## Effect of an acute blood donation on oxygen uptake kinetics in moderate and heavy domains over a period of 96 hours

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### Conflict of Interest

The authors state that they have no conflict of interest or financial involvement with this manuscript.

**Blood donation and oxygen kinetics**

## Abstract

**Background**

Studies determining the effects of blood donation (BD) on oxygen uptake kinetics are limited. This study aims to ascertain the effects of BD (~ 470ml) over a period of 96 hours on oxygen uptake kinetics in moderate and heavy exercise domains.

**Study Design and Methods**

Twelve participants (9 males and 3 females; 31.1 ± 11.7 years, mass 79.9 ± 12.8 kg, height 175.5 ± 7.5 cm), completed 4 consecutive days (24-96 h) of moderate and heavy V̇O2 on-kinetics trials pre BD and post BD. Visit one (0 h), pre BD established haematological levels, V̇O2max and Gas Exchange Threshold (GET). Subsequent visits comprised two 6-minute moderate (∆ 50 % rest-GET) and 1 heavy (∆ 20 % GET-V̇O2max) trial. Post BD 0 h the participants donated blood post haematological testing only.

**Results**

Despite non-significances for V̇O2 amplitude, time constant-2 (tau2) for V̇O2 showed significant decreases at 24 and 48 h, and tau3 significant increases at 72 and 96 h pre to post BD (P < 0.05). Haemoglobin (Hb) values reduced (P < 0.05), pre (14.48 ± 0.16 g.dL-1) to post BD (13.47 ± 0.66 g.dL-1). Hb significantly decreased at 24, 48, 72 and 96 h compared to 0 h post BD (P < 0.05).

**Conclusion**

BD has no effect on V̇O2 amplitude, but time-based components show sensitivity to reduced circulating O2, with a decreased PO2 a slower O2 exchange across the blood myocyte barrier could result in altering O2 kinetics.

### Key words

Blood donation (BD), oxygen uptake kinetics, Haemoglobin (Hb)

## Introduction

In the UK, for the National Health Service Blood and Transplant (NHS Blood and Transplant) to meet current life-saving demands 400 new blood donors are needed daily,1 while maintaining and recruiting new donors depends on the altruism of the general public. General advice is to avoid strenuous exercise the day of BD, beyond this time frame little is known regarding the effects of donation on exercise. Experimental work in this field to date has mainly concerned maximal oxygen uptake (V̇O2max) as opposed to sub-maximal exercise.2 It is possible this lack of research contributes to the difficult task of keeping or recruiting donors as 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.3 Guidance for high-performance competitive athletes suggests a marginal decrease in exercise tolerance may be noticed up to a week post BD,4 while prudence should be executed immediately after BD. 4-5 Although, the majority of donors are not high-performance but rather un-trained or moderately physically active. Yet despite there being various forms of advice across different countries, it appears there is currently no scientific evidence supporting the avoidance of exercise immediately following donation.6

At the commencement of exercise O2 intake (V̇O2) and CO2 output (V̇CO2) rise quickly with the achievement of steady state dependent on exercise intensity, with O2 supply meeting demand at moderate intensities of exercise more readily than at heavy or severe intensities. 7-8 A square-wave transition occurs in adenosine tri-phosphate (ATP) demand to sustain work at the onset of exercise and the desired rate of O2 uptake is delayed reaching steady state as supply does not immediately match demand. 7-8 At moderate and heavy intensities steady state is not achieved until 2-3 minutes7 and ~6-8 minutes8 respectively, thus creating an O2 deficit, describing the differences of V̇O2 and steady state values post exercise on-set. Furthermore, the transit of O2 across the muscle is a function of its diffusion (DO2) and saturation9 as reflected through use of near infrared spectroscopy.

There are three phases associated with O2 uptake kinetics. Phase I concerns cardio-dynamics, phase II muscle V̇O2 and phase III steady state attainment. Phase I is swift due to the immediate increase in V̇O2 from the prompt rise of cardiac output (Q̇) pushing blood through the lungs with little variation in the arterio-venous oxygen difference (a-vO2diff)10 as blood returning to the lung has not been exposed to augmented O2 extraction via the muscle.11 Phase II comprises the exponential rise of V̇O2, as Q̇ and a-vO2diff will increase, reflecting the kinetics of muscle O2 uptake.11 Phase III occurs at steady state and includes the slow component when exercising beyond gas exchange threshold (GET) and comprises a further increase in V̇O2. V̇O2 kinetics are a function of cardio-respiratory fitness, in-particular V̇O2max, suggesting those who are less aerobically fit may be sensitive to changes in Hb following donation similar to well-trained counter-parts.7

Maximal oxygen uptake (V̇O2max) shows a significant decrease proportional to the change in Hb following BD. 2,12-14 Yet, the majority of exercise undertaken by both recreational and competitive athletes is completed in the moderate and heavy exercise domains. To date only two studies have examined the effect of BD on oxygen uptake kinetics with mixed results8,13 while neither study investigated beyond 24 h post BD. Therefore, this study examined the effects of a standard BD (~470ml) on oxygen uptake kinetics in the moderate and heavy intensity exercise domains across a 96 h time period in a recreationally active population. It was hypothesised that VO2 kinetics would show a significant change in the moderate and heavy domains following BD after 24 hours and across the subsequent 96 hours.

## Method

### Participants

A statistical power calculation determined optimal participant numbers (n=6) by using previous research14 results to compare two Hb means pre BD (14.9 ± 0.8 g.dL-1) and post BD (14.0 g.dL-1), using a power of 80 % and critical value for normal distribution set at 1.96. Subsequent to ethical approval (Anglia Ruskin University, Faculty Research Ethics Panel) and NHS Blood and Transplant approval, 12 participants of differing fitness levels (9 males and 3 females; 31.1 ± 11.7 years, mass 79.9 ± 12.8 kg, height 175.5 ± 7.5 cm) volunteered. All procedures conformed to the World Medical Association Declaration of Helsinki (2013)15 and volunteers provided written informed consent.

Inclusion criteria were volunteers were able to donate blood and be regularly physically active, defined as >150 min of moderate intensity aerobic activity per week. Additionally, participants should have no contra-indications to exercise and could exercise at various intensities. Participants were requested to avoid strenuous exercise 24 hours prior to any test and were additionally asked to arrive hydrated having eaten a balanced meal 2-3 hours prior to all tests.

### Study Design

Testing occurred between 7.30am and 11am to reduce diurnal variation over two weeks, with a gap of one-two weeks between each condition. Week one and week two were pre and post BD respectively. Visit one (day 0) of pre BD established baseline levels for haematology, blood pressure and V̇O2max. Subsequent visits pre BD were for moderate and heavy domain testing, with post BD being almost identical with the exception of donating blood (~470 ml) on day 0 of week two post Hb testing. Figure 1 depicts the activity according to days.

### V̇O2max Protocol

Exercise tests were performed on a pre-calibrated electronically controlled cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). Seat and handlebar heights were established on the first visit and subsequently maintained. Participants selected a constant cadence (75-95 rpm), that they maintained throughout all subsequent trials. The test increased at a rate of 0.42 W.s-1, commencing at 100 W (males) or 50 W (females) for the first 60 s and concluded when the cadence declined by > 5 rpm from that which was self-selected or when volitional exhaustion ensued. V̇O2max was confirmed by ∆ V̇O2 over the final two consecutive 30 breath sample periods.16 A pre-calibrated metabolic cart (Cortex MetaLyzer 3B, UK) recorded gas exchange variables (V̇O2, V̇CO2) along with minute ventilation (V̇E) and respiratory exchange ratio (RER) on a breath-by-breath basis for all exercise trials. Heart rate (HR) was recorded continuously (Polar 810s, Electro, Kemple, Finland).

### Gas Exchange Threshold (GET)

GET was determined by combining three methods,17 excess CO2,18 ventilatory equivalents19 and V-slope.20

### On-Kinetics Trials

These 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. Two transitions were applied in the moderate domain ∆ 50 % (50 % of the difference between rest and GET) with a third in the heavy domain ∆ 20 % (20 % of the difference between GET and V̇O2max). Figure 2 provides an overview of the testing for the domains and the procedure.

### 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 prior to all exercise tests. Additionally, each participant made a standard blood donation (~470 ml) at a National Blood Transfusion Service within a few hours of haematological status being established at 0 h of post BD testing.

### Near Infrared Spectroscopy (NIRS)

The Portamon (Artenis Medical Systems) observed changes in oxy and de-oxy Hb/myoglobin during all trials. 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 confirming 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).21

The area was clean-shaven and the Portamon was placed in the same position at every test, on the right gastrocnemius muscle, on the right-hand side of the belly, at the point of the largest circumference. The skin surrounding the Portamon was marked with permanent pen to enable duplication for further testing. The probe was covered by dark material to avoid corruption from ambient light and elastic bandage held the device in place. A baseline value was recorded with the participant’s leg in the extended position for 2 minutes prior to the commencement of cycling and monitoring continued throughout all exercise. Variables recorded were oxygenated Hb (O2Hb), de-oxygenated Hb (HHb), total haemoglobin (tHb) and tissue oxygenation saturation index (TSI%) (= O2Hb-HHb), with a frequency of 10 Hz and expressed relative to baseline.

### Hydration Status

Participants arrived hydrated having consumed 500 ml of water an hour prior to testing and 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).

### Data Analysis

The V̇O2 (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 ≥ 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.

V̇O2(t) = V̇O2(bsl) + a1⊕(1 − e -(t - TD1)τ1) + a2⊕(1 − e –(t - TD2)/τ2) + a3⊕(1 − e − (t – TD3)/τ3)

Where V̇O2(t) denotes the total velocity of oxygen utilization at any given time (t). V̇O2(bsl) denotes the average velocity of oxygen uptake throughout the baseline recording (4 minutes). The other components denote respectively, the amplitude (a); the rise in V̇O2, time delay (TD); the time taken for the adjustment of V̇O2 and time constant (τ); the time taken for V̇O2 to attain the amplitude.8 Furthermore the equation contains exponential terms/functions for example (1 − e -(t - TD1)τ1), with e representing the base for the natural logarithm.22 Following modeling of the moderate trials the values were combined and averaged. Limits of agreement assessed bias and 95 % confidence intervals. With no proportional bias detected a one sample t-test was used for further indication of data reliability.

The NIRS data was initially smoothed with a 1 s moving average and to further reduce the “noise” 10 s rolling averages were applied. 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. As with breath-by-breath data, the moderate trials were combined and subsequently averaged.

### Statistical Analysis

Following tests for normality (Shapiro-Wilk) a combination of repeated measures t-tests, one-way repeated measures ANOVA for differences in trial/week 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 (ES) were also considered; Cohen’s d was adopted for t-tests and partial eta squared (p2) for ANOVA results. Cohens d is evaluated as 0.8 - > 2.0 (large), 0.3 - 0.5 (medium) and < 0.2 (small).23 Partial eta squared is evaluated as 0.14 (large), 0.06 (medium) and 0.01 (small).24 A level of statistical significance was set at P < 0.05 and statistical analysis was accomplished using SPSS version 20 (SPSS, Chicago, IL).

## Results

### On-Kinetics Trials Responses

When comparing moderate to heavy trials significant differences can be seen in most components (Table 1 and 2). In the moderate trial, tau2 (time taken to attain 63% of the rise in V̇O2) showed significant differences when comparing pre to post BD values at 24 h (ES = 1.10 (CI, -4.19, 4.14)) and at 48 h (ES = 0.86 (CI, -3.81, 3.80)). This was also the case for tau3 at 72 h (ES = 1.20 (CI, -9.94, 5.88)) and at 96 h (ES = -0.75 (CI, -7.30, 10.83)) and for TD3 at 72 h (ES = 1.27 (CI, -12.03, 11.48)) and at 96 h (ES = 0.98 (CI, -8.74, 12.56)). In the heavy trial, tau1 when comparing pre to post BD values at 72 h (ES = 0.76 (CI, -0.37, 1.92)), this was also the case for TD2 at 72 h (ES = 0.76 (CI, -1.04, 2.60)), tau3 at 24 h (ES = -0.69 (CI, -9.79, 7.84)) and TD3 at 24 h (ES = 0.66 (CI, -13.95, 13.64)) and at 48 h (ES = -0.37 (CI, -18.04, 14.09)).

Two-way ANOVA results in the moderate domain showed significance between pre to post BD regarding tau1 (P = 0.035, ES= 0.34 (CI, 0.06, 1.46)),tau3 (P = 0.019, ES= 0.41 (CI, -19.36, -2.14)), TD2 (P = 0.035, ES= 0.34 (CI, 0.10, 2.32)) and TD3 (P = 0.028, ES= 0.37 (CI, 2.04, 29.52)). One-way ANOVA results for the moderate domain showed significance in a3 post BD (P = 0.010, ES = 0.29), with pairwise significance between 72 and 96 h (P = 0.025, (CI, 0.10, 2.32)), while tau3 was significant (P = 0.041, ES= 0.22) post BD, but with no pairwise significance between days. Two-way ANOVA results in the heavy domain showed non-significance across all variables with a2 post BD 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).

### Haematological (Hb and Hct) Responses

Figure 3 shows the mean Hb and Hct values across each condition, with pre BD (14.48 ± 0.16 g.dL-1) and post BD (13.47 ± 0.66 g.dL-1). Comparisons across time points 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 same time points for Hb, with the decrements of 7.2, 2.3, 2.6 % and an increase of 1.9 % when compared to baseline.

### 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.

TSI decreases (Figure 4) 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. Significant decreases were observed from moderate to heavy intensity: 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). Pre to post BD revealed 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).

Resting baseline O2Hb 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, ES *=* 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, ES *=* -0.57 (CI-7.31, 3.88)) (Figure 5). HHb showed a cumulative nature as intensity increased from baseline unloaded cycling, with a significant increased from moderate to heavy intensity in pre BD at 48 h only (*P* =0.049 (CI, -7.92, -0.02). Significant differences between pre BD and post BD at 24 h(*P* = 0 .028, ES= 0.65 (CI, -5.28 -0.45)) and at 96 h (*P* = 0 .048, p2 = 0.58 (CI, -3.76 0.03)). Similarly to HHb, tHb increases from baseline unloaded cycling (Figure 6), with significant increases 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)). Pre to post BD showed a significant increase at 48 h for moderate intensity 2.28 ± 5.19 v 7.82 ± 4.81 µM.cm (*P* = 0.018, ES *=* -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, ES *=* -1.25 (CI-6.04, 2.52)).

## Discussion

This work was undertaken to determine the effects of BD (~ 470ml) over a period of up to 96 hours on oxygen uptake kinetics in the moderate and heavy exercise domains whilst reviewing cardiorespiratory and Hb changes in the muscle in a population of recreationally active participants. A group who contribute to BD and whose oxygen uptake kinetics may be sensitive to a decrease in Hb as a function of their level of aerobic fitness.

The 7.3 % reduction in Hb 24 h post BD resulted in significant decrease during phase II for tau2 in the moderate domain at 24 h compared to pre BD. In the heavy domain during phase III a significant increase occurred for tau3, although a significant decrease for TD3. Although in general agreement with Gordon and colleagues,8 who found no significant changes in any areas 24 h post BD, these particular findings are conflicting. A confounding factor could be the different training statuses of the participants. The V̇O2max values were 44.15 ± 8.31 ml.kg-1.min-1 for this study and 53.0 ± 4.1 ml.kg-1.min-1 for Gordon and colleagues,8 indicating that the cohort for the current study lacked aerobic power, indeed faster kinetics are associated with higher compared to lower V̇O2max values.25 Additionally, V̇O2 is a function of O2 delivery (Hb, Q̇, SaO2) and mixed venous partial pressure of O2 (V̇O2 = DO2 x PV̇O2), with convection and diffusion processes providing O2 transport or indeed limiting O2 uptake. Consequently, a high V̇O2max relies on efficient O2 delivery (Q̇) and utilisation to the engaged muscle. Individuals with enhanced capabilities to utilise O2 during sub-maximal exercise enable O2 supply to meet demand with rapidity, so sparing finite anaerobic contributions, thus a high V̇O2max services fast V̇O2 kinetics, leaving oxidative phosphorylation as the prime energy provider and limiting slow component development if at a heavy intensity. Thus, participants with higher V̇O2max would have faster O2 kinetics, as highlighted in tau2 between Gordon, et al.8 and this study pre BD (27.6 ± 7.2 s v 32.9 ± 9.4 s) respectively.

The time constant (tau) computes the degree of V̇O2 increase. For every multiple of tau V̇O2 rises by 63 % of the difference between the prior time value and the steady state requirement.26 Hence, post BD less time was taken for V̇O2 to reach 63 % of the total phase II rise in moderate exercise implying O2 availability did not influence O2 kinetics. However, more time was taken to reach 63 % of the amplitude of phase III in the heavy domain, implying O2 availability influenced O2 kinetics. Although anaerobic pathways dominate in this domain it is possible the relative contribution from aerobic sources was affected by the reduced O2 circulating to the working muscles. It is known that tau during phase II for V̇O2 and phosphocreatine (PCr) for both moderate and heavy intensity exercise reflect each other, with V̇O2 rising and PCr falling to their respective steady states.27

The PCr reaction depends on the degree of ATPase activity (catalysing ATP to/from ADP and Pi) and creatine kinase (CK) the rate limiting enzyme for PCr kinetics.28 Thus, for tau2, although PCr hydrolysis itself is not dependent on O2 availability, if there is a decrement in circulating O2 from BD, this requires anaerobic metabolism to be raised. Additionally, it is conceivable that PCr was used more rapidly and with large rises in ADP inhibiting the CK forward flux (to ATP) a speeding of V̇O2 kinetics occurred. With the rate of PCr catabolism increased, tau2 is attained more rapidly. However, it should be noted, despite the speeding of tau2 post BD the TD did not change, implying post speeding there was a slowing of the PCr kinetics reflecting changes in the V̇O2 response. Additionally, post BD tau3 significantly increased, implying a slowing in the V̇O2 response; delaying steady state attainment, again possibly due to the role of CK in oxidative phosphorylation.28

However, in circumstances where circulating O2 is reduced (BD), myoglobin could potentially act as a buffer as the O2 is instantly available, augmenting intracellular O2 transport. During hypoxia (12 % O2) diffusional conductance and offloading are elevated, myoglobin-facilitated O2 flux increases and intracellular resistance reduces.9 Myoglobin desaturation is rapid, within 20 s of exercise onset at 50 % of V̇O2max a partial desaturation can be noted,9 however the time of phase I and II (24.88 ± 5.38 s in this study post BD) are also rapid, thus maybe sufficient time to facilitate the speeding of tau2.

A further 2.3 % reduction in Hb 48 h post BD resulted in a significant reduction of tau2 in the moderate domain. In the heavy trial a significant increase was observed for TD3. Of note, tHb saw significant increases 48 h pre to post BD across both domains. During phase III V̇O2 remained unaffected by BD therefore it is conceivable that in order for tHb to increase, O2 extraction at the muscle also increased, possibly the result of enhanced O2 diffusional conductance29 reducing diffusion distance and increasing surface area for O2 exchange.30 Also, a blood flow redistribution from muscles that are less engaged is possible. Therefore, although the muscle maybe hypoxically threatened, the redistribution of blood from less active tissues prevents anaerobic metabolism dominating and moving beyond the so-called tipping point.31 At 72 h post BD another Hb reduction (2.1 %) resulted in a significant increase in the moderate domain for tau3 and a significant decrease for TD3, whilst in the heavy domain tau1 and TD2 showed significant decreases. However, NIRS showed non-significant increases similarly to the amplitude of V̇O2 implying that despite the decreased circulating O2 V̇O2 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 tau3 and a significant decrease for TD3, as seen in the previous collection point.

Limitations within this study concern the lack of female participants and the age range of the group. Furthermore, this study used a fixed BD of ~470 ml for all participants irrespective of body stature. Also, participants were not blinded to BD and the use of a sham donation could provide a control condition.

To conclude, with non-significant effects found on V̇O2 amplitude despite a significant Hb fall up to 96 h post BD, it is conceivable that (recreational) athletes could donate blood as it will not affect their sub-maximal exercise capacity and return to this form of exercise within 24 h. Future work should consider the impact of larger standard blood withdrawals (500 ml) and the physiological responses that may occur within the first 24 h period post BD.

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## Table 1

Mean ± SD for moderate oxygen uptake kinetics 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** |
| a1 (V̇O2)  (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 |
| tau1  (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 |
| a2 (V̇O2)  (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 |
| tau2  (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 |
| TD2  (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 |
| a3 (V̇O2)  (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 |
| tau3  (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\* |
| TD3  (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\* |

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

## Table 2

Mean ± SD for heavy oxygen uptake kinetics across a two week time period that represented 24, 48, 72 and 96 h pre and post BD.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **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** |
| a1 (V̇O2)  (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† |
| tau1  (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 |
| a2 (V̇O2)  (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  ††† |
| tau2  (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† |
| TD2  (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 |
| a3 (V̇O2)  (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  ††† |
| tau3  (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††† |
| TD3  (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  ††† |

*\* 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 (Table 1) to heavy kinetics*