-

N.B.

* denotes values in mmol/L and

^ denotes that this time period was 48-72hrs.

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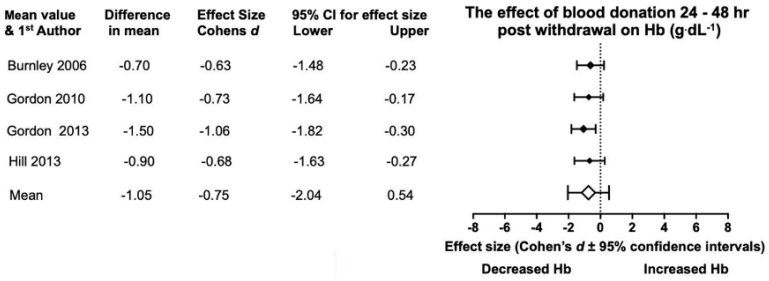

Fig 5. Statistical results from four studies of pre and 24–48 hr post BD in Hb. With the diamonds reflecting the effect size of the studies. The white diamond shows the overall mean effect size. All effect sizes are Cohen's *d* and 95% confidence interval, with heterogeneity of $I^2 = 0\%$.

https://doi.org/10.1371/journal.pone.0215346.g005

Table 6. Hct pre and post donation (Unbled and bled) values over varying time frames.

Study	Hct (%)	
	UnBled	Bled (24-48hrs)
Burnley, et al., 2006	44 ± 2	41 ± 3
Gordon, et al., 2010	43.55 ± 3.28	40.20 ± 2.63
Gordon, et al., 2013	48.76 ± 4.31	43.99 ± 3.91 ^
Krip, et al., 1997	41.1 ± 1.2	-
Meurrens, et al., 2016	45.8 ± 0.6	40.9 ± 0.9
Ziegler, et al., 2014	43.7 ± 0.6	-

N.B.

^ denotes that this time period was 48-72hrs.

https://doi.org/10.1371/journal.pone.0215346.t006

confidence intervals were to the left of 0 (1.82, -0.30). Cohen's *d* effects were moderate to high across all the studies. Burnley, et al. [18], Gordon, et al. [33] and Hill, et al. [19] were not shown to have statistical significance under meta-analysis scrutiny for Hb.

Hct. Hct pre and post donation evidence was recorded from four studies (Table 6). Across these studies there is a tendency for Hct to decline in a Bled state, revealing an 8.81% decrease in Hct levels. From Unbled to Bled the meta-analysis (Fig 6) across the three relevant studies

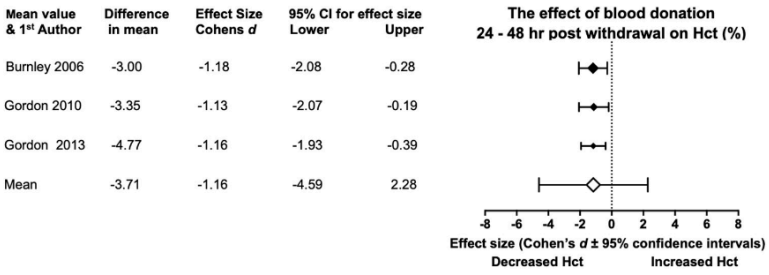

Fig 6. Statistical results from three studies of pre and 24–48 hr post BD in Hct. With the diamonds reflecting the effect size of the studies. The white diamond shows the overall mean effect size. All effect sizes are Cohen's *d* and 95% confidence interval, with heterogeneity of $I^2 = 0\%$.

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Table 7. RBC's pre and post donation (Unbled and bled) values over varying time frames.

Study	RBC's (Mio μL^{-1})	
	UnBled	Bled (24-48hrs)
Gordon, et al., 2013	5.28 ± 0.50	4.71 ± 0.59 ^
Meurrens, et al., 2016	5.2 ± 0.1	4.6 ± 0.1
Ziegler, et al., 2014	4.9 ± 0.07	4.6 ± 0.07

N.B.

^ denotes that this time period was 48-72hrs.

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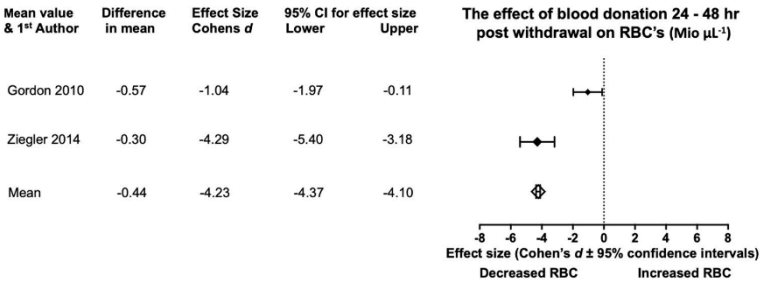

Fig 7. Statistical results from two studies of pre and 24–48 hr post BD in RBC's. With the diamonds reflecting the effect size of the studies. The white diamond shows the overall mean effect size. All effect sizes are Cohen's *d* and 95% confidence interval, with heterogeneity of $I^2 = 0\%$.

https://doi.org/10.1371/journal.pone.0215346.g007

[16,18,33] showed a mean reduction in Hct (-3.71%) and a large mean effect size (Cohen's *d*, -1.16). Statistical significance is not present at the mean meta-analysis level due to the 95% CI passing through 0 (-4.59, 2.28). All studies show large effect sizes and statistical significance.

RBC's. RBC's pre and post donation evidence was recorded from three studies (Table 7). Across all studies there is a tendency for RBC's to decline in a Bled state this reveals a 9.49% decrease in RBC's levels. From Unlbed to Bled the meta-analysis (Fig 7) across the two relevant studies [16,34] revealed a mean reduction in RBC's (-0.44 Mio μL^{-1}) and a very large mean effect size (Cohen's *d*, -4.23). Statistical significance is present at the mean meta-analysis level due to the 95% CI being placed notably to the left of 0 (-4.37, -4.10). All studies show large to very large effect sizes and statistical significance.

Discussion

Based on the hypothesis stated that blood donation produces either no changes (H_0) or produces significant changes (H_1) in haematology, $\dot{V}O_2$, heart rate (HR), exercising power and time, the following can be concluded. $\dot{V}O_{2max/peak}$ based on the mean Cohen's *d* ± 95% confidence intervals appears to accept the Null hypothesis, as the line passes through "0" and thus is not statistically significant; this is also the case for HR, TEx and W_{max}. For the blood variables the alternate hypothesis is accepted only for RBC's, rejecting the Null hypothesis, as the mean Cohen's *d* ± 95% confidence intervals favour donation.

Research papers have historically determined that BD decreases $\dot{V}O_{2max}$ [15,16,19,20] However, in this analysis the confidence intervals have not produced statistical significance. Individual studies have too much variance and therefore the pooled data using the Cohen's *d*

allows for comparisons across all papers. A potential confounding reason for the lack of significance could be the absence of statistical power due to low sample sizes. Indeed, this systematic review had the potential to include 20 papers, but due to quality issues, only 9 papers were considered to be of good quality, none of the papers were considered to be excellent quality, based on the adapted scoring method [26]. Reasons for exclusion were heterogeneous, but a reoccurring theme even in the good quality papers was lack of a power calculation, indeed only the Meurrens, et al. [20] and Judd, et al. [31] studies that were in the list examined provided this.

An overall mean reflection of blood variables for the systematic review data collected showed that under Bled conditions (24–48 hr) RBC's, Hct and Hb values were significantly reduced. Where data allowed there were three studies [16,18,32] in this systematic review that showed proportional reductions in Hct and Hb post donation. Mathematically for normal haematocrit (37–41/43% and 42–47% in women and men respectively [35,36]) of 15g Hb/100ml (blood), the Hb carrying capacity of O_2 = 1.34 x 15 = 20.1 mL O_2/100mL [37]. This would equate to loosing 94.47 mL O_2 and 70.5 g Hb in a 470 ml blood donation, which has been postulated to affect variables such as $\dot{V}O_{2max}$ decreasing [16,19,20]. Therefore, it seems prudent for all studies that involve blood donation and exercise together to collect relevant haematological variables, such as Hb, Hct, RBC's. Yet, various authors neglected to report these values fully.

One issue worth noting is that only one study [16] controlled for hydration adequately, in the other studies it was not controlled properly or sometimes not mentioned at all. It is important as; dehydration (or even over hydration) affects blood-based readings. Hct and Hb will alter according to hydration levels. It is thus suggested that for maintaining hydration (in a clinical setting) fluid intake should be 35 $ml.kg^{-1}$ for 18–60 yrs and 30 $ml.kg^{-1}$ for over 60 yrs [38]. Therefore, the variance in fluids could be attributed to the change in fat-free mass and adiposity that can alter with age, as fat-free mass is ~ 70–80% water [39] and muscle mass diminishes with age [40] particularly if there is inactivity.

Although in many of these studies Hb, Hct and RBC's were the considered blood variables only two studies [20,34] considered the iron storage protein, ferritin concentration. Approximately 25% of the body's iron is stored as ferritin (NHS, 2017) and repeated blood donations decrease its levels, as shown in the Meurrens, et al. [20] study where 3 blood donations (taken 3 months apart from one another) were observed. The results saw ferritin prior to any donation at 55 ± 8 μg/l to then reduce to 40 ± 6 μg/l ($p < 0.01$) before the second donation, and a value of 41 ± 5 μg/l ($p < 0.001$) was observed prior to the third donation, It is not known in some of the studies if the participants are new or regular donors, iron status according to ferritin levels could aid in indicating normal levels (41–400 μg/l and borderline 16–40 μg/l (NHS, 2017)). This might be important to help provide insight on differing results between studies.

Further consideration when comparing studies should be given to the heterogeneous mix of participants, for example a study declares the participants to be trained athletes [16], but fails to mention what their training history was, while another study reported to have physically active and healthy participants [18], but no quantification of this was provided. Quantification could come in the form of analysing individual $\dot{V}O_{2max}$ results, for example by using ACSM [41] guidelines and observing the combined values for Gordon and colleagues [16] study reveals the participants were of excellent fitness (in terms of aerobic power) and the participants for Burnley [18] and associates were of good fitness. Thus, greater transparency is required and authors should provide this type of information.

The only study in this systematic review to use a randomized controlled trial (sham bleed) was that of Meurrens and colleagues [20] and hence this study was not pooled into the relevant forest plots and subject of the meta-analysis. Thus, it can be thought with confidence that measures to reduce bias were adopted to test the effectiveness of the intervention (blood donation)

in this study, while the other studies involved opted not to operate in this manner. The Meurrens [20] group blinded the participants and they all went through the process of donation, however some were not aware that they had not donated blood (sham). The positive results that are associated with this type of approach should be appreciated. Blinding helps to ensure impartiality and that bias is eliminated or at least reduced.

Further analysis showed that with the exception of HR, Meurrens and associates [20] would have had clear statistical significance across all variables and very large effect sizes with for example a Cohen's *d*, -1.93 for VO_{2max} and -6.01 for Hb., if they were included in the meta-analysis.

However, the pooling of robust high quality randomized controlled studies with lower quality uncontrolled designs is not recommended and would possibly lead to type I errors.

Conclusion

In summary, it is clear from this systematic review that there are not enough high-quality papers to draw any definitive conclusions, regarding cardiorespiratory outcomes following BD. Although we have an understanding from individual study results of the raw data that $\dot{V}O_{2max}$ is reduced from donation, pooled results show that $\dot{V}O_{2max}$ is indeed not significantly reduced from blood donation 24–48 hrs post donation. Additionally sub-maximally data is limited for substantial comparatives. Moreover, there is a need for further exploration regarding post blood donation health and well-being in athletes and non-athletes alike. Generally the papers evaluated in this instance portrayed the participants as generally active/athletic and additionally they were all young, but this does not necessarily reflect the general public (who donate blood), thus this avenue needs to be investigated further encompassing a more reflective cohort of the people. To conclude, pending superior quality studies the current evidence cannot provide definitive answers regarding cardiorespiratory variables, but it can report the reductions in the blood variables investigated, which potentially impact sporting ability.

Supporting information

S1 Table. Search terms and hits table.
(DOCX)

S2 Table. PICO table.
(DOCX)

S3 Table. Variables extracted for analysis.
(DOCX)

S4 Table. Excluded papers information.
(DOCX)

S5 Table. PRISMA checklist.
(DOC)

Author Contributions

Conceptualization: Diane Maria Johnson.

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Validation: Diane Maria Johnson.

Writing – original draft: Diane Maria Johnson.

Writing – review & editing: Dan Gordon.

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Appendix 2. Publication – Influence of Whole Blood Donation on Peak Power Outputs across a 96-hr Period

Official Journal of the American College of Sports Medicine

A-37 Free Communication/Poster - Cardiovascular

Wednesday, May 29, 2019, 7:30 AM - 12:30 PM
Room: CC-Hall WA2

163 Board #1 May 29 9:30 AM - 11:00 AM

Influence of Whole Blood Donation (~ 470ml) on Peak Power Outputs Across a 96hr Period

Diane Johnson, Justin Roberts, Dirk Dugdale, Henry Hodgkins, Ronique Gordon, Madie Rowland, Ellen Lockwood, Viviane Merzbach, Antonio Femminile, Flora Veres, Dan Gordon. *Anglia Ruskin University, Cambridge, United Kingdom.*

(No relevant relationships reported)

PURPOSE: Previous studies investigating the effects of blood donation (BD) on power output (PO) concerning all out sprints appear elusive, thus this study determines the effects of BD (~ 470ml) over a period of up to 96 hours on power output during four repeated 15s sprints. **METHODS**: Following local institutional ethical approval 11 participants, 8 males (age 30.3 ± 13.1 years, mass 85.6 ± 10.5 Kg, height 177.8 ± 6.9 cm) and 3 females (age 34.7 ± 10.3 yrs, mass 69.1 ± 11.6 Kg, height 171.2 ± 9.1 cm) volunteered to participate. Testing was conducted in the morning over a two week block with a rest week between testing phases. Week one (W1) was pre-BD and week two (W2) was post BD. Visit one on W1 established haematological levels and $\dot{V}O_{2max}$. Subsequent visits during W1 were for Sprint Interval Testing (SIT),with a resistance of $0.07 kg \cdot kg^{-1}$of body mass, with 90s of unloaded pedalling prior to each effortusing a Lode Excalibur Sport cycle ergometer. W2 was identical except the participant donated blood on visit 1 post haematological testing only. Additionally throughout testing VO_2, cardiac output, stroke volume and heart rate using thoracic impedance cardiography, while Near Infrared Spectroscopy measured O_2delivery at the muscle. **RESULTS**: Pre-BD (Hb=14.61 ± $0.72 g \cdot dL^{-1}$, Hct=42.91 ± 2.12%) compared to post BD of day 1 (Hb=13.57 ± $0.97 g \cdot dL^{-1}$, Hct=39.91 ± 3.02%) reduced by 7.09%. Hb percentage decreases continued over days 1-2 (2.68%) and days 2-3 (2.55%), a percentage increase (1.98%) occurred between days 3-4, values yielded significance in all cases ($P< 0.0001$). ΔPPO (W) pre-BD on day 1 (46.2 ± 180.3), 2 (45.5 ± 150.7), 3 (18.7 ± 74.7) and 4 (31.9 ± 99.4) compared to post BD on day 1 (170 ± 153.0), 2 (156.6 ± 125.4), 3 (88.7 ± 106.6) with the exception of day 4 (84.9 ± 72.7) all revealed significance ($P= 0.006$, $P= 0.005$ and $P= 0.04$ respectively). There was no interaction for time for either pre or post BD periods for eitherΔPPO or mean PO ($P>0.05$). **CONCLUSIONS**: The data suggests that BD has significant effects on Hb and Hct up to 96 hours post BD. ΔPPO was also significantly influenced up to 72hours post BD, this is potentially attributed to a down regulation of the resynthesis of PCr between sprints from decreased O_2availability. These findings have significant implications for individuals wishing to undertake sprint interval-based exercise post BD.

Appendix 3. Publication – The Effect of a Standard Blood Donation on Oxygen Uptake

The effect of a standard blood donation on oxygen uptake

We read with interest the systematic review by Van Remoortel and colleagues,[1] concluding that a standard whole blood donation reduces hemoglobin (Hb) levels, VO_{2max}, and maximal exercise 24 to 48 hours after donation. We generally support the conclusions, but believe that some results are possibly skewed, particularly regarding VO_{2max} (24-48 hr after donation).

The article identifies that VO_{2max} decreases by 7% from donating a unit of blood. The forest plot used in the meta-analysis of this variable uses blood donations ranging from 400 to 500 mL. It is uncertain as to how this range of data in one forest plot could provide a robust analysis. For example Ekblom and coworkers[2] withdrew 400 mL and Birnbaum and coworkers[3] withdrew 500 mL (although the review incorrectly states that the amount is 450 mL in their Table 1). Thus, given that on average 100 mL of blood carries 15 g of Hb, if the mean values of Hb are used from these two studies which are 14.9 and 15.3 g/dL, respectively, and calculated accordingly per 100 mL of blood volume lost in donation then the values equate to 59.6 and 76.5 g Hb, equating to a 28.4% difference due to volume and as such will likely have differing effects on VO_{2max}. Indeed the decrease for VO_{2max} is 6.4% for Ekblom[2] and 9.8% for Birnbaum.[3] We contend that using studies of similar blood volumes would have provided a more robust analysis.

Moreover, it is felt that the previously mentioned article by Ekblom and colleagues[2] should not be in this forest plot, as the testing period (part of the Group II work that involved sequential venisections) was 3 days after each blood loss and not the 24- to 48-hour period as intimated in the forest plot. If indeed this period was to be included then it could be argued that the data from Gordon and coworkers[4] should be in the plot too, as their time span was 48-72 hours, thus deeming the article eligible.

Additionally we would question the inclusion of the study by Foster and colleagues,[5] partly as the donation amount is 500 mL (as previously discussed), but also due to additional confounding issues. First, it does not appear clear if the measure of pre– and post–blood donation $VO_{2max/peak}$ values were obtained from the first graded exercise test (GXT) or the second GXT (when the talk test [TT] is used). Second, the fact that it involves a TT (a method Foster and colleagues argue is associated with identifying ventilatory threshold) while possibly gaining $VO_{2max/peak}$ results compromises ecologic validity, as talking while using open circuit spirometry will affect the fidelity in expired air samples. Additionally, as the participants are exercising to volitional exhaustion, the validity of the test outcome is raised, as there is a compromise between talking and concentrating on the exercise component.

Finally there is an error where the amount of participant numbers is incorrectly stated (10 should be 11) for one of the studies (Burnley and colleagues[6]) represented in the discussed forest plot. The impact of this is that the statistics are wrong, such as affecting the confidence intervals.

We would very much welcome a response from the authors in this regard.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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Justin Roberts
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Cambridge, UK

REFERENCES

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Appendix 4. Chapter 6 Ethical Approval

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Diane Johnson
[redacted]

06th January 2017

Dear Diane,

Project Number:	FST/FREP/16/610
Project Title:	The impact of body stature on haematological parameters post blood donation.
Principal Investigator:	Diane Johnson

Thank you for supplying revisions to your application for ethical approval, as requested by the Faculty Research Ethics Panel (FREP) following its meeting on 19/12/2016.

I am pleased to inform you that your application has been approved by the Chair of the Faculty Research Ethics Panel under the terms of Anglia Ruskin University's *Research Ethics Policy*. Approval is for a period of three years from 06/01/2017.

It is your responsibility to ensure that you comply with *the Code of Practice for Ethical Approval at Anglia Ruskin University*, and specifically:

- The Participant Information Sheet and Participant Consent Form should be on Anglia Ruskin University headed paper.
- For online surveys it is recommended that the researcher turns off the IP logging software to ensure secure communication between the survey taker and server.
- The procedure for submitting substantial amendments to the committee, should there be any changes to your research. You cannot implement these changes until you have received approval from FREP for them.
- The procedure for reporting adverse events and incidents.
- The Data Protection Act (1998) and any other legislation relevant to your research. You must also ensure that you are aware of any emerging legislation relating to your research and make any changes to your study (which you will need to obtain ethical approval for) to comply with this.
- Obtaining any further ethical approval required from the organisation or country (if not carrying out research in the UK) where you will be carrying the research out. Please ensure that you send the FREP Secretary copies of this documentation.
- Any laws of the country where you are carrying the research out (if these conflict with any aspects of the ethical approval given, please notify FREP prior to starting the research).

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- Any professional codes of conduct relating to research or research or requirements from your funding body (please note that for externally funded research, a project risk assessment must have been carried out prior to starting the research).
- Notifying the FREP Secretary when your study has ended.

Information about the above can be obtained on our website at:

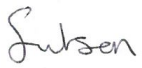
http://web.anglia.ac.uk/anet/rdcs/ethics/index.phtml/ and or
http://www.anglia.ac.uk/ruskin/en/home/faculties/fst/research0/ethics.html

Please also note that your research may be subject to random monitoring by the Committee.

Please be advised that, if your research has not been completed within three years you will need to apply to our Faculty Research Ethics Panel for an extension of ethics approval prior to the date your approval expires. The procedure for this can also be found on the above website.

Should you have any queries, please do not hesitate to contact me. I wish you the best of luck with your research.

Yours sincerely,



Sam Wilson

Secretary to the Faculty Research Ethics Panel (FREP)
Faculty of Science and Technology
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Email: FST-Ethics@anglia.ac.uk

Cc Dan Gordon (supervisor)

Appendix 5. Chapter 7 & 8 Ethical Approval

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Diane Johnson
[redacted]

02nd March 2018

Dear Diane,

Principal Investigator: Diane Johnson
FREP/DREP number: FST/FREP/17/727
Project Title: Influence of a standard UK blood donation on moderate and heavy intensity kinetics and beyond VO2max (~170%)

Thank you for supplying revisions to your application for ethical approval, as requested by the Faculty Research Ethics Panel (FREP) following its meeting on 21/02/2018

I am pleased to inform you that your ethics application has been approved by the Faculty Research Ethics Panel (FREP) under the terms of Anglia Ruskin University's Research Ethics Policy (Dated 8 September 2016, Version 1.7).

Ethical approval is given for a period of 3 years for doctorate students from 02/03/2018. If your research will extend beyond this period, it is your responsibility to apply for an extension before your approval expires.

It is your responsibility to ensure that you comply with Anglia Ruskin University's Research Ethics Policy and the Code of Practice for Applying for Ethical Approval at Anglia Ruskin University available at www.anglia.ac.uk/researchethics including the following.

- The procedure for submitting substantial amendments to the committee, should there be any changes to your research. You cannot implement these amendments until you have received approval from FREP for them.
- The procedure for reporting accidents, adverse events and incidents.
- The Data Protection Act (1998) and General Data Protection Requirement from 25 May 2018.
- Any other legislation relevant to your research. You must also ensure that you are aware of any emerging legislation relating to your research and make any changes to your study (which you will need to obtain ethical approval for) to comply with this.
- Obtaining any further ethical approval required from the organisation or country (if not carrying out research in the UK) where you will be carrying the research out. This includes other Higher Education Institutions if you intend to carry out any research involving their students, staff or premises. Please ensure that you send the FREP copies of this documentation if required, prior to starting your research.

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- Any laws of the country where you are carrying the research and obtaining any other approvals or permissions that are required.
- Any professional codes of conduct relating to research or requirements from your funding body (please note that for externally funded research, where the funding has been obtained via Anglia Ruskin University, a Project Risk Assessment must have been carried out prior to starting the research).
- Completing a Risk Assessment (Health and Safety) if required and updating this annually or if any aspects of your study change which affect this.
- Notifying the FREP Secretary when your study has ended.

Please also note that your research may be subject to monitoring.

Should you have any queries, please do not hesitate to contact me. May I wish you the best of luck with your research.

Yours sincerely,

Sam Wilson

Secretary to the Faculty Research Ethics Panel (FREP)
Faculty of Science and Technology
Tel: 01245 683917 or 0845 196 3917
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cc. Dr Dan Gordon (1st Supervisor)

NHS
Blood and Transplant

11th December 2017

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Professor Peter Bright
Chair: Faculty of Science and Technology Research Ethics Panel
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Dear Professor Bright

I am writing in response to Diane Johnson's email from 23/11/2017 that relates to the next stage of her PhD research work: "...my participants as part of the experimental intervention would be required to exercise (24, 48, 72 and 96hrs) after donating blood. My details will be going to Anglia Ruskin ethics board for approval, but if I could get an agreement from you prior to this the it would be appreciated and as you requested before I am involving you in my next study at an early stage to hopefully ensure smooth running. As before HRA or NHS REC should not be required."

I am a Consultant in Donor Medicine and lead the research subgroup of the BSCARE committee (Blood Supply Clinical Audit, Research, and Effectiveness) at NHS Blood and Transplant. I have discussed the proposed project with colleagues including
Dr Nicky Anderson (Associate Medical Director, Blood Donation) and Donna Cullen (Nurse Consultant - Practice Development).

The proposed study will include blood donors after they have left NHSBT premises. This research is not an NHSBT project and unfortunately no NHSBT resources will be available to support it.

However, we have no objection to this project proceeding but have some suggestions:

- The donors in this study should have a clear 24 hours post donation before doing any vigorous exercise. We can provide the investigators with some information for the blood donors.
- The donors should get specific instructions about rehydration etc after donation.
- We should be informed of any adverse or vasovagal event - such events might prevent donors from donating again.

Please feel free to contact me for any further information.

Yours sincerely

Dr Gavin Cho
Consultant in Donor Medicine

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