ARTICLE TITLE: The effect of a standard whole blood donation on oxygen uptake and exercise capacity: a systematic review and meta-analysis

RUNNING HEAD: The effect of blood donation on exercise

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ABSTRACT

BACKGROUND: Blood is a life-saving product for many people worldwide. Voluntary blood donation serves the demand for blood but there are concerns amongst potential donors about the impact of blood loss on exercise performance. This systematic review aimed to collect the best available evidence of the effect of a standard whole blood donation on aerobic exercise performance.

METHODS: Studies from 6 databases dealing with a standard whole blood donation (400-500 mL) followed by (sub)maximal exercise were retained. The outcomes included exercise-related blood parameters (hemoglobin concentration, hematocrit and red blood cell count) and endurance exercise parameters ((sub)maximal oxygen uptake, peak work rate, and time to exhaustion). Overall effects at different time-points post-donation were investigated by performing meta-analyses and calculating mean differences (and 95% CIs). The GRADE methodology (Grades of Recommendation, Assessment, Development and Evaluation) was used to assess the quality of evidence.

RESULTS: We identified 6237 references and finally included 18 before-after studies of low quality. 24-48 hours after a blood donation, (1) hemoglobin concentration was reduced (7% decrease) until 14 days after the blood donation (4% decrease), (2) maximal oxygen uptake (VO_{2max}) was lower (7% decrease) and (3) a reduction in maximal exercise capacity (10% decrease) was present.

CONCLUSION: The best available evidence indicates that a standard whole blood donation (400-500 mL) leads to small but potentially physiologically important reductions in hemoglobin levels, maximal oxygen uptake and maximal exercise capacity in the first 2 days after the blood donation.

1 INTRODUCTION

2 Blood services rely on voluntary (unpaid) donors and are permanently looking for ways to keep
3 the life-saving blood supply stable. It is therefore important to increase the number of blood
4 donors and retain as many donors as possible. In high-income countries, around 45 million
5 blood donations are collected annually. However, these donations come from only 4% of the
6 population (36 donations per 1000 population).¹

7 One possible explanation is that misconceptions about the effects of blood donation are
8 widespread even among educated people. Reasons for a negative attitude to blood donation
9 that are mentioned by non-donors are laziness, lack of time, fear of needles, inconvenience or
10 lack of knowledge.²⁻⁴ A smaller part of the population indicates that giving blood may have a
11 negative impact on their general health, their immune system or may lead to reduced energy
12 levels.^{3,4} Among this last group, people that perform regular exercises (ranging from leisure-
13 time to professional sports activities) are often afraid of the detrimental effects a blood donation
14 may have on their exercise performance. Indeed, the loss of red blood cells will logically reduce
15 oxygen delivery and may lower the energy production in the muscles. However, it remains
16 unclear to what extent and for how long exercise performance is impacted by a standard whole
17 blood donation.

18 It is therefore important to inform donors about the potential effects of a standard whole blood
19 donation (400-500 mL) on exercise performance. In 2006⁵ and 2010⁶, two reviews were
20 published by experts in the field showing that an acute reduction in hemoglobin concentration
21 has a pronounced effect on exercise responses. Until now, no systematic review (according to
22 the Cochrane methodology⁷) has been conducted about the effects of a 'standard' whole blood
23 donation (400-500 mL) on exercise capacity and aerobic power. Therefore, this systematic
24 review and meta-analyses aimed to collect the best available evidence to analyze the effects
25 of a standard whole blood donation (400-500 mL) on exercise performance.

METHODS

We carried out a systematic literature review according to a predefined protocol.⁸ We planned and reported the systematic review in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA checklist, **Online Resource 1**).⁹

Data sources and searches

A literature search was performed in CENTRAL, MEDLINE (via the PubMed interface), Embase (via Embase.com), CINAHL (using the EBSCOhost interface), Web of Science, PEDro and SPORTDiscus for eligible studies from the time of inception of the database until November 2013. We developed sensitive search strategies for each database including the use of index terms and free text terms (**Online Resource 2**). Where MeSH terms were not used (Web of Science), we identified search terms and all possible synonyms and spelling, and used them in the search strategy. Search yields were exported to a citation program (Reference manager version 12), duplicates were discarded and title and abstract screening was initiated.

Study selection

Experimental and observational studies were eligible for inclusion if they answered to the following PICO question: “Does a standard blood donation (I) influence exercise performance (O) in (recreational) athletes (P) in comparison to no blood donation (C)?”. The review was restricted to original articles published in English, German, French and Dutch. Full texts of potentially relevant articles were reviewed according to the following inclusion and exclusion criteria:

- *Population:* healthy adults performing aerobic exercise at a competitive or non-competitive level were included.
- *Intervention:* a standard whole blood donation (i.e. 400-500 mL), followed by aerobic exercise of light, moderate, vigorous or maximal intensity was included. We excluded interventions with blood loss or blood donations of more than 500 mL. Blood reinfusion

1 interventions or other interventions aimed at replacing the blood volume were also
2 excluded.

- 3 • *Comparison:* no blood donation, followed by exercise of light, moderate, vigorous or
4 maximal intensity
- 5 • *Outcome:* exercise-related blood parameters which are directly linked to anemia
6 (hematocrit, hemoglobin, and number of red blood cells), aerobic power (represented
7 by (maximal) oxygen uptake (VO₂)) and exercise capacity (represented by time to
8 complete a set amount of work and the peak work rate). Outcomes related to strength
9 (e.g. muscle force) or a combination of endurance and strength (e.g. number of push-
10 ups) and other exercise-related blood parameters (e.g. glucose, and blood lactate)
11 were excluded

12 Two reviewers independently performed the title and abstract screening followed by the full
13 text assessment according to these inclusion and exclusion criteria. Disagreements were
14 resolved by discussion. The reference lists of each study included in this review were screened
15 and the first 20 related citations in PubMed were checked to identify additional relevant studies.

16 **Data extraction and quality assessment**

17 Data concerning study and population characteristics, type of intervention and outcome
18 measures were extracted. The methodological quality of included studies as well as the overall
19 level of the body of evidence was assessed using the GRADE approach (Grading of
20 Recommendations, Assessment, Development and Evaluation).¹⁰ GRADE considers
21 limitations in study design of the included studies, inconsistency between the different studies
22 (due to differences in populations, interventions or outcomes), indirectness (of population,
23 intervention or outcome), imprecision and publication bias. Limitations in experimental study
24 designs were analyzed by evaluating the presence of a lack of allocation concealment, a lack
25 of blinding, incomplete accounting of outcome events and selective outcome reporting. The
26 quality of the evidence can be downgraded for each of the previous quality criteria and finally
27 results in a high, moderate, low or very low level of evidence.

Data management

Studies that reported endurance performance outcomes (VO_2 , time to exhaustion, work rate) were subdivided by exercise intensity (i.e. maximal or submaximal (vigorous, moderate, light or very light intensity)) (**Online Resource 3**). To come up with subcategories for submaximal exercise intensity, we first calculated the maximal predicted heart rate ($HR_{\max}$ predicted) using the formula $\langle HR_{\max} \text{ predicted} = 207 - 0.7 \times \text{age} \rangle$.¹¹ Subsequently, the heart rate during maximal exertion ($HR_{\max}$) was subtracted from the study and the relative intensity was calculated ($\%HR_{\max} = (HR/HR_{\max} \text{ predicted}) \times 100$). Studies were then classified according to their intensity ($\%HR_{\max}$), as proposed by Garber et al.¹² In studies where no heart rate data were available, exercise intensity was estimated from the available VO_2 data ($\%VO_{2\max}$).¹²

Meta-analysis

Relevant studies were stratified into subgroups for meta-analysis. Firstly, studies were stratified into two main groups of study designs: 1) controlled before-and-after studies (i.e. intervention group had a blood donation and the control group had sham blood donation) and 2) uncontrolled (i.e. no group that had sham blood donation) before-and-after studies. Secondly, data were stratified into two intervention categories (maximal exercise testing and submaximal exercise testing). The following continuous outcomes were considered for meta-analysis: exercise-related blood parameters (i.e. hemoglobin concentration ([Hb]), hematocrit percentage (Ht %) and number of red blood cell cells (RBC)), aerobic power outcomes (i.e. (maximal) oxygen uptake ($VO_2(\max)$) and exercise capacity outcomes ((peak) workload ($W(\max)$, time to exhaustion or time to complete a set amount of work). Separate random-effects meta-analyses at different time points after the blood donation (0-2 hours, 24-48 hours, 48-72 hours, 7 days, 14 days, 21 days and 28 days) were conducted for these continuous outcome measures when sufficient reported data were available from ≥ 2 studies. Many (before-after) studies with continuous outcomes did not report required individual standard deviations of the assessment before and after the intervention to allow the sampling variance of the effect size to be calculated. In these cases, we checked for the best available

estimates of the before-after correlation value from previous studies in the literature that examined similar outcomes in similar populations.^{7,13} Subsequently, these before-after correlation values were used to estimate the sampling variance of the effect size using the following formula: $v = 2*(1-r)/n + d^2/(2n)$ where v is the sampling variance of the effect size, r is the correlation between before and after-scores and n is the sample size of the group. Due to the lack of before-after correlations, sensitivity analyses were conducted for all scenarios by comparing before-after correlations of 0.2 and 0.9.^{7,13,14} When describing the meta-analyses results and formulating the recommendations, the more conservative results (before-after correlation of 0.2) were used in order to avoid overestimation of results. The effect sizes were reported as mean differences with 95% confidence intervals. Heterogeneity was expressed by the I^2 statistic, which estimates the percentage of total variation between studies that is due to heterogeneity rather than chance. I^2 is calculated from basic results obtained from a typical meta-analysis as $I^2 = 100% * (Q - df) / Q$, where Q is Cochran's heterogeneity statistic and df the degrees of freedom. The following thresholds for the interpretation of I^2 can serve as a rough guide: 0%-40% (might not be important), 30%-60% (may represent moderate heterogeneity), 50%-90% (may represent substantial heterogeneity) and 75%-100% (considerable heterogeneity).¹⁵ Comprehensive Meta-analysis software (version 3) was used to perform meta-analyses. A p-value <0.05 was considered as statistically significant.

RESULTS

Study selection

The systematic literature search resulted in a total of 4121 citations which were scrutinized by 2 reviewers independently. **Figure 1** represents the different processes used in the systematic review. About half of all studies (n=10, 55%) included were published in the past 10 years (2005-2015), and only 4 studies (22%) were performed before 1980. All 18 studies were experimental studies that used a before-and-after design with (n=1) or without (n=17) a control group. The design of 1 study was a controlled before-and-after study but was labeled as an uncontrolled before-and-after study since no outcome data of the control group were reported.¹⁶ The number of participants was rather low (between n=4 and n=85), consisted mainly of young men (89% (165/187) were males) with an overall mean age of 30 years and were considered as having an excellent level of physical fitness (overall mean $\text{VO}_{2\text{max}}$ before blood donation of 49.7 ± 7.0 mL/min/kg).¹⁷ Exercise before and after the blood donation was performed using maximal exercise testing in 12 studies (incremental exercise testing until exhaustion on a treadmill¹⁸⁻²¹ or on a cycle ergometer²²⁻²⁸, a constant workload test until exhaustion on a cycle ergometer^{29,30}) and submaximal exercise testing in 5 studies (a constant workload test on a cycle ergometer until 6 minutes duration³¹, incremental submaximal exercise testing on a treadmill (until heart rate 130 beats per minute^{16,19} or 180 beats per minute³²) or a cycle ergometer (until heart rate 170 beats per minute³³ or until 60 minutes duration³⁴). One study tested participants both during an incremental treadmill test until exhaustion and a constant work load test at 30% and 60% of $\text{VO}_{2\text{max}}$ until 6 minutes of duration.²⁹ Details on the characteristics of the included studies can be found in **Table 1**.

Effect of a standard whole blood donation on performance during maximal exercise testing

Exercise-related blood parameters

Hemoglobin concentration ([Hb]), hematocrit percentage (Ht%) and the number of RBC were assessed after donation in 7 studies^{18,21-23,25,26,30}, 5 studies^{19,21,22,24,26} and 2 studies^{21,26},

respectively (see Table S1-S3 in Online Resource 4). Absolute values are available in Online Resource 5.

[Hb] was reduced at 24-48 hours (-7% (95%CI, -10 to -4), $p=0.0001$), 48-72 hours (-10% (95%CI, -14 to -7), $p=0.0001$), 7 days (-6% (95%CI, -12 to -0.3), $p=0.038$) and 14 days (-4% (95%CI, -8 to -0.6), $p=0.023$) after blood donation.

Ht% was reduced 24-48 hours (-5% (95%CI, -6 to -3), $p=0.0001$) and 48-72 hours (-11% (95%CI, -17 to 5, $p=0.0001$) whereas no difference could be demonstrated 14 days after donation. A decreased number of RBC was present at 48-72 hours (-11% (95%CI, -17 to -4), $p=0.001$) and 14 days (-5% (95%CI, -9 to -0.27), $p=0.037$) after blood donation.

Maximal oxygen uptake

Post-donation VO_{2max} was measured in 10 studies^{18-20,22,23,26-30} and 4 studies²³⁻²⁶, respectively (see Table S4-S5 in Online Resource 4).

Evidence from 8 studies showed that VO_{2max} was reduced 24-48 hours after blood donation (-7% (95%CI, -11 to -3), $p=0.0001$) (Figure 2). No difference in VO_{2max} was found at other time points after blood donation (Online Resource 5).

Exercise capacity

Time until exhaustion at all different time points (from 0-2 hours until 28 days after blood donation) and W_{max} (from 24 hours until 72 hours after blood donation) was documented in 6 studies^{20-22,26,28,29} and 4 studies²³⁻²⁶, respectively (see Table S5-S6 in Online Resource 4). A reduction in time until exhaustion (-10% (95%CI, -18 to -2), $p=0.01$) was present 24-48 hours after donation (Figure 3). W_{max} tended to be lower 24-48 hours after blood donation (-6% (95%CI, -13 to 1), $p=0.11$, Figure 4).

Overall, no heterogeneity in results was observed ($I^2 = 0\%$, see Online Resource 5), except for the VO_{2max} measured immediately after ($I^2 = 65\%$) and 48-72 hours after blood donation ($I^2 = 20\%$).

Effect of a standard whole blood donation on performance during submaximal exercise testing.

Exercise-related blood parameters

[Hb], Ht% and number of RBC was assessed after donation in 3 studies^{31,33,34}, 3 studies^{16,31,33} and 2 studies^{31,33}, respectively (see Table S1-S3 in Online Resource 4). Absolute values are available in Online Resource 6. All exercise-related blood parameters were reduced, or at least tended to be reduced, from immediately after (0-2 hours) until 24-48 hours after donation (0-2 hours: [Hb]: -3% (95%CI, -5.5 to 0.1), $p=0.06$, Ht%: -1% (95%CI, -2 to -0.4), $p=0.003$, RBC volume: -3% (95%CI, -6 to 0.04), $p=0.053$; 24-48 hours: [Hb]: -7% (95%CI, -9 to -4), $p=0.0001$, Ht%: -3% (95%CI, -4 to -1), $p=0.0001$, number of RBC: -8% (95%CI, -11 to -5), $p=0.0001$). No difference in [Hb] was found at 7 days after donation.

Oxygen uptake

Post-donation VO_2 during light, moderate and vigorous intense exercise was assessed in 1 study²⁹, 1 study²⁹ and 3 studies^{16,31,33}, respectively (see Table S4 in Online Resource 4). No post-donation differences in VO_2 during light (at 24-48 hours), moderate (at 24-48 hours) or vigorous intense exercise (at 0-2 hours, 24-48 hours) could be demonstrated (Online Resource 6).

Exercise capacity

The only controlled before-and-after study measured the exercise time until the heart rate reached 180 beats per minute during a graded submaximal treadmill test³²(see Table S7 in Online Resource 4). No difference in exercise performance after blood donation (at 0-2 hours, at 24-48 hours and at 7 days) was observed (Online Resource 6).

Overall, no heterogeneity in results was observed ($I^2 = 0\%$, see Online resource 6), except for the number of RBC measured 24-48 hours after the blood donation ($I^2 = 42\%$).

Sensitivity analysis

1 In general, the application of different correlation values revealed that the analyses were robust
2 to these values and changing the correlations had a negligible impact on the results. Indeed,
3 sensitivity analyses revealed that the significance of estimates only changed in 11% of the
4 cases (i.e. VO_{2max} 7 days after blood donation, W_{max} 24-48 hours after blood donation and time
5 until exhaustion 0-2 hours after donation).

6 7 **Level of evidence**

8 The quality assessment for each included study is detailed in **Online Resource 7**. No high-
9 quality studies (i.e. well-designed randomized controlled trials) were found. All included
10 experimental studies were “before-and-after studies” with limitations in study design. Firstly,
11 94% (17/18) of the studies did not include a control group (i.e. no blood donation). Secondly,
12 the study using a control group, did not use randomization techniques to allocate participants
13 to an intervention or control group.³²

14 Thirdly, as participants were not blinded (e.g. a sham intervention as proposed by Howell³²)
15 and no studies successfully blinded assessors, there was a high risk of performance bias and
16 detection bias across studies. Additionally, the sample sizes in all studies were generally small
17 (between $n=4$ and $n=85$), leading to imprecise estimates of the effect. Therefore, the quality of
18 evidence, initially started at a high level (because of the experimental study design), was
19 downgraded by one level (from high to moderate quality) for serious study limitations (-1) and
20 by one level for imprecision (from moderate to low quality) (-1), resulting finally in a low-quality
21 level of evidence. This implies that it is difficult to infer whether the impact of these interventions
22 would last when further research of higher quality becomes available, according to the GRADE
23 methodology.

DISCUSSION

The present systematic review collected the best available evidence on the effects of a standard whole blood donation (400-500 mL) on exercise performance. Based on a sensitive literature search with inclusion of 18 “before-and-after” studies, we found that during maximal intense exercise (i) hemoglobin was significantly reduced from 24-48 hours (7% decrease) until 14 days after the blood donation (4% decrease), (ii) maximal oxygen uptake ($\text{VO}_{2\text{max}}$) was significantly lower (7% decrease) 24-48 hours after blood donation and (iii) a significant reduction in time until exhaustion (10% decrease) was present 24-48 hours after donation. In contrast, during submaximal intense exercise, no differences in oxygen uptake or exercise capacity were observed at any time point after blood donation.

Until today, numerous exercise studies and narrative reviews have already reported that maximal oxygen uptake and exercise capacity are decreased for every g/dL loss of hemoglobin mass.^{5,35-38} To our knowledge, the present review is the first systematic review and meta-analysis that has investigated the effects of a standard whole blood donation on maximal and submaximal performance. Placing the focus on a standard whole blood donation (400-500 mL) is important and relevant because evidence-based conclusions and recommendations on a potential donor fear (i.e. negative effect on exercise performance) may improve blood donor recruitment. The key finding of our review is that pooled results of 8 studies showed a 4-7% reduction in $\text{VO}_{2\text{max}}$ from 24-48 hours until 14 days after blood donation. In contrast to recreational athletes, this reduction could be labeled as “physiologically meaningful” for competitive/professional endurance athletes since even very small decreases in maximal oxygen uptake (i.e. 1% reduction or less) could have a meaningful effect in terms of ranking, prize money and/or glory. Therefore, separate recommendations for competitive and non-competitive athletes seem appropriate. The current recommendation of Belgian Red Cross-Flanders is that (non-competitive) athletes and casual exercisers should avoid strenuous physical activity for at least 24 hours after donation. The American Red Cross-Blood service suggests that after blood donation, blood

1 donors “do not do any heavy lifting or vigorous exercise for the rest of the day”.³⁹ Additional
2 but similar recommendations are made for student athletes by stating that “student athletes
3 should wait about 12 hours before resuming strenuous exercise after blood donation,
4 depending on how they feel. As a precaution, they should not give blood on the same day of
5 a competition or strenuous practice.”⁴⁰

6 The only blood service that has a recommendation explicitly and specifically for professional
7 and elite athletes is the Australian Red Cross-Blood service: they suggest professional and
8 elite athletes refrain from donating whole blood during the competitive period or season due to
9 a possible, minor, temporary impact on performance.⁴¹ Iron is an essential micronutrient
10 involved in oxidative metabolism and critical to exercise performance. Because poor iron status
11 (after donating blood) has been shown to be detrimental to physical performance, iron
12 supplementation in iron-depleted athletes may improve parameters of exercise performance.⁴²
13 In addition, athletes should consider becoming plasma donors as there is also an increased
14 need for plasma worldwide and plasma donation does not affect their hemoglobin levels at all.
15 However, Hill et al. demonstrated that plasma donation could also affect exercise performance
16 because of reduced anaerobic capacity.³⁰

17 In our systematic review, a difference in (sub)maximal exercise performance (i.e. time until
18 exhaustion or the time to complete submaximal exercise) after a blood donation could not be
19 demonstrated. Due to the limited number of studies and with a low number of participants,
20 these results were considered as imprecise, which makes random error probable and implies
21 “no evidence of effect” rather than “evidence of no effect”. Indeed, a limitation of the present
22 review is that the 18 included before-and-after studies were considered as “low-quality” due to
23 imprecision and limitations in study design. Therefore, future well-designed research studies
24 (randomized controlled trials of sufficient power) of high methodological quality are needed to
25 make stronger evidence-based recommendations regarding blood donation and exercise
26 performance. Since our conclusions have been drawn using a rather specific population (i.e.
27 young physically active males), it remains to be elucidated whether similar effects would be

1 found in women and older adults with a low physical fitness level. We also need to emphasize
2 that our conclusions can only be applied to continuous sporting activities with a high dynamic
3 component (such as long-distance running or cycling) using mainly the oxidative energy
4 system. Future studies are needed to confirm whether these effects are also applicable to
5 intermittent sporting activities that use the ATP-Phosphocreatine or the glycolytic system (such
6 as sprint running, volleyball, gymnastics, etc).^{43,44} A final aspect that needs further investigation
7 is the cumulative impact of repeated donations on exercise performance, since people are
8 generally allowed to donate blood several times per year.

9 In conclusion, this systematic review and meta-analysis indicates that a standard whole blood
10 donation (400-500 mL) leads to small but potentially important reductions in hemoglobin levels
11 and maximal oxygen uptake and maximal exercise capacity. Pending larger high-quality
12 studies, the current evidence can be used by decision makers involved in formulating blood
13 donor deferral criteria.

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FIGURE LEGENDS

Figure 1. Study identification and selection process of the systematic review

Figure 2. Study-specific mean differences (24-48 hours after donation versus before donation) in VO_{2max} . Each black square represents the mean difference of the respective study together with a 95% confidence interval (CI). The size of the square represents the weight of the study in the meta-analysis. Weights are from random effects analysis. The grey box indicates the mean overall effect together with a 95% CI.

Figure 3. Study-specific mean differences (24-48 hours after donation versus before donation) in time until exhaustion. Each black square represents the mean difference of the respective study together with a 95% confidence interval (CI). The size of the square represents the weight of the study in the meta-analysis. Weights are from random effects analysis. The grey box indicates the mean overall effect together with a 95% CI.

Figure 4. Study-specific mean differences (24-48 hours after donation versus before donation) in maximal power output. Each black square represents the mean difference of the respective study together with a 95% confidence interval (CI). The size of the square represents the weight of the study in the meta-analysis. Weights are from random effects analysis. The grey box indicates the mean overall effect together with a 95% CI.

Table 1. Characteristics of the included studies.

Author, year Country	Study design	Population	Comparison	Exercise	Protocol
Birnbaum 2006, USA	Experimental: (uncontrolled) before-and- after study	10 individuals (male) Age: 23±4 years Weight: 74.4±12.3 kg Height: 178±7.6cm	Before and 24 hours after donation Donation: 450 mL blood	Incremental treadmill test until exhaustion	Treadmill (Bruce protocol) 10 minutes warm-up started with a speed of 2.74 km/h and an incline of 10% and speed of the belt increased each 3 minutes
Burnley 2006, UK	Experimental: (uncontrolled) before-and- after study	10 volunteers (9 male, 1 female) Age: 23±6 years Weight: 77.2±11.0 kg	Before and 24 hours after donation Donation: 450 mL blood	Incremental cycle ergometer test until exhaustion	Cycle ergometer 3 minutes pedalling at 0 watt, continuous ramped increase in work rate of 25 or 30 watts/minute, maintain cadence of 90±2 rpm when rpm ≤ 80 test was stopped
Dellweg 2008, Germany	Experimental: (uncontrolled) before-and- after study	11 volunteers (7 male, 4 female) Age: 36 years Weight: 78±10 kg Height: 182±10 cm BMI: 23.8±3.0 kg/m ²	Before and 24-48 hours after donation Donation: 500 mL blood	Incremental cycle ergometer test until exhaustion	Cycle ergometer continuous ramped increase in work rate of 20 or 30 watts/minute, maintain cadence of 50 rpm when rpm ≤ 45 test was stopped
Duda 2003, Poland	Experimental: (uncontrolled) before-and- after study	12 individuals (male) Age: 23.2±2.6 years Height: 178±7.6 cm BMI: 23.6±2.0 kg/m ²	Before and 24 hours after donation Donation: 450 mL blood	Incremental cycle ergometer test until exhaustion	Cycle ergometer starting at 20 watts, gradual increase in work rate of 20 watts every 3 minutes, maintain cadence of 70 rpm
Ekblom 1972, Sweden	Experimental: (uncontrolled) before-and- after study	4 students (male) Age: 23.8±1.3 years Weight: 68.3±4.3 kg Height: 177.8±4,1 cm	Before and 72 hours after donation Donation: 400 mL blood	Incremental treadmill test until exhaustion and a constant workload testing on a cycle ergometer until 6 minutes duration	The constant workload was set at 30% and 60% of VO _{2max} (which was based on an incremental exercise test until exhaustion)
Foster 2008, USA	Experimental: (uncontrolled) before-and- after study	10 individuals Male (n=not reported): Age: 22.2±1.8 years Weight: 83.3±25.7 kg Height: 184±4 cm	Before and 48 hours after donation Donation: 500 mL blood	Incremental treadmill test until exhaustion	Treadmill (Modified Balke protocol) 4 minutes warm up, the slope was set at 0%, for men the speed was set to 5.3 km/h and for women to

		Female (n=not reported): Age: 23.8±1.3 years Weight: 62.0±6.1 kg Height: 169±2 cm			4.5 km/h, the slope increased every 2 minutes by 2%
Fritsch 1993, Germany	Experimental: (uncontrolled) before-and-after study	16 individuals (10 male, 6 female) Age: 25.3±2.1 years	Before and 48 hours after donation Donation: 450 mL blood	Incremental cycle ergometer test until exhaustion	Cycle ergometer, continuous ramped increase in work rate of 20 watts/minute
Gordon 2010, UK	Experimental: (uncontrolled) before-and-after study	10 individuals (6 male, 4 female) Age: 21±2 years Weight: 66.4±2.8 kg Height: 175.2±5.1 cm	Before and 24 hours after donation Donation: 450 mL blood	Constant work load testing on a cycle ergometer until 6 minutes duration	The constant workload was set at 80% of the respiratory exchange ratio (test 1) or 50% of the difference between the respiratory exchange ratio and the VO_{2max} (test 2).
Gordon 2013, UK	Experimental: (uncontrolled) before-and-after study	15 athletes Age: 23.3±4.5 years Weight: 77.4±13.1 kg Height: 180.1±6.0 cm	Before and 48-72 hours after donation Donation: 450 mL blood	Incremental cycle ergometer test until exhaustion	Cycle ergometer, starting at 100 watts continuous ramped increase in work rate of 25 watts/minute maintain cadence of 80 rpm when rpm ≤ 75 test was stopped
Hill 2013, USA	Experimental: (uncontrolled) before-and-after study	9 volunteers: 2 female: Age: 23±1 years Weight: 60±8 kg Height: 170±11 cm 7 male: Age: 25±3 years Weight: 92±15 kg Height: 182±2 cm	Before and 2 hours, 48 hours and 7 days after donation Donation: 450 mL blood	Constant work load testing on a cycle ergometer until exhaustion	Cycle ergometer, 4 minutes pedalling at 79±15 watts, 4 minutes rest, maintain cadence of 80 rpm at 274±2 watts, when rpm ≤ 75 test was stopped
Hollmann 1969, Germany	Experimental: (uncontrolled)	17 healthy students: Age: 22 years Weight: 73 kg	Before and 2 hours, 24 hours, 48 hours and	Incremental cycle ergometer test until exhaustion	Cycle ergometer, starting at 3mkp/sec, gradual increase in

	before-and-after study	Height: 177 cm	72 hours after donation		work rate of 4mpk/sec every 3 minutes
			Donation: 430ml blood		
Howell 1964, Canada	Experimental: (controlled) before-and-after study	12 subjects: 6 underwent blood donation (intervention group) and 6 underwent a sham intervention (control group) Age: first year university students	Before and immediately, 24 hours, 7 days after donation Donation: 500ml blood	Incremental treadmill test until 180/min heart pulse	Treadmill, speed was 5.4 km/h and remained constant, the slope was set to 0%, and increased 1% per minute
Janetzko 1998, Germany	Experimental: (uncontrolled) before-and-after study	24 elderly donors (mean age 65)	Before and 24h after donation Donation: 450 mL blood	Incremental treadmill test until 130/min heart pulse	Treadmill (Bruce protocol), the slope and speed of the belt increased every 3 minutes
Judd 2011, Canada	Experimental: (uncontrolled) before-and-after study	12 individuals (10 male, 2 female) Age: 24.3±5.2 years Weight: 87.5±15.3 kg Height: 181±8cm	Before and 24 hours, 7 days, 14 days, 21 days and 28 days after donation Donation: 450 mL blood	Incremental cycle ergometer test until exhaustion	Cycle ergometer, initial resistance 1,5 kp (male) or 1,0 kp (female), continuous ramped increase in work rate of 0,5 kp/2 minutes, maintain cadence of 80 rpm
Markiewicz 1981, Poland	Experimental: (uncontrolled) before-and-after study	18 male volunteer blood donors: Age: 21.6±1.1 years	Before and 1 hour, 24 hours after donation Donation: 400 mL blood	Incremental cycle ergometer test until 170/min heart pulse	Cycle ergometer, increasing workload of 10, 20, 30 N, maintain cadence of 60 rpm
Mora-Rodriguez 2012, Spain	Experimental: (uncontrolled) before-and-after study	8 male individuals Age: 27±7 years Weight: 74.4±9.7 kg Height: 180±7cm	Before and 2 hours, 48 hours, 7 days after donation Donation: 450 mL blood	Incremental cycle ergometer test at 60% VO _{2max} until 60 minutes duration	Cycle ergometer, graded exercise test, 60min of pedaling at 60%VO _{2max}
Strandenes 2013, Norway	Experimental: (uncontrolled)	25 male soldiers Median age: 29 years Mean BMI: 24.7 kg/m ²	Before and immediately after donation	Incremental treadmill test until exhaustion	Treadmill (Bruce protocol), slope and speed of the belt increased every 3 minutes

	before-and-after study		Donation: 450 mL blood		
Williams 1978, USA	Experimental: (uncontrolled) before-and-after study	16 male volunteers Age: 31.5±6.8 years Weight: 72.2±6.9 kg Height: 178.2±4.9cm	Before and 14 days after donation Donation: 460 mL blood	Incremental treadmill test until exhaustion	Treadmill, initial speed 9,6km/h, after 5 minutes increased to 11,2km/h, additional increases every 5 minutes with 0,8km/h