INVESTIGATING DIFFERENCES IN HAEMATOPOIETIC STEM CELL YIELD THROUGHOUT CONTINUOUS VS. INTERVAL-BASED EXERCISE BOUTS.

by

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ABSTRACT

It is well established that bouts of exercise increase the concentration of haematopoietic stem and progenitor cells (HSPCs) within peripheral blood. Thus, exercise has been proposed as a potential adjuvant therapy for HSPC mobilisation in healthy, allogenic donors to assist in the HSPC donation process. Despite evidence indicating that both exercise volume and intensity are key drivers of HSPC mobilisation, the optimal exercise protocol to maximise HSPC concentration after exercise, remains unclear. The aim of the present study was to investigate differences in exercise-induced HSPC mobilisation kinetics throughout moderate intensity continuous training (MICT), high-volume high intensity interval training (HV-HIIT) and low-volume high intensity interval training (LV-HIIT) bouts of cycling. We hypothesised that both HV-HIIT and LV-HIIT would evoke greater increases in HSPC concentrations vs MICT. In a randomised design, five healthy males (mean ± SD: age 25 ± 4 years; BMI 26.9 ± 2.6 kg.m^2) undertook three cycling bouts: (i) MICT (30 min at 65-70% HR_{max}) (ii) HV-HIIT (4 × 4 min at 80-85% HR_{max} and (iii) LV-HIIT (4 × 2 min at 90-95% HR_{max}). Flow cytometric data were analysed using repeated measures analysis of variance (ANOVA). HSPC concentration (cells/μL) increased over time in all three trials ($p < 0.001$, $\eta p^2 = .85$), with a significant interaction effect (Trial * Time) between trials ($p < 0.001$, $\eta p^2 = .59$). At post-exercise, HSPC concentrations were greater in LV-HIIT and HV-HIIT compared to MICT (LV-HIIT: 45.19 ± 2.56 vs. 30.51 ± 3.96, $p < 0.001$; HV-HIIT: 38.56 ± 3.12, $p = 0.032$), with no difference between HV-HIIT and LV-HIIT ($p = 0.416$). The present study also reports that HSPCs were mobilised alongside all leukocytes as part of a uniform mobilisation response rather than being preferentially recruited. In summary, our findings indicate that HIIT evokes greater increases in HSPC concentration compared to MICT. Thus, further investigation is warranted into the utility of HIIT as a potential adjuvant therapy in the HSPC allogenic donation process to treat patients suffering from haematological malignancies.

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INTRODUCTION

Stem cells are unspecialised cells with the ability to self-renew or differentiate into a specific cell type for embryonic development or replacement of cells that have been lost or damaged in adult organisms [1]. Haematopoietic stem cells (HSCs) have been extensively studied in the literature due to their ability to retain multilineage potential, thus possessing a greater capacity for differentiation within adult organisms [2]. HSCs were first functionally characterised in mice by performing bone marrow cell transplants [3], subsequently laying the groundwork to phenotypically and functionally characterise HSCs in humans [4]. The differentiation pathway of HSCs to haematopoietic progenitor cells (HPCs) ultimately results in the replenishment of mature blood cells in the circulation (~10^9 per day), such as cells of the innate and adaptive immune system [5]. In humans, haematopoietic stem and progenitor cells (HSPCs) can be identified using flow cytometry as $CD34^+$ cells in the bone marrow (1 – 3% of total bone marrow cells) and peripheral blood (0.01 – 0.1% of total blood cells) [6]. $CD34^+$ expression is lost as cells continue to mature along their lineage and differentiate into lymphoid or myeloid progenitors [7]. Additional surface markers, such as CD38 (expressed on ~90% of $CD34^+$ cells), are required to specifically identify HSCs as $CD34^+/CD38^-$ cells, which is the most primitive HSC population [7].

Approximately 60,000 HSPC transplants are performed worldwide every year for the treatment of various genetic disorders and blood cancers [8], with the primary aim of increasing patient survival rates and reducing the common symptom burdens associated with these conditions, such as fatigue, insomnia and pain [9]. The capacity for HSPCs to regenerate the haematopoietic system cells has made HSPC transplantation a primary curative approach for cell therapy interventions [10]. HSPCs can be obtained from the transplant recipient (autologous transplant) or a healthy donor (allogenic transplant) [8]. Mobilised peripheral blood is the preferred site for HSPC collection for transplantation due to its

ease of access and faster recipient reconstitution compared to HSPC collection from the bone marrow or umbilical cord blood [11]. Mobilisation of sufficient amounts and specific types of HSPCs from the bone marrow is vital to extract a blood graft from the donor of adequate quality to increase the likelihood of transplant success [8]. Blood grafts with a greater content of the aforementioned primitive $CD34^+/CD38^-$ HSC population in particular have shown to increase recipient survival [12].

HSPCs are commonly mobilised from the bone marrow following granulocyte-colony stimulating factor (G-CSF) therapy [13]. G-CSF is a growth factor which has potent mobilisation properties, stimulating HSPCs to move from the bone marrow to the peripheral blood [13]. Apheresis usually occurs when $CD34^+$ quantity reaches $2–4 \times 10^6$ $CD34^+$ cells/kg body weight, which is associated with more complete engraftment and increased transplant success [14]. Apheresis involves removing blood through a needle or catheter, separating it into components using centrifugation and extracting the HSPCs, while returning the other blood components e.g. plasma, red blood cells and platelets back to the patient [15]. However, apheresis is an arduous procedure that can last up to 4 hours, and therefore can be a painful, time-consuming process for the donor [15]. In addition, there are common negative side effects to using pharmaceutical approaches like G-CSF including mild bone pain, fatigue, headaches and nausea [16] and even life-threatening effects like splenic rupture [17]. Furthermore, G-CSF treatments have a 30% failure rate among healthy allogenic donors and >60% failure rate in high-risk autologous donors e.g. older individuals, those with underlying diseases or prior chemotherapy and radiotherapy exposure [10]. Evidently, there is a need for improved strategies to facilitate HSPC mobilisation, particularly in healthy individuals, for donations to patients with haematological malignancies. Plerixafor administration is a strategy utilised for people deemed as ‘poor mobilisers’, where G-CSF therapy produces little success [10]. Plerixafor blocks the chemokine ligand 12 (CXCL12)/ chemokine receptor 4 (CXCR4) chemotaxis pathway to facilitate HSPC mobilisation [10].

However, given that Plerixafor administration is an expensive procedure, the prescription of exercise has been proposed as a potential low-cost approach to increase HSPC mobilisation [11].

In 1978, it was first demonstrated that acute bouts of aerobic exercise can induce a significant increase in HSPC concentration in the blood [18]. Following on from this, Heal and Brightman (1987) established a time course for this exercise-induced mobilisation, displaying a peak in HSPC concentration in peripheral blood immediately following exercise cessation (~50% increase) but a return to baseline levels after 15 minutes [19]. This transient increase in HSPC concentration was observed following a bout of maximal stair sprints but not observed following a bout of submaximal cycling. This was the first suggestion that exercise could assist with increasing the number of HSPCs for donation, with the intensity of exercise playing a role in this HSPC mobilisation response [19]. Table 1 provides an overview of the previous findings in the literature on the effect of acute exercise on HSPC mobilisation.

Further studies have examined the relationship between maximal intensity exercise and HSPC mobilisation into peripheral blood using a variety of exercise modes in individuals of different age, gender, and training status. A study in male and female competitive rowers reported comparable increases in HSPC concentration after 1000m of maximal rowing [20]. Furthermore, a study in trained and untrained younger and older men reported similar increases in HSPC concentration in both groups following maximal cycling to exhaustion [21]. These findings have been replicated in studies employing maximal treadmill running [22], with one study confirming similar responses for trained and untrained males and females [23]. Taken together, previous studies indicate that HSPCs are mobilised in response to maximal exercise and that this is independent of age, gender, and training status. Therefore, using

exercise as a means of increasing HSPC concentration appears to be appropriate for a wide range of allogenic donor populations, thus increasing the donor pool available for HSPC transplants.

Studies have also examined the relationship between prolonged, submaximal endurance exercise and HSPC mobilisation. A study found no significant changes in HSPC concentration in peripheral blood immediately after both a half and full marathon but found a significant decrease in HSPC concentration compared to baseline in both groups in the morning post-race [24]. Following on from this, another study reported a significant decrease in HSPC concentration in peripheral blood immediately after a full marathon [25]. These findings highlight that submaximal, prolonged exercise fails to induce increases in HSPC mobilisation, further indicating that HSPC mobilisation is dependent on exercise intensity. Furthermore, a resistance exercise study only produced significant increases in HSPC concentration after 3 hours post-exercise, whereas significant increases in HSPC concentration were observed immediately after aerobic exercise [26], supporting the view that aerobic exercise is the favoured protocol to increase HSPC concentration.

In the aforementioned studies, the increase in peripheral blood HSPC concentration was transient, increasing immediately following maximal exercise but returning to baseline values within ~30 minutes post-exercise. There was little information on HSPC mobilisation during exercise as extraction of blood was usually at pre- and post-exercise. A 4-hour cycling study reported that HSPC concentration increased significantly above rest during the trial after 3-hours and peaked during the exercise bout at 3-hours 30 minutes [27]. More recently, a 60 minute treadmill running study reported that HSPC concentration significantly increased above rest, and peaked, during the exercise bout at 20 minutes [28]. Agha et al., (2018) detected a 68% increases in HSPC concentration compared to

baseline at 15 minutes of a 30 minute vigorous treadmill running bout, but no increases in HSPC concentration following 90 minutes of moderate exercise [29]. These findings suggest that the HSPC concentration in peripheral blood may increase and even peak much earlier during the exercise bout rather than immediately following exercise cessation as previous studies have reported. These findings also confirm that exercise intensity, not duration, is possibly the critical factor in driving peak HSPC concentrations in peripheral blood.

Recently, studies have reinforced the notion that HSPC mobilisation is dependent on exercise intensity. Baker et al., (2017) reported a significant increase in HSPC concentration immediately after cycling at 70% work rate peak until exhaustion, whilst no increases in HSPC concentration were observed after 30% work rate peak cycling [30]. Nederveen et al., (2020) followed up these findings by adding comparisons between older and younger individuals. The study reported significant increases in HSPC concentration immediately post-exercise following the 70% work rate trial for both the older and younger group, but no significant increases in HSPC concentration following the 30% work rate trial [31]. This confirmed the heterogeneous nature of using maximal intensity exercise for HSPC mobilisation in various donor populations. The incorporation of a high intensity interval training (HIIT) trial consisting of a lower total exercise volume in the Nederveen et al., (2020) follow-up study, however, resulted in no increases in HSPC concentration post-exercise [31]. Therefore, it was suggested for the first time that the optimal exercise protocol to maximise HSPC mobilisation may require a combination of both sufficient exercise intensity and volume [31].

Studies have also looked at the effect of exercise on the primitive $CD34^{+}/CD38^{-}$ HSC population, as these cells are suggested to improve patient responses to haematopoietic engraftment [12]. Morici et al.,

(2005) observed no change in $CD34^+/CD38^-$ cells following 1000m of maximal rowing, suggesting that exercise may instead mobilise more mature populations of HSPCs, evidenced by the 2-fold increase in $CD34^+$ cells [20]. Indeed, cycling studies incorporating higher exercise volumes have been shown to also elicit increases in $CD34^+/CD38^-$ cells [30,31], implying that acute exercise does mobilise clinically relevant $CD34^+/CD38^-$ HSCs, which also relates to both exercise volume and intensity. Evidently, there is a clear interplay between exercise intensity and volume that is not yet established and understanding this relationship is key for the appropriate implementation of exercise to complement HSPC donations. Indeed, more work is required examining this intensity and volume trade-off and analysing the time course of mobilisation during an exercise bout to address these knowledge gaps and find the ideal timepoint to harvest HSPCs at peak concentration to increase the likelihood of transplant success.

Despite continuous training consisting of a higher exercise volume and intensity seeming efficacious, the majority of these protocols have largely been too long ($\geq$ 30 minutes) to feasibly implement to assist with the HSPC donation process. Therefore, the utility of HIIT may serve as a practical alternative to support HSPC donations. HIIT is defined as brief, intermittent bursts of vigorous activity (e.g. 10 seconds to 5 minutes at $\geq$ 90% $VO2_{max}$) interspersed with periods of rest or low-intensity activity [32]. The most common HIIT intervention is the Wingate test, which consists four to six bouts of 30 second all-out maximal cycling sprints against resistance, separated by a minimum of 4 minutes of recovery [32]. However, sprint interval training (SIT), defined as short 30-60 second bouts at supramaximal intensities [33], such as the Wingate protocol, may not be tolerable for much of the population due to its demanding nature [34] and may also be too low in total volume of exercise to achieve peak HSPC concentrations. Hence, more practical HIIT protocols with a manageable intensity have been designed, which are aimed at clinical populations. Examples include, a low

volume HIIT (LV-HIIT) protocol consisting of 10 × 1 minute bouts at ~90% of maximal heart rate with 1 minute of recovery between bouts [35], which was utilised in the Nederveen et al., (2020) study, or a high volume HIIT (HV-HIIT) protocol of 4 minute intervals at ~85% of maximal heart rate with 3 minutes of recovery between bouts [33]. HV-HIIT protocols have been defined as high intensity intervals accumulating to ≥15 minutes, with HIIT protocols below this duration being defined as LV-HIIT [34]. These protocols have been utilised in clinical settings; therefore, they are more accessible for a larger proportion of the population to complete alongside an apheresis session. This is a crucial consideration when choosing the optimal protocol to assist with HSPC donations. Considering the time demands of apheresis and MICT utilised in previous studies, the time-saving aspect of HIIT suggests that it may serve as a feasible option to increase HSPC concentration for donation.

There is currently very little known regarding the effects of HV-HIIT, LV-HIIT and SIT on HSPC mobilisation, as highlighted in a recent meta-analysis [36]. The manipulation of exercise volume and intensity in different interval-based exercise trials may enable the identification of a feasible protocol, to maximise HSPC concentration for donation in clinical transplant settings, which could occur after as little as one HIIT interval. A study consisting of 6 × 20 second all-out cycling sprints reported significant increases in HSPC concentration post-exercise [37], although similar to the Wingate test, this SIT protocol may not be manageable for the general population. It appears that the exercise conditions in the clinical-based 10 × 1 minute LV-HIIT protocol failed to produce a sufficient stimulus to induce an increase in HSPC mobilisation [31]. However, this is not certain as blood draws were only taken immediately post-exercise and during the recovery period and not throughout the exercise session, thus the HSPC mobilisation kinetics during LV-HIIT bouts are still unclear. A HV-HIIT protocol of 5 × 3-minute cycling bouts at 90% peak power output reported greater increases in HSPC concentration post-exercise compared to 30 minutes of continuous cycling at 70% $VO2_{max}$ [38],

suggesting that HV-HIIT may be the ideal approach to maximise HSPC concentration in clinical donation settings, whilst still being a tolerable protocol for the general population to complete. Indeed, more research is required to further analyse this relationship between exercise volume and intensity underpinning HSPC mobilisation across different interval-based protocols. Furthermore, taking blood draws at frequent periods throughout the exercise session would provide additional information on the effect of individual interval bouts on the HSPC mobilisation kinetics.

The specific mechanisms underlying the observed increase in HSPC mobilisation following acute exercise are still being established. The increase in HSPCs appears to relate to both physiological and biochemical factors driving both uniform mobilisation of all leukocytes, as well as preferential mobilisation of HSPCs (Figure 1). Increases in cardiac output and blood flow during exercise are key mechanisms underlying uniform leukocyte mobilisation during exercise [39]. The haemodynamic shear stress from the increased cardiac output and blood flow causes the detachment of these immune cells from the vascular endothelium [39]. It is suggested in the literature that blood flow through the bone marrow niche is increased during high intensity exercise [40]. Therefore, it is plausible that the increased blood flow and subsequent shear stress may also mobilise immune cells (i.e. HSPCs) residing in the vascular niche of the bone marrow as well as those attached to the vascular endothelium. In addition to these uniform physiological changes, exercise-induced changes in catecholamines, cytokines and certain growth factors may play a crucial role in recruiting specific immune cell populations during exercise [39].

It is well established that exercise-induced increases in adrenaline evoke a preferential recruitment of leukocytes from the vascular endothelium and marginal pools within the circulation [41]. Lymphocytes

and natural killer cells with high functional capacity (i.e. high cytotoxicity, antigen experience and tissue migration potential) have high surface expression of β2-adrenergic receptors (β2-AR), which leads to their preferential mobilisation into the bloodstream in an adrenaline-dependent manner [39,41]. These immune cells, which include natural killer cells and cytotoxic T-cells [42], migrate from the circulation towards various tissues to survey the body for damage and/ or infection [43]. Agha et al., (2018) reported that the mobilisation of HSPCs is dependent on β2-AR signalling, as the administration of a β2-AR antagonist resulted in no increases in exercise-induced HSPC mobilisation [29]. It was concluded that the rapid mobilisation of HSPCs in response to exercise is likely due to adrenaline activating β2-AR signalling and causing α-AR-induced increases in haemodynamic shear stress that accompany sustained elevations in cardiac output and blood flow during exercise [29]. The nature of this preferential mobilisation response indicates that the exercise-induced mobilisation of natural killer cells and cytotoxic T-cells could also be harvested alongside HSPCs. The addition of these immune cells would help prevent post-transplant viral infections and graft-versus-host disease through heightened immune surveillance, thus increasing the likelihood of patient survival post-transplant [8].

Exercise-induced increases in growth factors e.g. vascular endothelial growth factor (VEGF) [20,27,44] and hepatocyte growth factor (HGF) [20,22] and increases in cytokines such as interleukin-6 (IL-6) [45] and muscle-derived myokines i.e. stem cell factor (SCF) and transforming growth factor beta 1 (TGF-β1) [20,44] have been suggested to increase cell-signalling to promote preferential recruitment of HSPCs into the bloodstream. However, the direct experimental evidence for demonstrating the role of these proteins is lacking. It is suggested that the transient nature of this preferential recruitment of HSPCs to the peripheral blood is likely due to these chemoattractants facilitating HSPC homing from the peripheral circulation to extramedullary sites of tissue damage and inflammation, such as skeletal

muscle and spleen, to aid in repair following the exercise bout [20,22,23,27,44]. It was also previously suggested that increases in markers of muscle damage and the circulating growth factor G-CSF may evoke the mobilisation of HSPCs to peripheral blood to facilitate tissue repair hours after exercise cessation [26,46]. Agha et al., (2018) observed an independent relationship between circulating G-CSF and HSPC mobilisation during exercise. Thus, further indicating that increases in systemic growth factors post-exercise may cause a delayed recruitment of HSPCs from the bone marrow to repair damaged cells and tissues caused by exercise hours after exercise cessation [29]. This viewpoint is supported by studies reporting a recruitment of HSPCs from the bone marrow to the peripheral blood in response to physiological stress e.g. acute exercise [44,47], albeit in mice, and a significant increase in circulating HSPCs 3 hours and 24 hours post-exercise [26,38].

Mobilisation of HSPCs for stem cell donations can also occur in response to disruption of the CXCL12/CXCR4 axis within the bone marrow through either Plerixafor administration to antagonise CXCR4 [10], or increased plasma CXCL12 levels [11]. CXCR4 is expressed on HSPCs and binds to CXCL12 in the bone marrow, retaining them in the bone marrow niche unless this interaction is disrupted [48]. There is conflicting evidence regarding whether increases in plasma CXCL12 concentration released by muscle after exercise play a role in recruiting HSPCs into the circulation from the bone marrow after it was recently reported that exercise-induced HSPC mobilisation was unrelated to plasma concentrations of CXCL12 [28]. Evidently further work, employing more of an experimental approach, is required to address the conflicting findings and determine the exact mechanisms responsible for the observed increase in HSPC mobilisation and the factors responsible for the removal of HSPC from circulation post-exercise. The exact location where these exercise-induced circulating HSPCs are being mobilised from, whether that be from the bone marrow or the

walls of the vascular endothelium, and whether it is a uniform or preferential mobilisation response, also warrants further investigation.

Majority of the studies mentioned previously have demonstrated a similar relative 2-3-fold increase in HSPC concentration above resting values following exhaustive exercise. However, the absolute numbers of HSPCs mobilised varies markedly between studies. For example, most studies only elicit modest increases in HSPC numbers of 2 – 16 cells/μL [20,22,26,27,29–31], whereas Zaldivar et al., (2007) observed significantly greater increases in HSPCs of 150 – 185 cells/μL [46]. The equivocal findings in the literature on the absolute increases in HSPC mobilisation is likely due to the wide range of subject characteristics, markers used to define HSPCs, timing of analysis, exercise protocol, and most notably, staining methods used e.g. whole blood vs peripheral blood mononuclear cells (PBMCs). While majority of the previous studies have employed a dual-platform approach to count HSPCs, which involves isolation of PBMCs before counting, the gold standard measure for HSPCs analysis should follow the International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines with the utility of the single-platform flow cytometric approach staining whole blood instead of PBMCs [49]. This method was utilised in the Zaldivar et al., (2007) study and may explain the significantly higher HSPC numbers reported in that study compared to the other studies employing the dual-platform approach. However, the Zaldivar et al., (2007) study was conducted in pubertal boys only and the HSPC numbers reported do not fit into the typical single-platform HSPC reference ranges [50]. The ISHAGE guidelines have been updated since the Zaldivar et al., (2007) study, thus, future studies should employ the most up-to-date single-platform methodology to further investigate exercise-induced HSPC mobilisation.

Evidently, a knowledge gap in the research area exists concerning the ideal exercise conditions for maximal HSPC mobilisation. The safety, feasibility and benefits of exercise in the HSPC transplantation setting has previously been described [51], thus providing the foundation and rationale to identify the optimal exercise protocol for these clinical settings. Based on previous findings, and our evolving knowledge of exercise-induced HSPC mobilisation being intensity and volume dependent, the optimal protocol could be a HV-HIIT type protocol. Furthermore, common cofounders in the literature have been accepted to suppress immune function, such as lifestyle habits (e.g. poor sleep quality) and psychological factors (e.g. high anxiety levels and unpleasant moods during exercise) [52]. Indeed, the suppression of immune function due to these factors could lead to the modulation of HSPC mobilisation. Therefore, future work should focus on controlling for these variables to mitigate their effect on the exercise-induced HSPC mobilisation. A thorough, high-quality experimental design is key to obtain an accurate HSPC count following exercise, which would be imperative when assessing the further applications of exercise as a non-invasive therapy in HSPC donations.

The ~2-3-fold transient increase in HSPC concentration following exercise reported in previous studies is largely inferior to the ~10-15-fold increase in HSPC mobilisation following G-CSF treatment [53] or after the addition of Plerixafor [54]. Evidently, the use of exercise is insufficient to completely replace exogenous G-CSF therapy and CXCR4 antagonists as a means for increasing HSPC mobilisation for transplantations. However, exercise may provide a safe, feasible, low-cost approach to be utilised as an adjuvant therapy alongside pharmaceutical agents to further increase HSPC mobilisation prior to apheresis and improve the donor blood graft, through mobilisation of $CD34^{+}/CD38^{-}$ cells and cytotoxic lymphocytes, to increase transplant success [11]. Considering it can take a number of days for pharmaceutical agents to increase circulating HSPC numbers [55], exercise

could provide a means for rapidly increasing the mobilisation of HSPCs to the peripheral blood. Exercise may also assist in reducing the donor burden from the arduous apheresis session, decrease the likelihood for donors to undergo a painful bone marrow HSPC harvest procedure and reduce the side-effects from pharmaceutical administration [11]. Further work in patient populations is needed to conduct direct comparisons between pharmaceutical approaches and exercise for HSPC mobilisation and the effect of combining both approaches should also be investigated.

The present study is a preliminary investigation comparing the kinetics of HSPC mobilisation in healthy allogenic donors before, during and after MICT, HV-HIIT and LV-HIIT bouts of cycling. By employing serial blood sampling throughout the trials, this study will pinpoint the peak HSPC concentrations across each exercise protocol. It is hypothesised that higher peak HSPC concentrations will be achieved following the HIIT trials vs MICT. The incorporation of HV-HIIT and LV-HIIT protocols that are deemed appropriate for the general population, will work to establish a feasible exercise protocol that can maximise peripheral blood HSPC concentration for a lower total exercise dose and time commitment relative to previously mentioned utilised MICT protocols. The study will employ a single-platform flow cytometric approach, whilst controlling for covariate immune function suppressants, to obtain an accurate HSPC count. The study will also address the equivocal findings on whether it is a uniform or preferential mobilisation response and the effect of exercise on numbers of clinically relevant $CD34^{+}/CD38^{-}$ cells. Moreover, these advances will assist with the development of a practical and safe exercise protocol to complement HSPC donations and optimise the yield of HSPCs available for patients.

Figure 1: *Schematic overview of the potential mechanisms underlying the increase in exercise-induced HSPC mobilisation between pre- and post-exercise. Potential mechanisms include exercise-induced increases in blood pressure and shear stress, adrenaline and circulating chemokines and growth factors.*

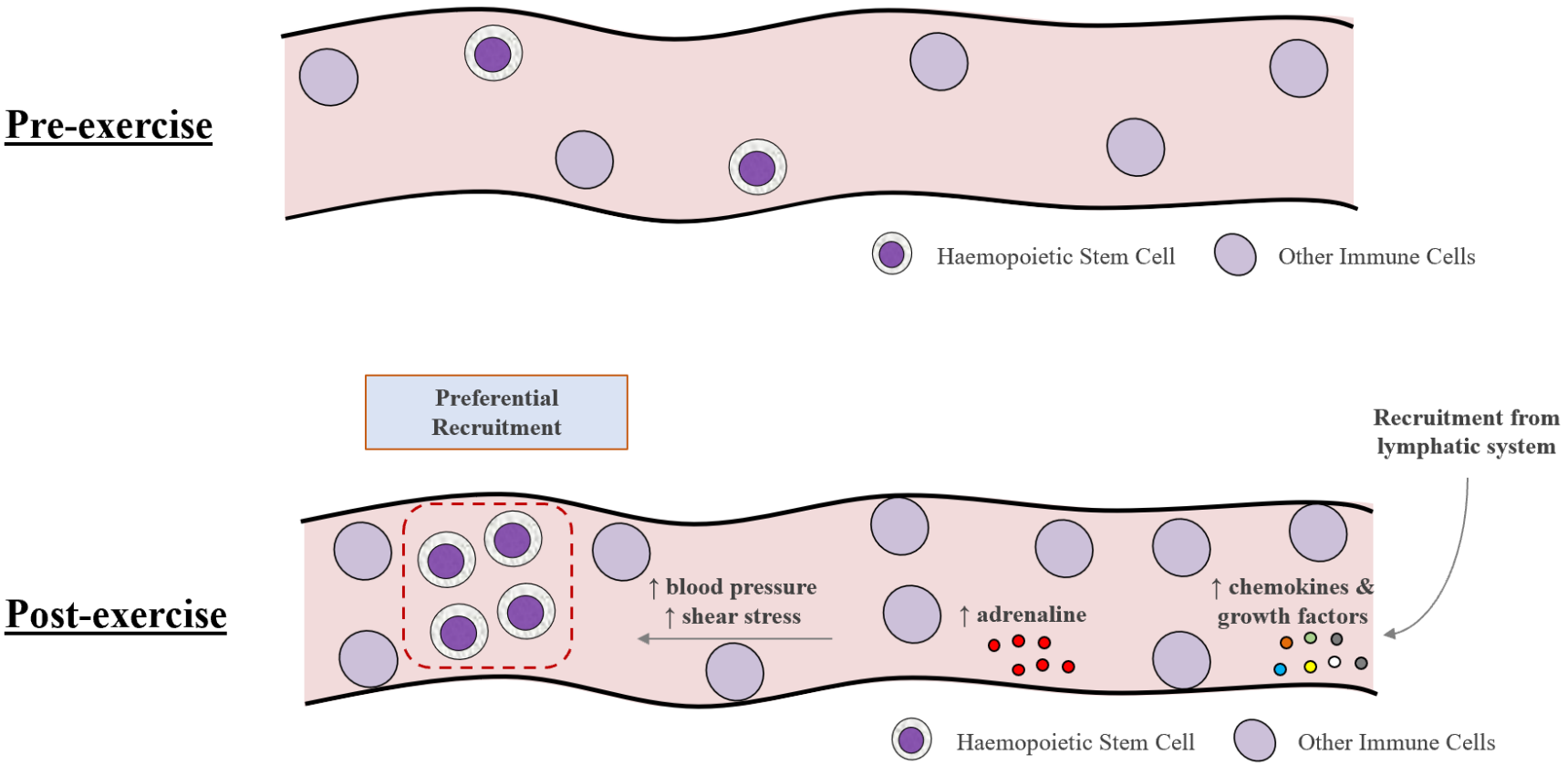


Table 1: *Literature review table summarising the previous findings on the effect of acute exercise on HSPC mobilisation.*

Study	Participants	Methods	Results
Bonsignore et al., (2002)	16 M: 41.8 ± 13.5 yr.	Marathon or HM. BW: Pre, IP, morning after.	**Marathon:** ↔ $CD34^+$ cells at IP and 9.5 ± 3.6 $CD34^+$ cells/μL (↓39%) at morning after. **HM:** ↔ $CD34^+$ cells at IP and 9.8 ± 6.6 $CD34^+$ cells/μL (↓58%) at morning after.
Morici et al., (2005)	13 M, 7 F: 17.1 ± 2.1 yr.	All-out 1000m rowing. BW: Pre and IP.	16.3 ± 9.1 $CD34^+$ cells/μL (~↑100%) and ↔ $CD34^+/CD38^-$ cells at IP.
Thijssen et al., (2006)	8 YM: 19-28 yr. 8 OM: 67-76 yr.	Max cycling test to fatigue. BW: Pre and 10-min post.	**Untrained OM:** ~200 $CD34^+$ cells/ml (~↑100%) at post. **Trained OM:** ~100 $CD34^+$ cells/ml (~↑75%) at post. **Untrained YM:** ~800 $CD34^+$ cells/ml (~↑100%) at post. **Trained YM:** ~600 $CD34^+$ cells/ml (~↑75%) at post.
Zaldivar et al., (2007)	14 EP: 10.3 ± 0.3 yr. 13 LP: 16.5 ± 0.4 yr.	20-min cycling at 70% WR peak. BW: Pre and IP.	**EP:** 182 ± 30 $CD34^+$ cells/μL (↑62.5%) at IP. **LP:**152 ± 21 $CD34^+$ cells/μL (↑141%) at IP.
Adams et al., (2008)	68 M & F: 57 ± 6 yr.	Marathon. BW: Pre and IP.	1175 ± 75 $CD34^+$ cells/ml (↓36%) at IP.
Möbius-Winkler et al., (2009)	18 M: 32.4 ± 2.3 yr.	240-min cycling at 70% AT. BW: Pre, during (5, 10, 15, 30, 60, 90, 120, 150, 180 and 210-min), IP and 30, 60, 120 and 1440-min post.	~2250 $CD34^+$ cells/ml (~↑200%) at 210-min (peak $CD34^+$ quantity).

Bonsignore et al., (2010)	10 M: 43.5 ± 11.3 yr.	Marathon or 1500m sprint. BW: Pre, IP, morning after.	**Marathon:** ↔ $CD34^+$ cells. **1500m:** ~12000 $CD34^+$ cells/ml (~↑100%) at IP and ↔ $CD34^+$ cells at morning after.
Krüger et al., (2015)	36 M: 25.9 ± 3.4 yr.	CET: Cycling at 80% VO2max to fatigue. EET: Downhill running at 80% VO2max to fatigue. RT: RT at 75% at 1RM BW: Pre, IP and 1-hr, 3-hr, 24-hr, and 48-hr post.	**CET:** ~3250 $CD34^+$ cells/ml (~↑117%) at IP, ~2500 $CD34^+$ cells/ml (~↑67%) at 3-hr post and ↔ $CD34^+$ cells at 24-hr post. **EET:** ~3000 $CD34^+$ cells/ml (~↑100%) at IP, ↔ $CD34^+$ cells at 3-hr post and ~2750 $CD34^+$ cells/ml (~↑83%) at 24-hr post. **RT:** ↔ $CD34^+$ cells at IP, ~2250 $CD34^+$ cells/ml (~↑80%) at 3-hr post and ↔ $CD34^+$ cells at 24-hr post.
Krüger et al., (2016)	23 M: 25.7 ± 3.2 yr.	5 × 3 min cycling HIIT bouts at 90% WR peak or 30-min cycling at 70% V02 max. BW: Pre, IP and 3-hr and 24-hr post.	**HIIT:** ~8 $CD34^+$ cells/μL (↑33%) at IP, ~11 $CD34^+$ cells/μL (↑83%) at 3-hr post and ↔ $CD34^+$ cells at 24-hr post. **70%:** ↔ $CD34^+$ cells/μL at IP, ~8 $CD34^+$ cells/μL (↑60%) at 3-hr post and ↔ $CD34^+$ cells at 24-hr post.
Niemiro et al., (2017)	7 M: 25.3 ± 2.4 yr.	60-min treadmill running at 70% V02 max. BW: Pre, during (20 and 40-min), IP and 15, 60 and 120-min post.	908.3 ± 255.5 $CD34^+$ cells/ml (↑165%) at 20-min (peak $CD34^+$ quantity).
Baker et al., (2017)	11M: 23.5 ± 2.9 yr.	Cycling at 70% or 30% WR peak to fatigue. BW: Pre, IP and 10, 30 and 60-min post.	**70%:** ~12000 $CD34^+$ cells/ml (↑98.4%) and ~4500 $CD34^+/CD38^-$ cells/ml (↑88%) at IP, ↔ $CD34^+$ and $CD34^+/CD38^-$ cells at 10, 30 and 60-min post. **30%:** ↔ $CD34^+$ and $CD34^+/CD38^-$ cells.

Agha et al., (2018)	18 M, 9 F: 29.5 ± 7.1 yr.	Part 1: 90-min MOD or 30-min VIG treadmill running. Part 2: 30-min VIG cycling after ingesting: β1-AR ANT, β1+ β2-AR ANT or placebo. BW: Pre, during (15 and 60-min), IP and 60, 120 and 180-min post.	**VIG:** 4.2 ± 1.4 $CD34^+$ cells/μL (↑68%) at 15 min and 4.4 ± 1.4 $CD34^+$ cells/μL (↑76%) at IP, ↔ $CD34^+$ cells at 1-hr, 2-hr and 3-hr post. **MOD:** ↔ $CD34^+$ cells. **Placebo:** 4.4 ± 2.0 $CD34^+$ cells/μL (↑90.5%) at IP and ↔ $CD34^+$ cells at 1-hr post. **β1-AR ANT**: 4.4 ± 1.6 $CD34^+$ cells/μL (↑60%) at IP and ↔ $CD34^+$ cells at 1-hr post. **β1+ β2-AR ANT:** ↔ $CD34^+$ cells.
O'Carroll et al., (2019)	8 M, 4 F: 29 ± 2 yr.	6 × 20 second maximal cycling HIIT sprints or 30-min cycling at 70% V02 max. BW: Pre, IP, 2-hr, and 24-hr post.	**HIIT:** 2496 ± 443 $CD34^+$ cells/ml (↑65%) at IP and ↔ $CD34^+$ cells/ml at 2-hr and 24-hr post. **70%:** ↔ $CD34^+$ cells/ml.
Nederveen et al., (2020)	8 YM: 21.5 ±0.8 yr. 8 OM: 69.9 ± 2.0 yr.	Cycling at 70% or 30% WR peak to fatigue or 10 × 1 min HIIT bouts at 90% HR max. BW: Pre, IP and 10, 30 and 60-min post.	**70% OM:** ~3500 $CD34^+$ cells/ml (↑101%) and ~2000 $CD34^+/CD38^-$ cells/ml (↑56%) at IP, ↔ $CD34^+$ and $CD34^+/CD38^-$ cells at 10, 30 and 60-min post. **70% YM:** ~6000 $CD34^+$ cells/ml (↑352%) and ~750 $CD34^+/CD38^-$ cells/ml (↑251%) at IP, ↔ $CD34^+$ and $CD34^+/CD38^-$ cells at 10, 30 and 60-min post. **30% OM & YM:** ↔ $CD34^+$ and $CD34^+/CD38^-$ cells. **HIIT OM & YM:** ↔ $CD34^+$ and $CD34^+/CD38^-$ cells.

Ages are presented as means ± SD or age range. M – Male, F – Female, OM – Older men, YM – Younger men, EP – Early puberal boys, LP – Late pubertal boys, HM – Half-marathon. ET – Endurance training, BW – Blood withdrawal, IP – Immediately post, WR – Work rate, AT – Anaerobic threshold, HIIT – High intensity interval training, HR – Heart rate, CET – Concentric endurance training, EET – Eccentric endurance training, RT – Resistance training, RM – Rep max, VT – Ventilatory threshold, AR – Adrenergic receptor, MOD – Moderate, VIG – Vigorous, VO2max – Maximal oxygen consumption, ANT – Antagonist, ↔ - No change, ↓ - Decrease, ↑ Increase.

METHODS

Participants

Following ethical approval from the University of Birmingham (#ERN_19-1574P2), 5 healthy males (mean ± SD: age 25 ± 4 years; BMI 26.9 ± 2.6 $kg.m^2$; $Watt_{max}$ 3.67 ± 0.68 $W.kg^{-1}$) were recruited onto this study. Participants were included in the study if the following criteria were met: (i) free from COVID-19 symptoms, (ii) aged 18 – 45 years, (iii) healthy and physically active. Participants were excluded from the study if they (i) had a history of cardiovascular, respiratory, metabolic, or neuromuscular illnesses, (ii) were a regular smoker, (iii) donated blood in the last 3 months, (iv) had taken anti-inflammatory drugs within the last 2 weeks. Participants were required to refrain from any strenuous exercise and consumption of alcohol or caffeine for two days prior to the experimental sessions. All participants completed online questionnaires, as part of an initial screening procedure at the start of the study, addressing health history and habitual levels of weekly physical activity using a University of Birmingham General Health Questionnaire (GHQ) and General Practice Physical Activity Questionnaire (GPPAQ) [56]. Participant anxiety levels and sleep quality were also assessed on the morning prior to each experimental session using a State Trait Anxiety Inventory (STAI) Questionnaire [57] and Consensus Sleep Diary (CSD) [58]. STAI score scale ranged from < 35 (low level anxiety) to > 65 (high level anxiety). A sleep efficiency ≥ 80% indicated sufficient sleep quality. Participants completed a pre-screening COVID-19 questionnaire to confirm their absence of any symptoms 24 hours prior and on the morning before arrival for every visit. All participants gave their written informed consent, and the study was carried out in accordance with the Declaration of Helsinki (2013).

Experimental sessions

All experimental sessions took place in the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham in the morning (9:00am – 9:30am start time). Participants reported to the laboratory on four separate occasions, all carried out under stable climate conditions (18 - 20°C and humidity between 60-65%). Following a 30-minute period of rest, body mass (*Ohaus CD-31, USA*), resting heart rate (*Polar M430, Finland*), resting blood pressure (*Thuasne BP 3W1-A, Switzerland*) and height (*Seca Alpha, Germany*) were recorded on each visit. A Perspex screen was installed between participants and experimenters during each visit to ensure a 2-metre distance was maintained where possible to abide by the COVID-19 safety guidelines.

Participant's first visit to the laboratory included a standard incremental $Watt_{max}$ (W_{max}) test on an electronically braked cycle ergometer (*Lode Excalibur Sport, Netherlands*) to determine maximal power output. The W_{max} test consisted of an initial 5-minute warm-up until the participant indicated a rating of perceived exertion (RPE) of 11, indicating 'fairly light' on the 6 - 20 Borg Scale [59], followed by the participant pedalling at 70 W for the first minute, and the workload being increased by 25 W every minute until volitional exhaustion was reached, or the participant was not able to maintain a consistent cadence above 50 revolutions per minute (RPM) [60]. Continuous measures of heart rate were collected throughout the W_{max} test for the determination of heart rate max (HR_{max}) which was utilised to calculate the participants target workloads for the subsequent exercise trials. Following the W_{max} test, participants underwent a familiarisation session to ensure the specific $\%HR_{max}$ range would be achieved in each of the ensuing exercise trials.

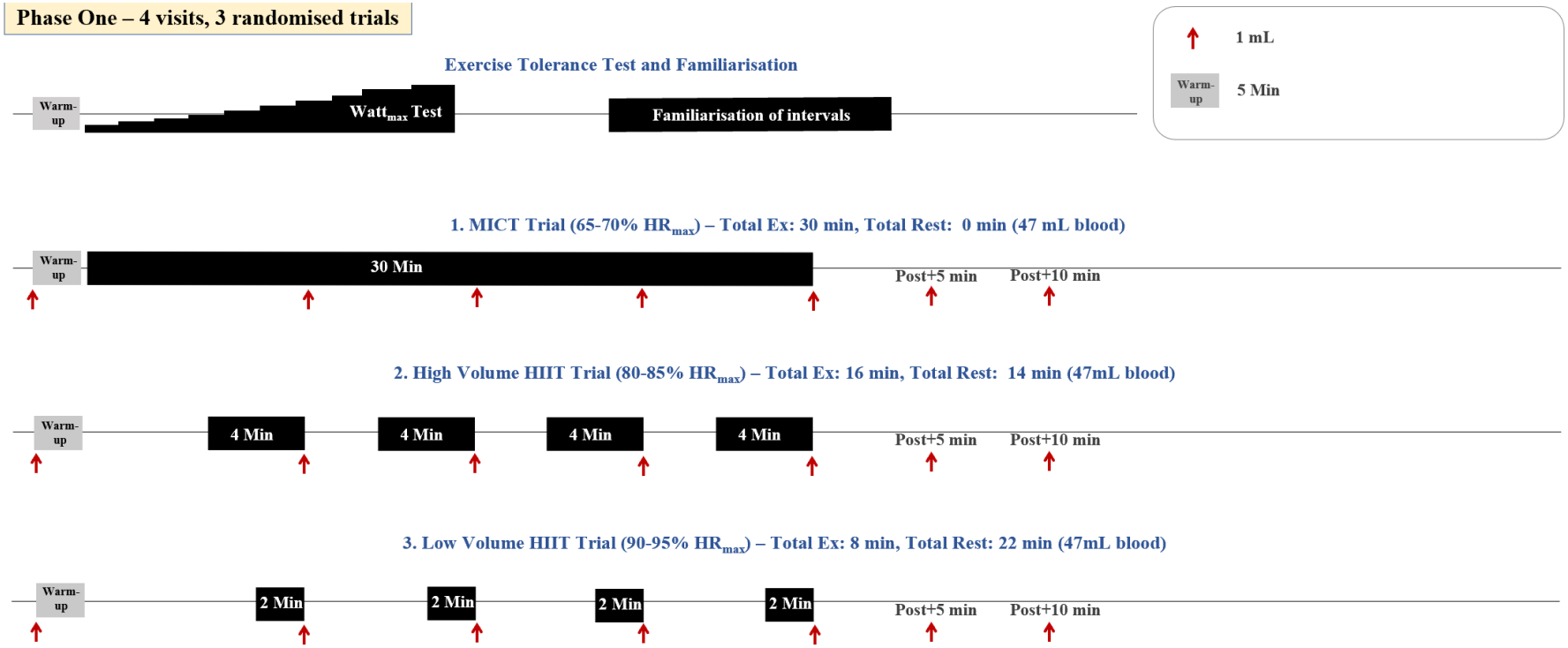
At least one week following the W_{max} test, participants undertook three time-matched cycling trials in a randomised order: a moderate intensity bout of continuous cycling at 65-70% HR_{max} for 30

minutes (MICT), a high volume HIIT bout consisting of 4 × 4-minute intervals at 80-85% HR_{max} with three minutes of rest between each interval (HV-HIIT) and a low volume HIIT bout consisting of 4 × 2-minute intervals at 90-95% HR_{max} with five minutes of rest between each interval (LV-HIIT). All trials took place following an identical overnight fast (starting at 9:00pm the night before) separated at least one week apart. Each exercise trial was carried out at a self-selected cadence, included a 5-minute warm-up identical to that used during the W_{max} test, and ended with a seated 10-minute passive recovery period. During all exercise protocols, heart rate was continuously recorded, verbal encouragement was given frequently to the participant and music of the participant's choice was provided. RPE [59] and ratings of perceived mood during exercise ranging from -5 (very bad) to +5 (very good) using the Feeling Scale (FS) [61] were measured at the end of each bout for the HIIT trials and at the corresponding time points for the MICT trial. An initial resistance on the cycle ergometer was set, which was calculated to induce the desired workload for the respective trial according to each participant's HR_{max} data. Heart rate was then continuously monitored across the MICT and HIIT trials to ensure the respective intensity was maintained and cycling resistance was adjusted as required to ensure heart rate remained in the target range. The HV-HIIT trial consisted of an initial 5-minute period where the participant remained stationary on the bike following the warm-up, this period was extended to 7 minutes for the LV-HIIT trial. The stationary period was included to ensure all three trials were time-matched at 30-minutes in order for each bout of exercise to finish at the same timepoint. For both HIIT trials, the workload was ramped up 10 seconds prior to each interval in order to account for the 10 second delay in resistance on the cycle ergometer.

Blood sampling

Trials were time-matched to match up the timing of blood samples between trials. In all three exercise trials (MICT, HV-HIIT and LV-HIIT), a catheter (*20G BD Venflon Pro, United Kingdom*) was inserted into the antecubital vein of the forearm to obtain 1mL blood samples after a minimum of 10 minutes of rest (Pre-Ex), during the trials at the end of each interval bout: 9 min (Ex-9 min), 16 min (Ex-16 min), 23 min (Ex-23 min), as well as immediately post exercise (Post-Ex) and 5 min (Post-Ex +5 min) and 10 min (Post-Ex +10 min) post-exercise. At each timepoint, blood was collected in separate 1mL vacuette tubes containing ethylene diaminetetraacetic acid (EDTA) for anticoagulation (*Greiner Bio-One Vacuette, United Kingdom*). The catheter was flushed between samples with isotonic saline solution containing 0.9% sodium chloride (*BD Posiflush, United Kingdom*) to prevent blood clotting and 2mL of blood was discarded between each sample to minimise the risk of sample dilution. 20μL of whole blood at each timepoint was inserted into an automated cell counter (*Horiba Yumizen H500, Japan*) to determine total leukocyte count. An overview of the study design is presented in Figure 2.

Figure 2: *Schematic overview of the overall study design.*



Development of a whole blood method for enumeration of HSPCs

Whole blood from each timepoint was stained with fluorescently conjugated antibodies and flow cytometry used to enumerate (*Beckman Coulter CytoFlex, USA*) viable HSPCs cells per μL of blood, using the single-platform ISHAGE approach [49]. In accordance with this standardised protocol, 10μL of both CD34-PE (*clone: 581, Biolegend, United Kingdom*) and CD45-FITC (*clone: 2D1, Biolegend, United Kingdom*) were added to 5mL falcon round-bottom tubes (*Fisher Scientific, United Kingdom*), followed by 2.5μL of 7-AAD viability dye (*Biolegend, United Kingdom*). Following an antibody titration experiment to calculate the optimal staining index (Figure 3A and 3B), the present study incorporated 2.5μL of fluorescently conjugated CD38-BV421 antibody (*clone: HB7, Biolegend, United Kingdom*) into the staining procedure, to also determine the number of viable $CD34^+/CD38^-$ cells per μL of blood. 100 μL of well-mixed blood was then added and the sample was incubated for 20 minutes at room temperature in the dark. Following this, 2mL of red blood cell lysis buffer (*Biolegend, United Kingdom*) was added, and the sample was incubated for 10 minutes at room temperature in the dark before proceeding to flow acquisition.

For flow acquisition, a time titration experiment confirmed that all samples must be analysed within 1 hour of lysis, due to beyond this timepoint, $CD34^+$ count was significantly reduced (Figure 4A) and lymphocyte cell death was significantly increased (Figure 4B). The software CytExpert (*Beckman Coulter version 2.4, USA*) was used, and a medium flow rate was run (30 μL/min), with at least 100 HSPC events captured and analysed as suggested by the ISHAGE protocol [49]. Compensation was adjusted daily by using single stained controls (Figure 5). UltraComp compensation beads (*Fisher Scientific, United Kingdom*) were used for FITC, PE and BV-421 compensation. 7-AAD compensation involved a heat-treat experiment of 50uL of blood being heated for 20 minutes before

being treated with 50uL of unheated blood to create an approximate 50:50 separation between live and dead cells. Positive population gates were established using fluorescence minus one (FMO) control for CD34-PE and CD38-BV421. The quantification of viable $CD34^+$ and $CD34^+/CD38^-$ cells was calculated using volumetric cell counting instead of using CountBright counting beads (*Fisher Scientific, United Kingdom*) after a volumetric vs. bead counting experiment (Figure 6) confirmed previous findings that there is no significant difference between the two counting methods [50]. The average coefficient of variation (CV) for the method validation experiments (i.e. the CD38-BV421 antibody titration, the $CD34^+$ time titration and the volumetric vs bead $CD34^+$ count experiment) was ~7%, which aligns with the ISHAGE single-platform method validation CV range of 5 – 9% [62].

HSPC enumeration is a rare event analysis requiring specific gating instructions for detection. Thus, the gating strategy employed in the present study (Figure 7) was based on the ISHAGE protocol [49]. The protocol utilises a Boolean gating technique to identify HSPCs as cells expressing both CD34 and CD45, with dim CD45 expression and low side scatter characteristics. The addition of a $CD34^+/CD38^-$ gate in the present study was derived from previous literature [63].

Statistical analysis

Statistical calculations were performed on SPPS (*IBM version 27, USA*). Assumptions of normality were assessed by the Shapiro-Wilk test of scale data at all time points. Variables with a non-normal distribution were log transformed, if necessary, prior to running parametric tests. Resting baseline data (i.e. height and weight) and covariate data (i.e. feeling scale, sleep quality and anxiety levels) were compared between trials (MICT, HV-HIIT and LV-HIIT) by one-way analysis of variance (ANOVA). Comparison of heart rate and RPE were analysed over time at the end of each bout (Ex-

9 min, Ex-16 min, Ex-23 min and Post-Ex) and between trials by repeated measures ANOVA. HSPC and $CD34^+/CD38^-$ cell concentrations, total white blood cell (WBC) counts, and lymphocyte cell counts, were analysed over time at each blood sampling timepoint (Pre-Ex, Ex-9 min, Ex-16 min, Ex-23 min, Post-Ex, Post-Ex +5 min, and Post-Ex +10 min) and between trials by repeated measures ANOVA. The difference in relative fold changes from resting values for HSPC cell concentration and total WBC count were compared within each trial over time at each blood sampling timepoint by repeated measures ANOVA. If sphericity assumptions were not met under Mauchly's Test, then Greenhouse-Geisser corrections were utilised for ANOVA results. Post hoc tests were carried out where significant interaction effects were found (Trial*Time), with significance being set at $p<0.05$. Effect sizes, using partial eta squared (ηp^2), were calculated to assess the magnitude of effects where the repeated measure ANOVA was run, using Cohen's definition of ηp^2 of 0.01, 0.06 and 0.14 for 'small', 'medium' and 'large' effects, respectively. All values are presented as mean ± standard error (mean ± SE*)*, unless otherwise stated.

Figure 3A: *Schematic overview of the CD38-BV421 antibody titration experiment. Dot plots display the separation between the negative and positive population at 0 µL (A), 0.15625 µL (B), 0.3125 µL (C), 0.625 µL (D), 1.25 µL (E), 2.5 µL (F), 5 µL (G) and 10 µL (H). Separation was greatest at the 2.5 µL concentration (F).*

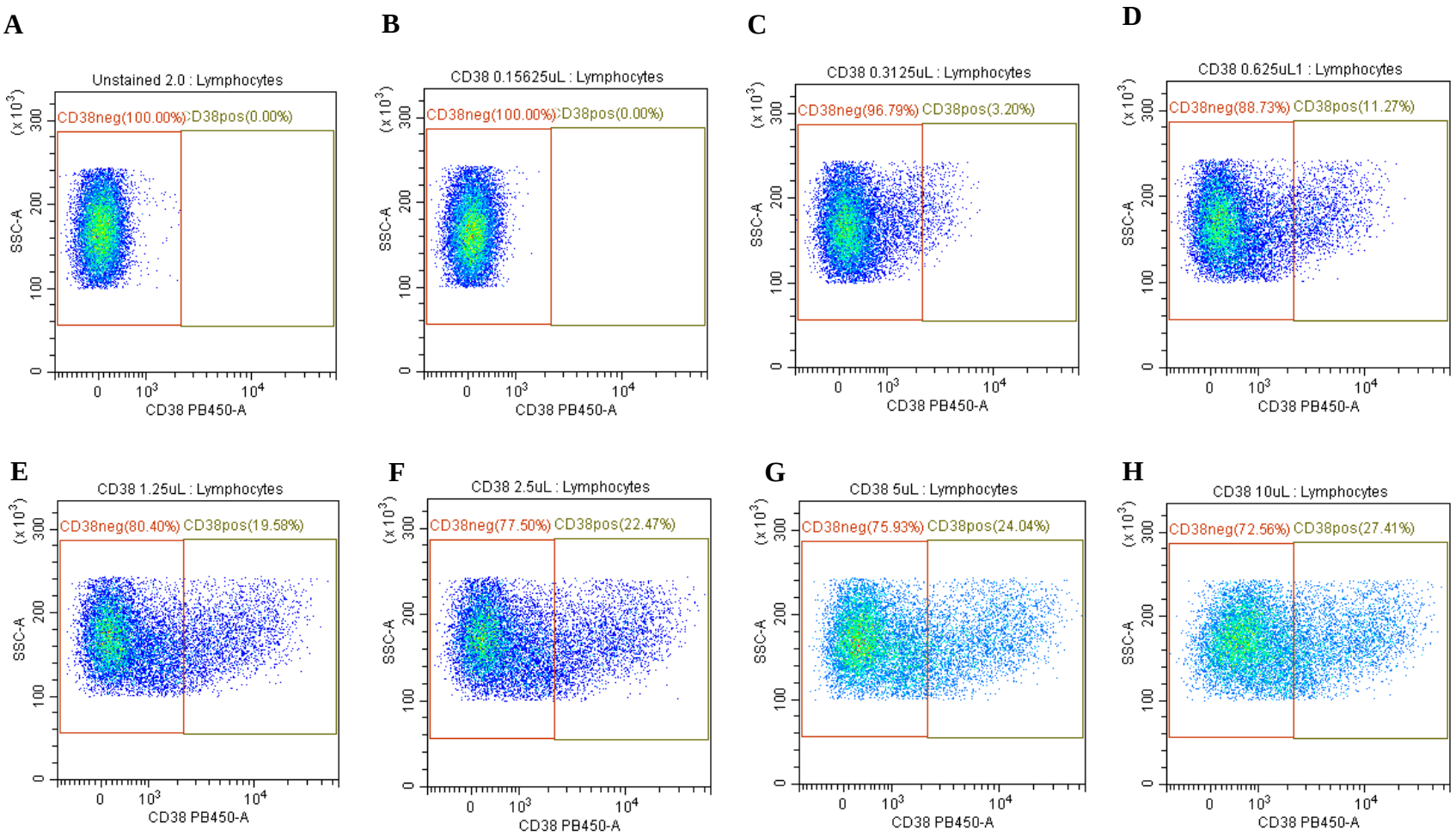


Figure 3B: *A graphical representation of the CD38-BV421 titration staining index. * indicates the concentration which had the greatest separation between the negative and positive population and therefore produced the highest stain index, which was 2.5µL.*

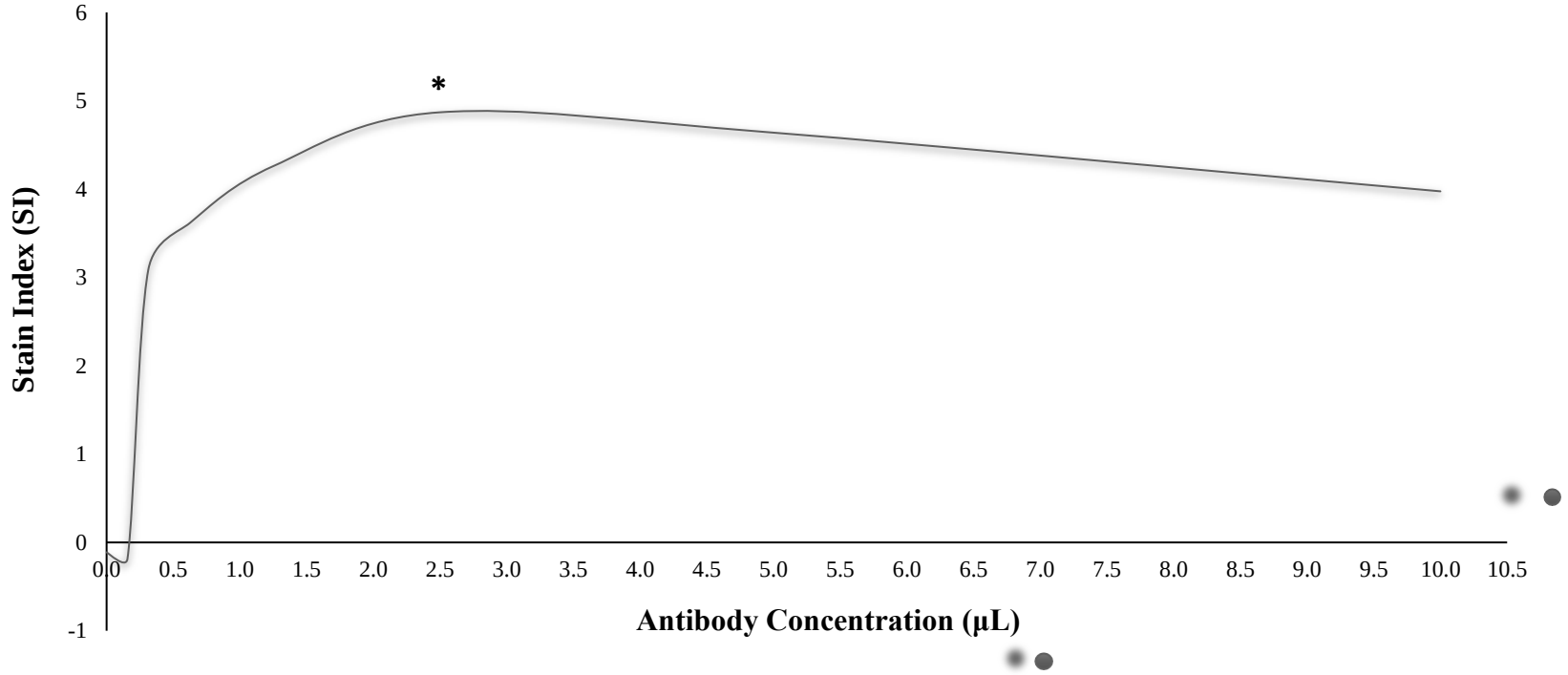


Figure 4A: *Schematic overview of the time titration experiment to examine the effect of time on CD34$^+$ count. Significance was set at p<0.05.* indicates significantly lower CD34$^+$ count compared to the 0-10 min timepoint (N=1).*

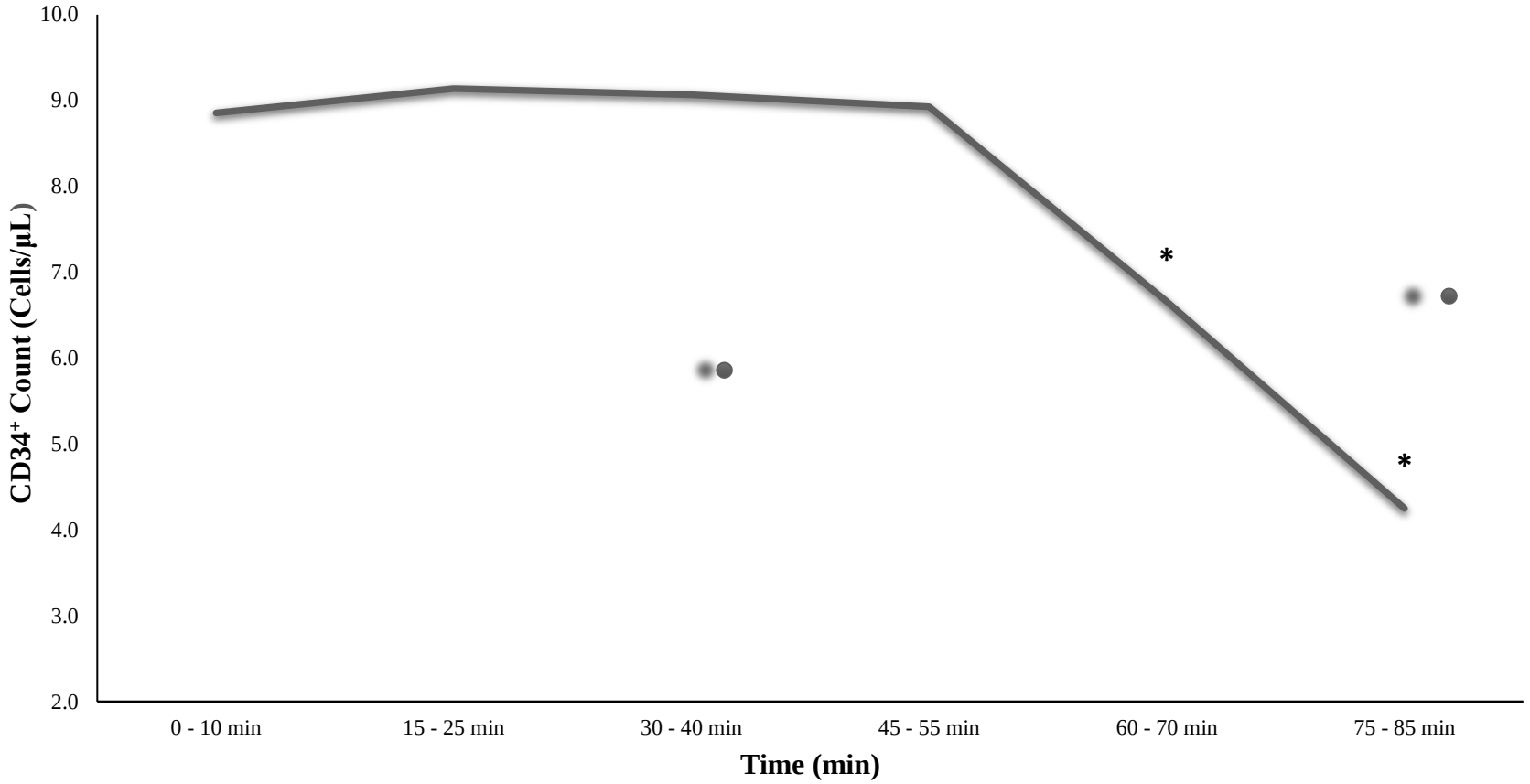


Figure 4B: *Schematic overview displaying increasing lymphocyte cell death at 0 min (A), 60 min (B) and 90 min (C). P2 gate – percentage of dead cells.*

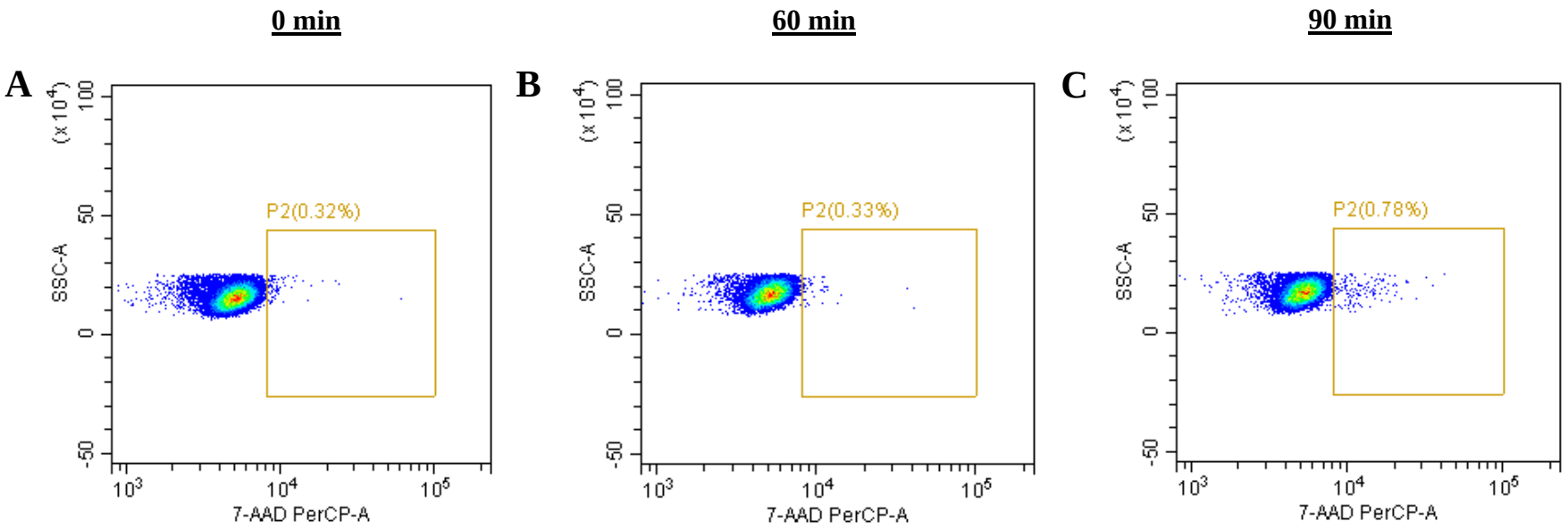


Figure 5: *Visual representation of the single stained compensation experiments for FITC (A), BV421 (PB450 - B), PE (C) and 7-AAD (PerCP - D).*

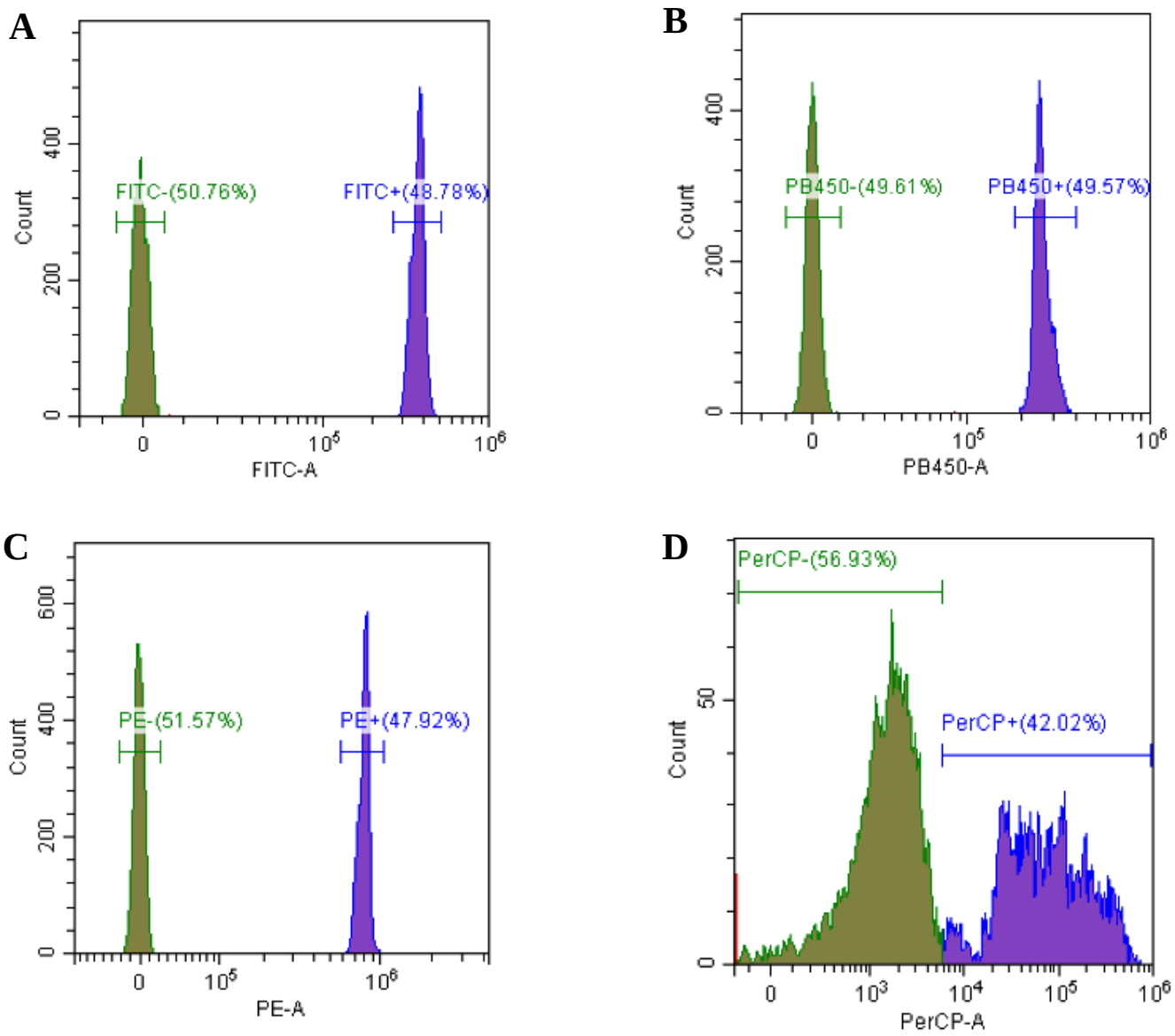


Figure 6: *Schematic overview of the bead vs. volumetric CD34$^+$ counting experiment. Data presented as mean ± SD. Significance was set at $p<0.05$. There was no significant difference in CD34$^+$ count between groups (N = 3).*

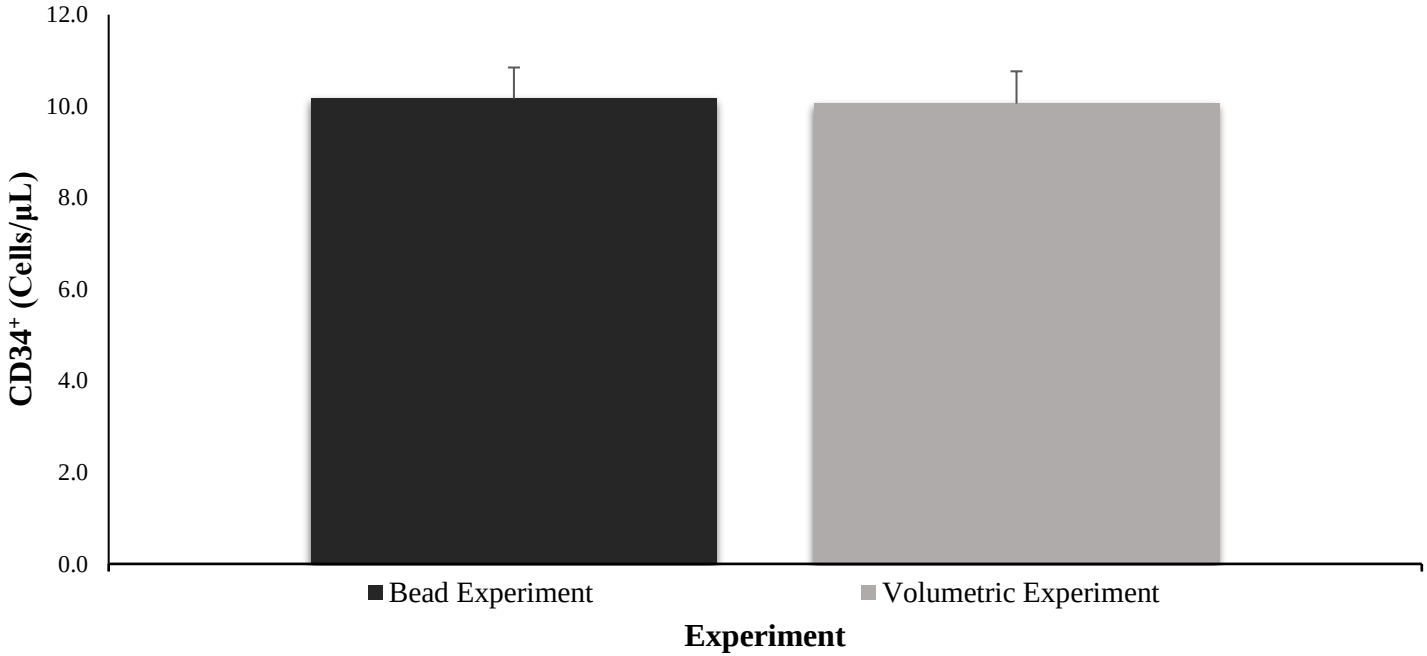
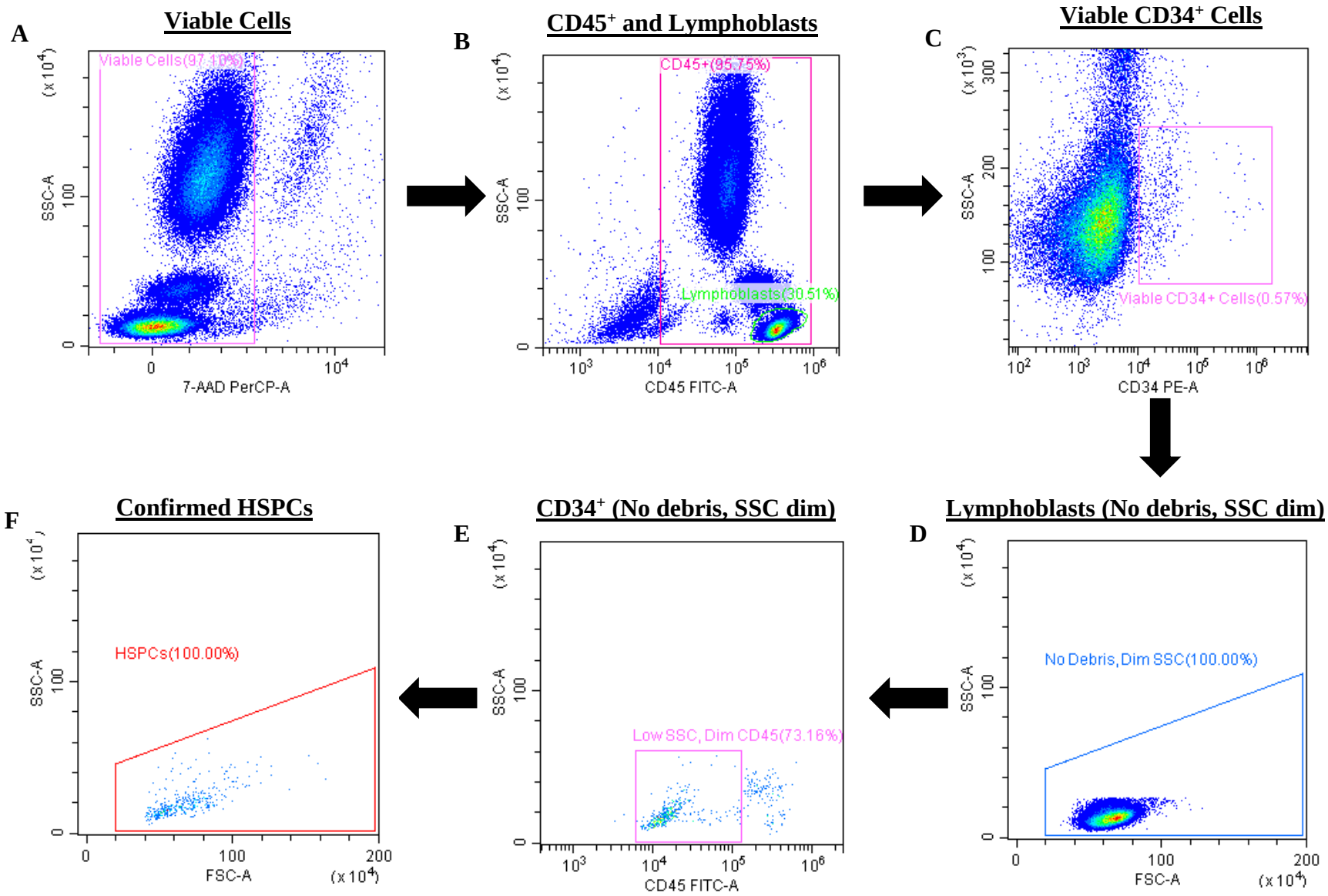


Figure 7: *Schematic overview of the overall Boolean gating strategy (A – F).*



RESULTS

Covariate response

There were no significant differences in the height (cm) or weight (kg) of participants ($p > 0.05$ for all). There were no significant differences in mean scores for feeling scale (2.86 ± 0.76 vs. 2.80 ± 0.66 vs. 2.82 ± 0.62, $p > 0.05$ for all) between the MICT, HV-HIIT and LV-HIIT trials respectively, correlating to a score of 'good' on the feeling scale. There were no significant differences in mean scores for anxiety levels (44.20 ± 2.11 vs. 43.60 ± 1.81 vs. 43.80 ± 2.04, $p > 0.05$ for all) between the MICT, HV-HIIT and LV-HIIT trials respectively, correlating to a score of 'medium' on the anxiety scale. There were no significant differences in mean scores for sleep efficiency (%) (82.00 ± 4.00 vs. 81.80 ± 5.58 vs. 82.00 ± 4.00, $p > 0.05$ for all) between the MICT, HV-HIIT and LV-HIIT trials respectively. All three sleep efficiency percentages correlate to a sufficient quality of sleep.

Physiological response

Heart rate (BPM) was significantly increased over time in all three trials ($p < 0.001$, $\eta p^2 = .83$). There was a significant interaction effect (Trial * Time) between trials over time ($p < 0.001$, $\eta p^2 = .58$) (Figure 8). Post hoc analysis revealed that heart rate was significantly greater in the LV-HIIT trial compared to both the HV-HIIT trial and MICT trial at the end of each bout ($p < 0.001$ for all). Heart rate was also significantly greater in the HV-HIIT trial compared to the MICT trial at the end of each bout ($p < 0.001$ for all).

RPE was significantly increased over time in all three trials ($p < 0.001$, $\eta p^2 = .82$). There was a significant interaction effect between trials over time ($p < 0.001$, $\eta p^2 = .43$) (Figure 9). Post hoc

analysis revealed that RPE was significantly greater in the LV-HIIT trial compared to both the HV-HIIT trial and MICT trial at the end of each bout ($p < 0.001$ for all). RPE was also significantly greater in the HV-HIIT group compared to the MICT group at the end of each bout ($p = 0.013$ for Ex-9 min, $p = 0.045$ for Ex-16 min, $p = 0.033$ for Ex-23 min and $p = 0.002$ for Post-Ex).

Total leukocyte and lymphocyte response to MICT, HV-HIIT and LV-HIIT

Total WBC count (cells/μL) and lymphocyte count (cells/μL) significantly increased over time in all three trials ($p < 0.001$, $\eta p^2 = .91$ for both). There was a significant interaction effect in total WBC count between trials over time ($p < 0.001$, $\eta p^2 = .68$) (Figure 10) and a significant interaction effect in lymphocyte count between trials over time ($p < 0.001$, $\eta p^2 = .72$) (Figure 11). Post hoc analysis revealed at Pre-Ex and Ex-9 min, there was no significant difference in WBC count and lymphocyte count between trials ($p > 0.05$ for all).

At Ex-16 min, WBC count was significantly greater in the LV-HIIT trial compared to the MICT trial (10058 ± 546 vs. 8126 ± 812, $p < 0.001$) and the HV-HIIT trial (8408 ± 848, $p = 0.007$). There was no significant difference between the HV-HIIT and MICT trials ($p = 0.904$). Lymphocyte count was significantly greater in the LV-HIIT trial compared to the MICT trial (4110 ± 347 vs. 2830 ± 345, $p = 0.027$). There was no significant difference between the HV-HIIT and MICT trials (3321 ± 365, $p = 0.322$) or between the HV-HIIT and LV-HIIT trials ($p = 0.470$). At Ex-23 min, WBC count was significantly greater in the LV-HIIT trial compared to the MICT trial (10458 ± 856 vs. 7978 ± 797, $p < 0.001$) and significantly greater in the HV-HIIT trial compared to the MICT trial (9368 ± 817, $p = 0.031$). Lymphocyte count was significantly greater in the LV-HIIT trial compared to the MICT trial (4304 ± 564 vs. 2630 ± 302, $p < 0.001$) and significantly greater in the HV-HIIT trial compared

to the MICT trial (3646 ± 402, p = 0.007). At Post-Ex, WBC count was significantly greater in the LV-HIIT trial compared to the MICT trial (10572 ± 1093 vs. 8076 ± 791, p < 0.001) and significantly greater in the HV-HIIT trial compared to the MICT trial (9344 ± 737, p = 0.036). Lymphocyte count was significantly greater in the LV-HIIT trial compared to the MICT trial (4270 ± 643 vs. 2660 ± 293, p < 0.001) and significantly greater in the HV-HIIT trial compared to the MICT trial (3642 ± 378, p = 0.008). There was no significant difference in WBC count and lymphocyte count between the HV-HIIT and LV-HIIT trials at Ex-23 min and Post-Ex (p > 0.05 for all).

At Post-Ex +5 min, WBC count was significantly greater in the LV-HIIT trial compared to the MICT trial (8722 ± 689 vs. 6874 ± 690, p < 0.001). There was no significant difference between the HV-HIIT and MICT trials (7536 ± 585, p = 0.087) or between the LV-HIIT and HV-HIIT trials (p = 0.052). Lymphocyte count was significantly greater in the LV-HIIT trial compared to the MICT trial (3464 ± 468 vs. 2082 ± 259, p = 0.009) and HV-HIIT trial (2714 ± 371, p = 0.015). There was no significant difference between the HV-HIIT and MICT trials (p = 0.056). At Post-Ex +10 min, WBC count was significantly greater in the LV-HIIT trial compared to the MICT trial (8262 ± 964 vs. 6230 ± 572, p < 0.001) and the HV-HIIT trial (6712 ± 535 p = 0.019). Lymphocyte count was significantly greater in the LV-HIIT trial compared to the MICT trial (3212 ± 597 vs. 1878 ± 179, p = 0.037) and HV-HIIT trial (2328 ± 358, p = 0.043). There was no significant difference in WBC count and lymphocyte count between the HV-HIIT and MICT trials (p = 0.414).

Haematopoietic stem and progenitor cell response to MICT, HV-HIIT and LV-HIIT

HSPC concentration (cells/μL) significantly increased over time in all three trials (p < 0.001, ηp^2 = .85). There was a significant interaction effect in HSPC concentration between trials over time (p <

0.001, ηp^2 = .59) (Figure 12). Post hoc analysis revealed at Pre-Ex, Ex-9 min and Ex-16 min, there was no significant difference in HSPC concentration between trials ($p > 0.05$ for all). At Ex-23 min, HSPC concentration was significantly greater in the LV-HIIT trial compared to the MICT trial (45.92 ± 2.99 vs. 31.51 ± 2.93, $p = 0.004$) and significantly greater in the HV-HIIT trial compared to the MICT trial (35.44 ± 2.91, $p = 0.016$). At Post-Ex, HSPC concentration was significantly greater in the LV-HIIT trial compared to the MICT trial (45.19 ± 2.56 vs. 30.51 ± 3.96, $p < 0.001$) and significantly greater in the HV-HIIT trial compared to the MICT trial (38.56 ± 3.12, $p = 0.032$). At Post-Ex +5 min, HSPC concentration was significantly greater in the LV-HIIT trial compared to the MICT trial (38.08 ± 3.01 vs. 27.60 ± 3.52, $p = 0.013$) and significantly greater in the HV-HIIT trial compared to the MICT trial (34.28 ± 4.22, $p = 0.025$). At Post-Ex +10 min, HSPC concentration was significantly greater in the LV-HIIT trial compared to the MICT trial (35.28 ± 1.99 vs. 26.60 ± 4.14, $p = 0.038$) and significantly greater in the HV-HIIT trial compared to the MICT trial (29.81 ± 3.37, $p = 0.049$). There was no significant difference in HSPC concentration between the HV-HIIT and LV-HIIT trials at Ex-23 min ($p = 0.120$), Post-Ex ($p = 0.416$), Post-Ex +5 min ($p = 0.341$) and Post-Ex +10 min ($p = 0.166$).

The average number of $CD34^+/CD38^-$ cells as a percentage of total population of cells across the three trials was ~0.02%. Therefore, the concentration of these cells in the peripheral blood was too low to incorporate into the gating strategy to accurately calculate and compare the effect of each trial on the number of $CD34^+/CD38^-$ cells/μL.

Comparison of HSPC and total WBC fold changes

There was no significant difference in the relative fold changes in HSPC and total WBC counts from resting values at any timepoint across the MICT trial ($\eta p^2 = .13$), HV-HIIT trial ($\eta p^2 = .43$) and LV-HIIT trial ($\eta p^2 = .57$) ($p > 0.05$ for all) (Figure 13, 14 and 15). The peak HSPC and WBC fold change in the MICT trial was 1.42 ± 0.05 and 1.27 ± 0.27, respectively, at Ex-16 min. The peak HSPC and WBC fold change in the HV-HIIT trial was 1.74 ± 0.23 and 1.42 ± 0.05, respectively, at Post-Ex. The peak HSPC and WBC fold change in the LV-HIIT trial was 2.05 ± 0.20 and 1.66 ± 0.06, respectively, at Ex-23 min.

Figure 8: *Heart rate (BPM) at the end of each exercise bout for the MICT, HV-HIIT and LV-HIIT trials. Data presented as mean ± SE. Significance was set at p<0.05.* indicates significantly greater than MICT at the same timepoint.* $^{+}$ *indicates significantly greater than HV-HIIT at the same timepoint.*

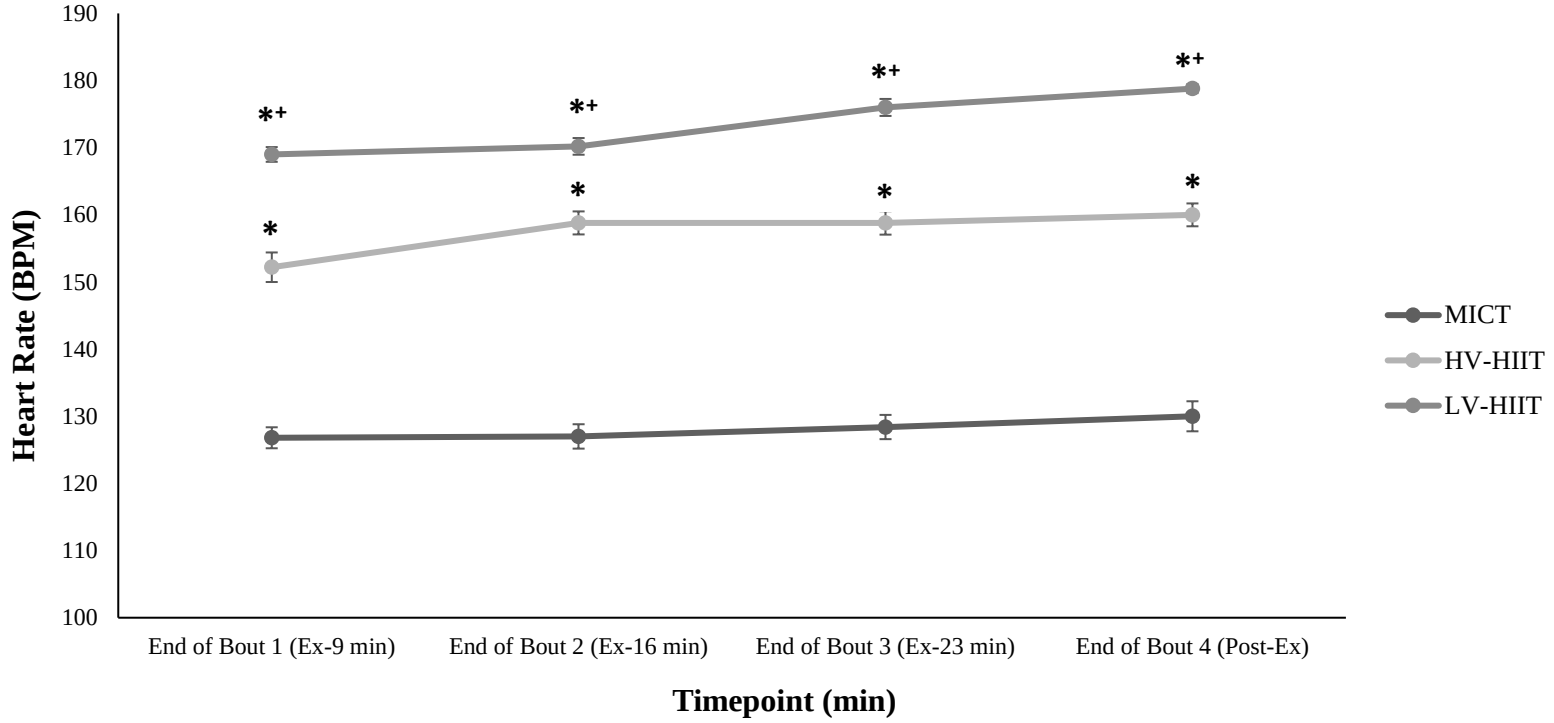


Figure 9: *RPE at the end of each exercise bout for the MICT, HV-HIIT and LV-HIIT trials. Data presented as mean ± SE. Significance was set at p<0.05.* indicates significantly greater than MICT at the same timepoint.* $^{+}$ *indicates significantly greater than HV-HIIT at the same timepoint.*

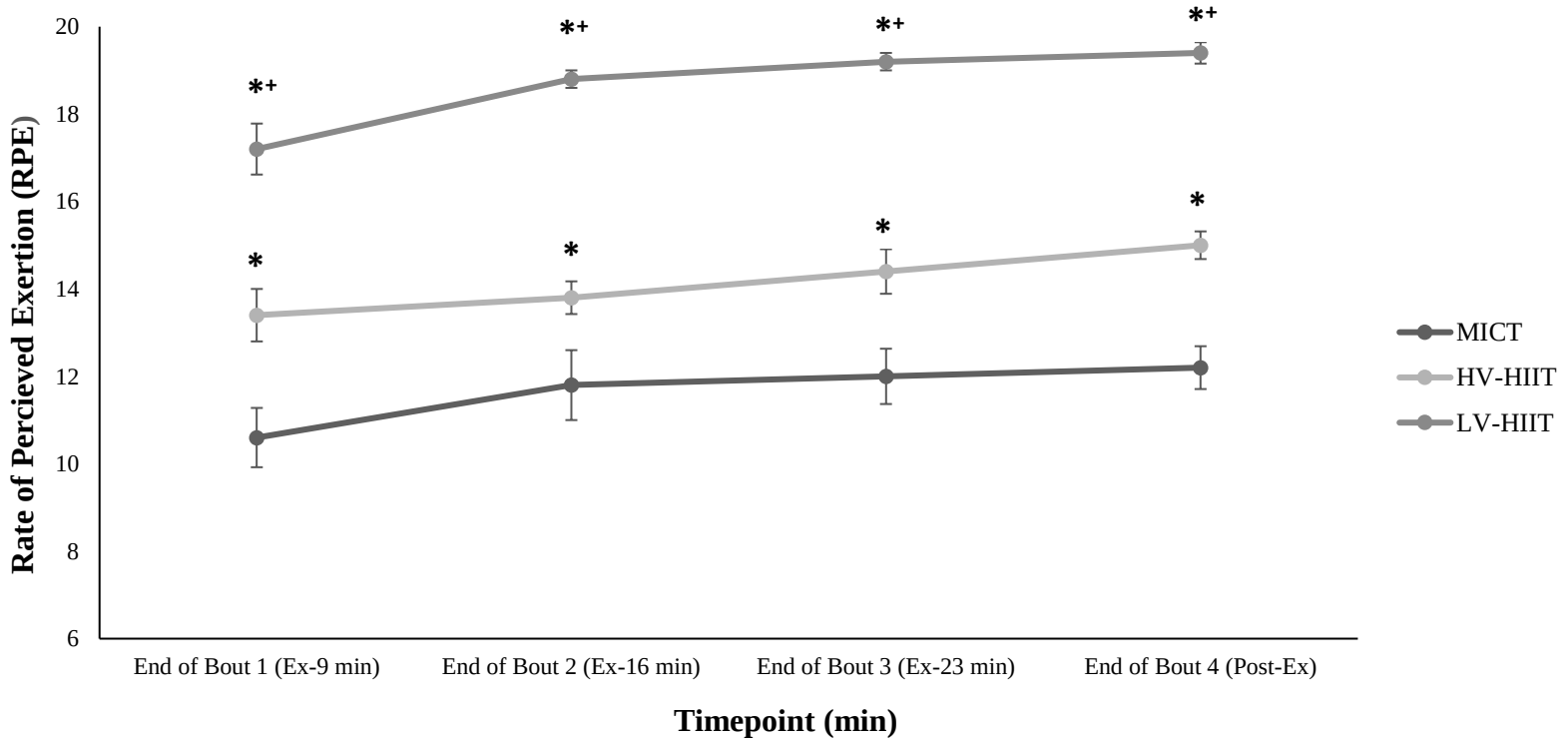


Figure 10: *Mean total white blood cell (WBC) count (cells/μL) across the MICT, HV-HIIT and LV-HIIT trials. Data presented as mean ± SE. Significance was set at p<0.05.* [#] *indicates significantly greater than Pre-Ex for the respective trial. * indicates significantly greater than MICT at the same timepoint.* [+] *indicates significantly greater than HV-HIIT at the same timepoint.*

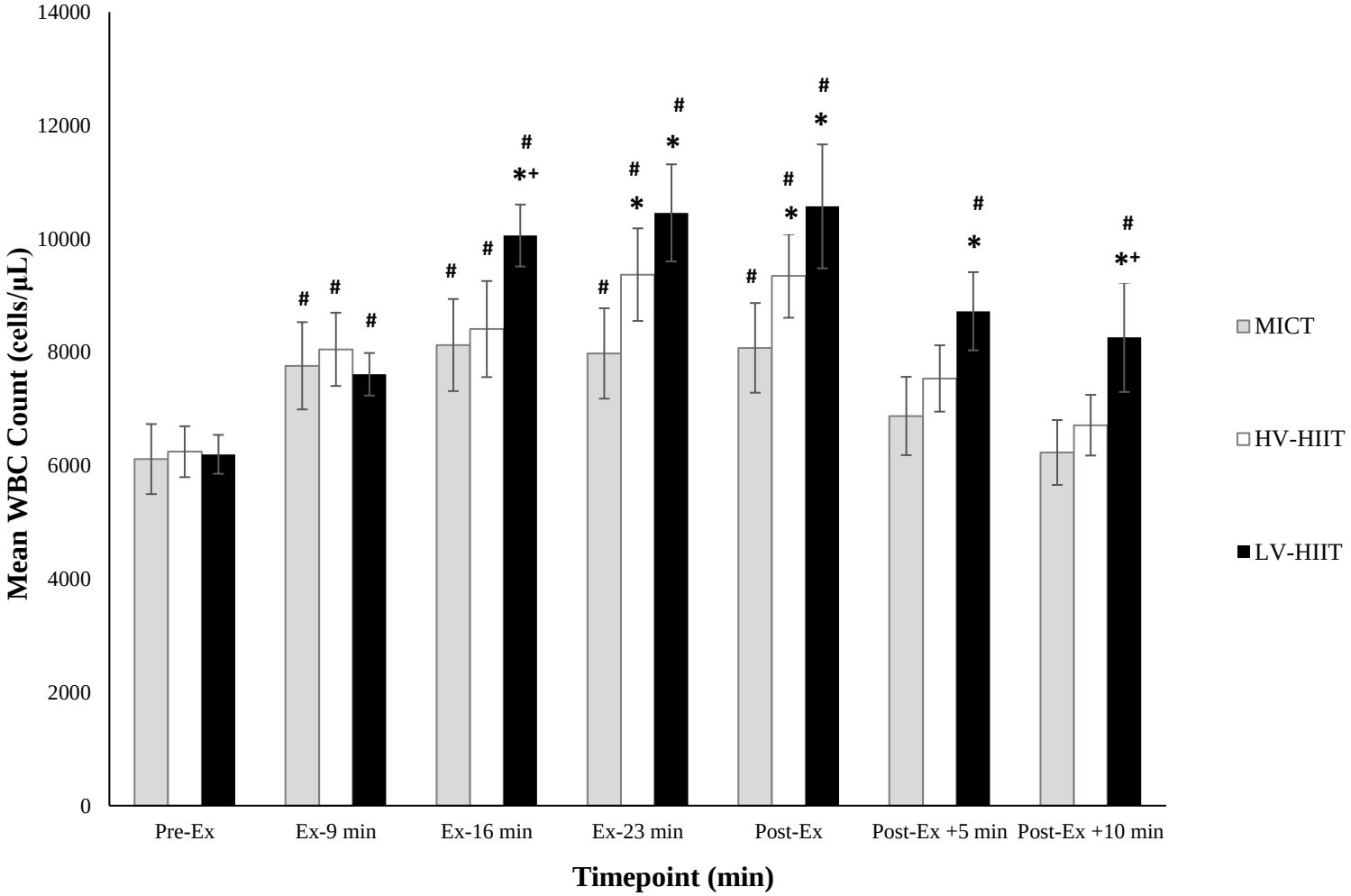


Figure 11: *Mean lymphocyte count (cells/μL) across the MICT, HV-HIIT and LV-HIIT trials. Data presented as mean ± SE. Significance was set at p<0.05.* [#] *indicates significantly greater than Pre-Ex for the respective trial. * indicates significantly greater than MICT at the same timepoint.* [+] *indicates significantly greater than HV-HIIT at the same timepoint.*

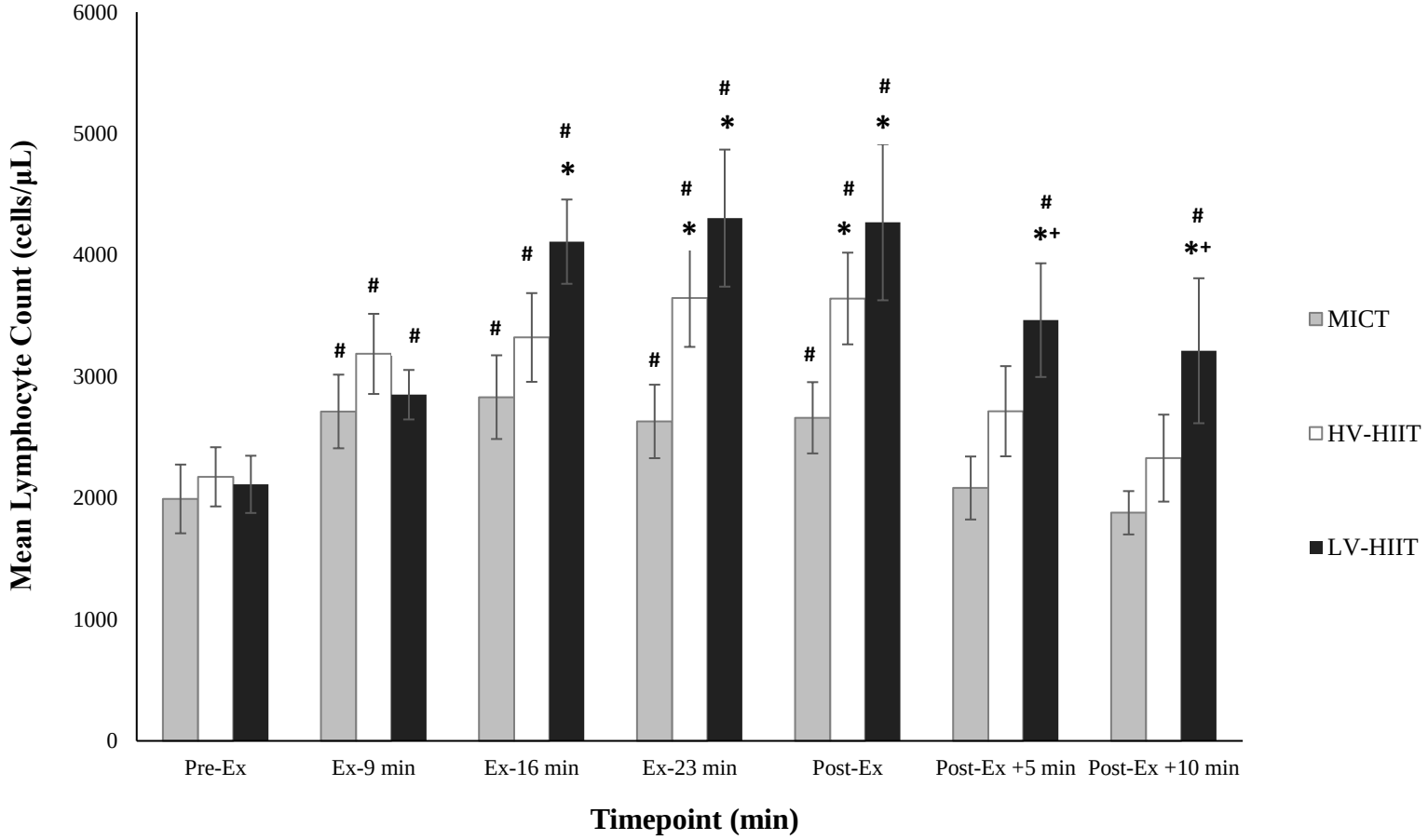


Figure 12: *Mean HSPC concentration (cells/μL) across the MICT, HV-HIIT and LV-HIIT trials. Data presented as mean ± SE. Significance was set at p<0.05.* # *indicates significantly greater than Pre-Ex for the respective trial. * indicates significantly greater than MICT at the same timepoint.*

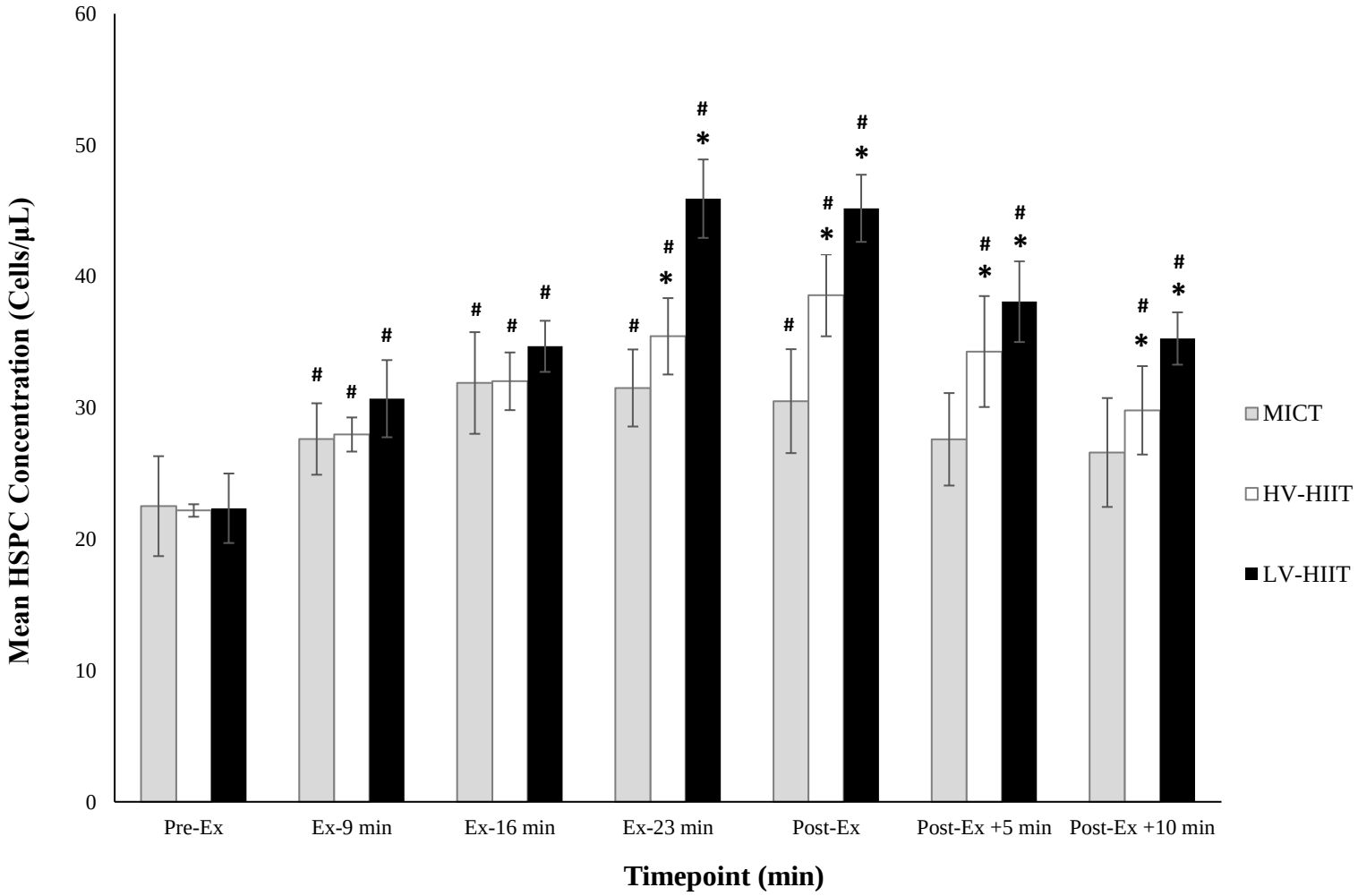


Figure 13: *Comparison of the WBC count and HSPC count relative fold changes from resting values (red dotted line) across the MICT trial. Data presented as mean fold changes ± SE. Significance was set at p<0.05. There was no significant difference in the mean relative fold change of WBC and HSPC counts at any timepoint.*

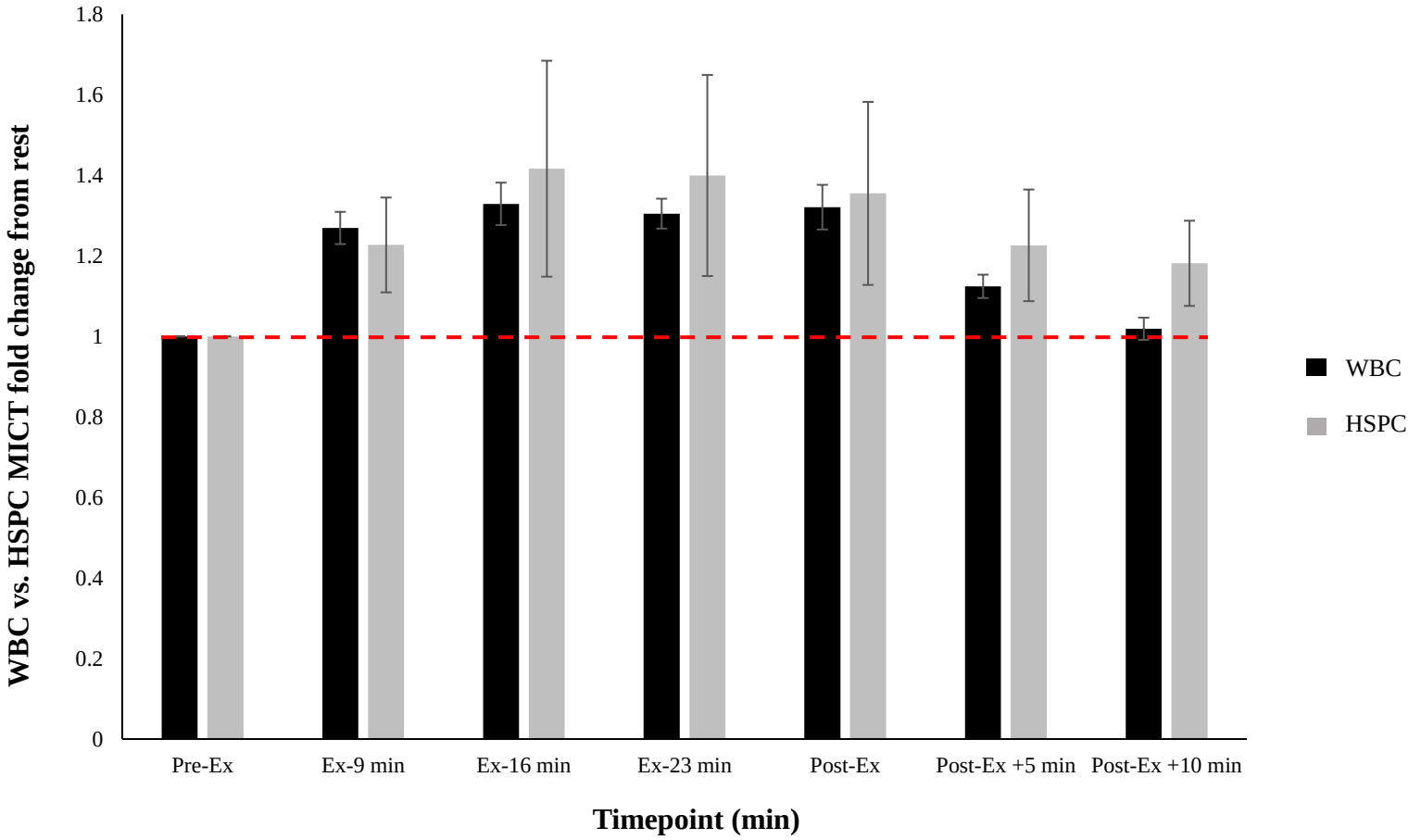


Figure 14: *Comparison of the WBC count and HSPC count relative fold changes from resting values (red dotted line) across the HV-HIIT trial. Data presented as mean fold changes ± SE. Significance was set at p<0.05. There was no significant difference in the mean relative fold change of WBC and HSPC counts at any timepoint.*

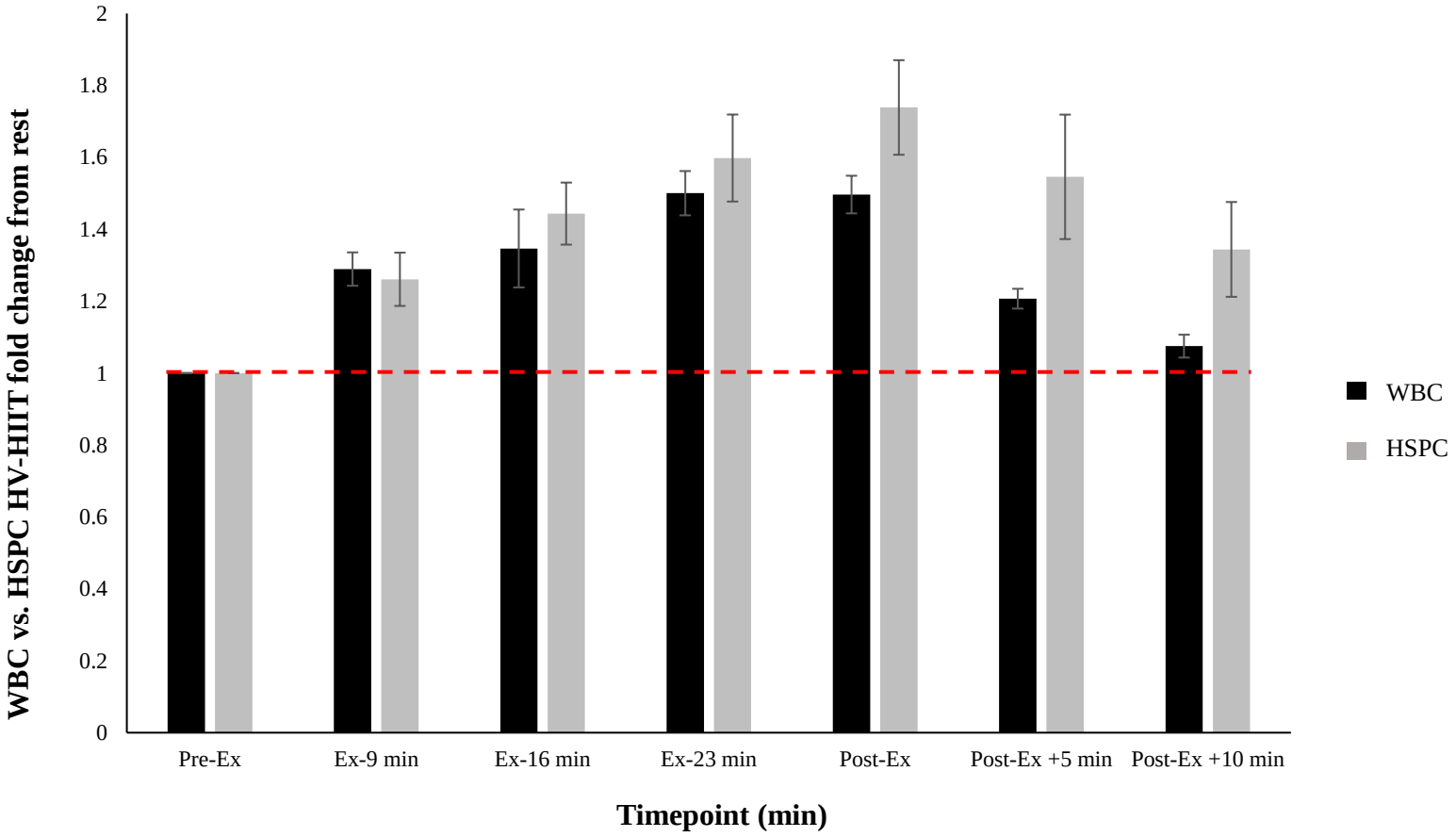
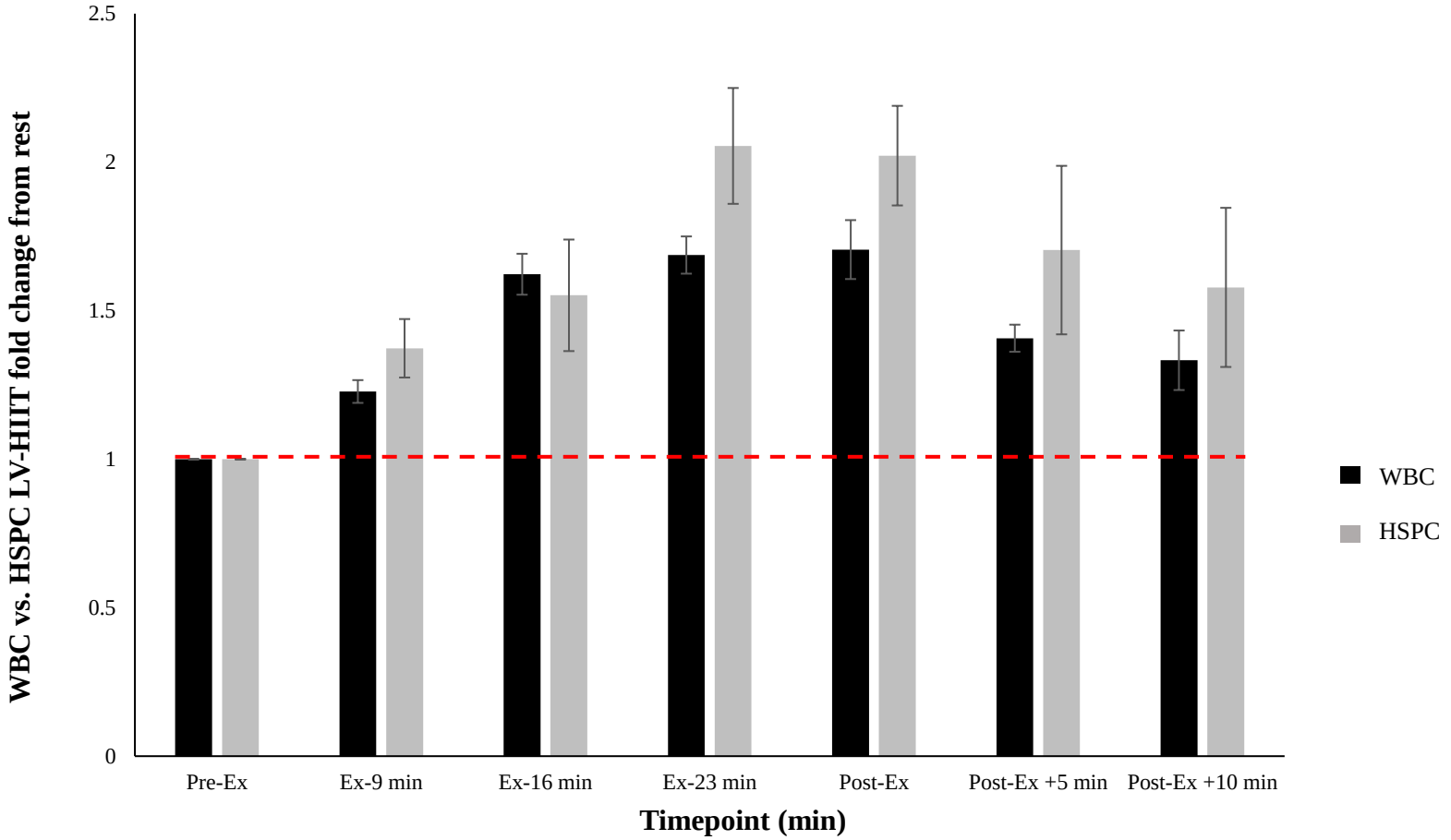


Figure 15: *Comparison of the WBC count and HSPC count relative fold changes from resting values (red dotted line) across the LV-HIIT trial. Data presented as mean fold changes ± SE. Significance was set at $p<0.05$. There was no significant difference in the mean relative fold change of WBC and HSPC counts at any timepoint.*



DISCUSSION

It is well established that acute exercise increases HSPC concentration in peripheral blood. However, despite evidence indicating that both exercise volume and intensity are key drivers of HSPC mobilisation, the optimal exercise protocol to maximise HSPC concentration after exercise, remains unclear. The purpose of the present study was to compare differences in HSPC concentrations after four successive bouts of HV-HIIT and LV-HIIT, compared to a bout of MICT using ISHAGE guidelines. The primary finding from our study was that HIIT evoked higher peak HSPC concentrations compared to MICT (Figure 12). Indeed, our findings complement a recent study highlighting that HIIT evokes a greater HSPC mobilisation response compared MICT [37]. Furthermore, there was no significant difference in HSPC concentration between LV-HIIT and HV-HIIT, despite a reduction in the total volume of work performed in LV-HIIT. The implication of this finding is that, in an already time-consuming, arduous donation procedure, LV-HIIT could be an effective protocol to increase peripheral blood HSPC concentration for a lower total exercise dose and exercise time commitment. HSPC concentration was significantly greater in LV-HIIT compared to MICT after Ex-23 min, which only equated to 6 minutes of cycling in the LV-HIIT trial compared to 23 minutes of cycling in the MICT trial. Thus, LV-HIIT appears to be more practical to use in a donor setting. Indeed, our findings align with other studies indicating that exercise intensity, not exercise volume, may be the main driver of HSPC mobilisation [29,30].

HSPC concentration significantly increased over time in all three trials. Further investigation into the HSPC mobilisation kinetics across all 7 time points reveals that at Ex-23 min, HV-HIIT and LV-HIIT evoked greater HSPC concentrations compared to MICT and sustained this significantly greater response in HSPC concentration up to Post-Ex +10 min. Therefore, it can be assumed that the cumulative response from multiple HIIT bouts, whether that be the clinical 4 × 4-min HV-HIIT model

[33] or the novel 4 × 2-min LV-HIIT model is sufficient to induce a significantly greater, longer-lasting elevation in HSPC concentration compared to MICT. The HSPC concentration in LV-HIIT also peaked at Ex-23 min, echoing previous studies that also employed serial blood sampling, demonstrating that HSPC concentrations can peak during the exercise bout rather than immediately at exercise cessation as many previous studies have reported [27–29].

The approximate 2-fold increase in HSPC concentration for the HIIT trials observed in the present study is consistent with previous studies reporting a similar relative 2 – 3-fold increase above resting levels [28–30]. However, the absolute numbers of HSPCs/μL observed in the present study varies drastically from the 2 – 16 HSPCs/μL reported in majority of previous studies [20,22,26,27,29–31]. This is likely due to the different methodology used in the present study i.e. the single-platform ISHAGE protocol for the enumeration of HSPCs [49], compared to the dual-platform approach used in previous studies. The single-platform approach mitigates the potential for errors due to the reduction in sample handling and calculation of results [49], making it a more accurate measure of HSPC concentration. A multicentre study comparing the CV's across different laboratories further demonstrated the increased proficiency of the single-platform method for the enumeration of HSPCs [62]. The single-platform approach also accounts for the lymphocyte/monocyte ratio to produce a more accurate HSPC count, therefore, the failure of the dual-platform approach to account for this ratio in previous studies may also explain the variability in HSPC concentration between studies [64]. It is important to note that the inability of the present study to accurately calculate and compare the effect of each trial on the concentration of $CD34^{+}/CD38^{-}$ cells/μL is likely due to the samples being too dilute following the single-platform ISHAGE approach. Therefore, the effect of exercise on this most primitive HSC population remains unclear following equivocal findings [20,30].

The present study measured total leukocyte (Figure 10) and lymphocyte (Figure 11) mobilisation in response to the three exercise trials to determine whether HSPCs were preferentially recruited or mobilised uniformly with all leukocytes. The total leukocyte and lymphocyte mobilisation response was greater following HIIT compared to MICT, agreeing with previous findings that exercise-induced leukocytosis is also intensity-dependent [65]. LV-HIIT induced greater total WBC and lymphocyte concentrations compared to both MICT and HV-HIIT during exercise at Ex-16 min and at Post-Ex +5 min and Post-Ex +10 min. Therefore, it is important to note that whilst HV-HIIT and LV-HIIT induced similar HSPC mobilisation responses, LV-HIIT appeared to have a more profound effect on exercise-induced leukocyte mobilisation, particularly post-exercise, compared to HV-HIIT. The comparison between relative fold changes for WBC and HSPC concentrations revealed no significant differences across all three trials (Figure 13, 14 and 15). Therefore, the mobilisation of HSPCs in response to the exercise protocols employed in this study appears to be a reflection of the general exercise-induced leukocyte mobilisation response, as reported in recent studies [30,31], indicating a uniform mobilisation response. However, the 'large' fold change effect sizes for HV-HIIT ($\eta p^2 = .43$) and LV-HIIT ($\eta p^2 = .57$) suggests a trend towards a preferential HSPC recruitment with HIIT. The statistical power is a limitation of our study; thus, we may have been underpowered to detect significant differences in magnitude of response between WBC and HSPC. An exercise-induced preferential mobilisation response would mean that natural killer cells and cytotoxic T-cells could be mobilised into the peripheral blood alongside HSPCs. It is well established that these cell types are mobilised in an intensity-dependent manner due to their antiviral function e.g. high cytotoxicity and tissue migration potential [66]. Indeed, in the context of HSPC transplants, the addition of these natural killer cells and cytotoxic T-cells alongside HSPCs would help improve the donor blood graft [11] and

prevent post-transplant viral infections and graft-versus-host disease through heightened immune surveillance, thus increasing the likelihood of patient survival post-transplant [8].

From a mechanistic perspective, as previous studies have already suggested, there is likely no single mechanism responsible for the observed exercise-induced HSPC mobilisation [11]. Instead, there is likely to be a combination of factors working simultaneously. Exercise-induced increases in blood flow and subsequent shear stress are the key mechanisms underlying general leukocyte mobilisation during exercise [39]. Thus, as the present study suggests a uniform mobilisation response, indicated by the fold change comparisons, it is entirely plausible that this increased blood flow and subsequent shear stress may also simultaneously mobilise HSPCs residing in the walls of the vascular endothelium [39] and potentially from the bone marrow niche [40]. This would explain why HIIT induced a greater HSPC mobilisation response compared to MICT as blood flow and shear stress is increased with greater intensities of exercise [67]. Our findings support this as heart rate was significantly greater in HIIT compared to MICT at the end of each bout, indicating that HIIT induced a greater cardiovascular response (Figure 8). Nonetheless, the 'large' effect sizes for the fold change comparisons during HIIT suggests that HSPCs could be preferentially mobilised with greater intensities of exercise. Exercise-induced increases in adrenaline evokes a preferential recruitment of cytotoxic lymphocytes with greater β2-AR expression from the vascular endothelium and marginal pools within the circulation [41]. A previous study investigating the expression of adrenergic receptors on HSPCs revealed that HSPCs also express these β2-AR [68]. Therefore, it is entirely possible that exercise preferentially mobilises HSPCs via a β2-AR mediated pathway in an adrenaline-dependent manner, similar to the mobilisation of cytotoxic lymphocytes, as suggested in a recent study reporting that the mobilisation of HSPCs is dependent on β2-AR signalling [29].

Interleukins (e.g. IL-6), chemokines (e.g. CXCL12) and other cytokines (e.g. G-CSF) are thought to be potential mediators of exercise-induced HSPC mobilisation [11]. The production of these cytokines in extramedullary tissues is increased with greater exercise intensities, which is thought to cause a brief preferential recruitment of HSPCs to the peripheral circulation, before facilitating HSPC homing to extramedullary sites of tissue damage and inflammation to aid in repair [44]. Therefore, the increased HSPC concentration in the HIIT trials may be a result of the increased production these cytokines and would also explain the transient nature of the increase in HSPC concentration observed in the present study and majority of the previous studies reported in Table 1. However, direct experimental evidence indicating a direct role of these cytokines in exercise-induced HSPC mobilisation is lacking. Thus, we recommend that future studies employ a more experimental approach to unravel the mechanisms underlying the exercise-induced HSPC mobilisation kinetics, albeit an apparent transient response, and confirm whether it is a preferential or uniform increase. The present study collected and froze PBMCs (Appendix 1) and plasma samples (Appendix 2) with the intention to conduct a follow-up study with a more mechanistic focus. However, the restrictions due to the Covid-19 pandemic hindered the completion of the follow-up study. Furthermore, the exact location where these exercise-induced circulating HSPCs are being mobilised from, whether that be from the bone marrow [44] or the walls of the vascular endothelium [29], is still unknown and warrants further investigation. Given that HSPC concentration was significantly greater in the HIIT trials at Post-Ex +10 min, it is entirely possible that HSPCs could be recruited to the peripheral blood from the bone marrow beyond exercise cessation, to combat the increased physiological stress posed by the HIIT protocols and aid in tissue repair, as previous studies have suggested [26,38,44,47].

Practical applications

The current HSPC donation procedure involves several barriers which prevents the process working optimally. Firstly, as previously mentioned, G-CSF therapy and Plerixafor administration can be expensive treatments and cause a number of harmful side-effects. Secondly, when a donor is deemed a 'poor mobiliser', they often have to come back for a second donation day and repeat the arduous procedure or even undergo a bone marrow aspirate, which can be painful [10]. Another issue is that due to allogenic donation being anonymous, if the donor is of significantly smaller stature than the patient, more HSPCs may be required as apheresis only occurs when a sufficient number of HSPCs per kg of body weight is obtained (usually $2–4 \times 10^6$ $CD34^+$ cells/kg body weight) [14]. Therefore, maximising HSPC yields is of paramount importance and any adjuvant therapies which can complement existing therapies would be beneficial. Previous studies have discussed the efficacy of utilising exercise as a potential adjuvant therapy alongside pharmaceutical administration to increase HSPC mobilisation and optimise the yield of HSPCs available for allogenic transplants in clinical settings [11]. The practicality of introducing the use of exercise for increasing HSPC mobilisation in clinical settings is only now being considered [51], and is still a very topical subject as highlighted in a recent meta-analysis by Martha et al., (2021) [69]. Short bursts of exercise throughout a 4-hour donation session may provide a safe, feasible, low-cost approach to rapidly increase HSPC mobilisation prior to apheresis whilst reducing the donor burden and side-effects from pharmaceutical administration and decreasing the likelihood for donors to undergo a painful bone marrow aspiration [11].

The feeling scale is designed to measure how one feels psychologically during exercise [61]. All three trials in the present study scored 'good' on the feeling scale, suggesting that the HIIT trials are as tolerable as the MICT trial. This findings supports a previous study which reported that HIIT is

perceived as more enjoyable than MICT [70]. Therefore, the incorporation of HIIT into the HSPC donation setting, rather than MICT as previous studies have recommended, may encourage increased patient adherence to the exercise sessions, improving the HSPC donation process. Furthermore, the HIIT trials evoked greater increases HSPC concentration compared to MICT despite a lower total exercise dose and time commitment. Specifically, the HSPC concentration was significantly greater in the HIIT trials at Post-Ex +10 min compared to Pre-Ex, whilst the HSPC concentration had returned to baseline levels at Post-Ex +10min in the MICT trial. Thus, it is suggested that while LV-HIIT involves a shorter duration of total exercise, it can induce similar increases in HSPC mobilisation as HV-HIIT and can sustain this effect for a longer period of time than MICT. Therefore, LV-HIIT serves promise as an effective, time-saving, feasible protocol to be conducted throughout the arduous 4-hour apheresis procedure [15]. An example of how LV-HIIT could be utilised as an adjuvant therapy is incorporating evenly spaced HIIT intervals, i.e. every 1 hour, across the 4-hour apheresis session to repeatedly spike HSPC concentrations, maximising the yield for donation. The greater, longer-lasting elevation in lymphocyte concentration, particularly post-exercise, and the 'large' effect size indicating a preferential recruitment, also suggests that LV-HIIT could improve the donor blood graft through the increased mobilisation of cytotoxic lymphocytes to be harvested alongside HSPCs following exercise cessation.

HIIT programmes such as the novel LV-HIIT protocol utilised in our study should be incorporated into a typical 4-hour mock HSPC donation day. This would enable researchers to put the theoretical applications of HIIT for HSPC donations into practice to identify if it is both feasible and effective in clinical settings. If deemed impractical to prescribe, a future study could explore the idea of attaching cycling pedals to the end of the typical donation chair to encourage patients to complete some form of exercise, albeit at an inevitably lower intensity. Increasing the period of time that HSPC

concentration remains elevated could extend the timeframe available for the clinician to harvest these HSPCs, thus, increasing the practicality of utilising exercise in the clinical donation setting. An example of exercise being successfully incorporated into a clinical setting is the use of intradialytic cycling for patients with kidney disease at Leicester General Hospital to improve physical function and reduce the risk of mortality [71]. Furthermore, it is evident that the HSPC concentrations following the exercise trials are significantly lower than the HSPC concentrations achieved following pharmaceutical administration, hence, exercise cannot be the sole method for promoting HSPC mobilisation. However, the potential for exercise to reduce required drug dosage and subsequent side-effects and provide a safe, feasible, low-cost approach to enhance HSPC mobilisation warrants further work to analyse the effect of combining both approaches of HIIT and pharmaceutical administration on HSPC mobilisation.

In the context of autologous transplants, any exercise protocol intervention prescribed would need to be tailored accordingly, i.e. reducing the exercise intensity, as traditional exercise guidelines for healthy individuals are intolerable for patients with haematological malignancies [72]. Nevertheless, it has been suggested that exercise in autologous transplant patients, albeit at a lower exercise intensity, may optimise the stem cell niche to receive transplanted HSPCs [73]. Thus, the prescription of exercise offers promise in both allogenic and autologous transplant settings. Indeed, the COVID-19 pandemic has encouraged researchers to explore the use of home-based exercise protocols in studies. Wood et al., (2020) recent pilot clinical trial assessing the use of home-based exercise prescription before HSPC transplantation reported that exercise preconditioning could increase the likelihood of post-transplant survival [74]. Therefore, patients completing exercise programmes at home prior to attending HSPC donation sessions is certainly a potential window of opportunity for future studies to investigate further.

Experimental considerations

The present study is not without its limitations. The low participant numbers that completed the study and were included in the data analyses means that the statistical power of the present study suggests we may have been underpowered. In addition, given that the study was a cycling study conducted in all young, healthy, and active males, we cannot specifically extrapolate our findings to older adults, females, inactive individuals, or other forms of exercise modes. Therefore, we recommend that future research should aim to include a higher number of participants of a varied age range and fitness level, which may reveal differences in WBC and HSPC fold change comparisons as suggested by the effect sizes. Furthermore, future research should explore the effects with different exercise modes – both in an acute and chronic context, conduct the study with female participants and incorporate more HIIT protocols to obtain a greater comprehensive analysis on the effect of HV-HIIT and LV-HIIT on HSPC mobilisation.

Furthermore, another limitation the inability of the current study to detect $CD34^{+}/CD38^{-}$ cells/μL due to sample dilution following the single-platform ISHAGE approach. Previous studies employing the dual-platform approach have been able to detect $CD34^{+}/CD38^{-}$ cells/μL [20,30,31], thus, future studies should consider employing PBMC isolation techniques, similar to those conducted in previous studies, in order to study this most primitive HSC population in further detail, but using a dual-platform approach. The present study fails to determine when HSPC concentration returns to baseline following the HIIT trials. Therefore, additional blood samples beyond Post-Ex +10 min would help ascertain exactly when HSPC concentration returns to baseline following the HIIT session and determine if there is a biphasic response before baseline levels are reached. Moreover, blood samples

at both before and after each HIIT bout would allow researchers to conduct area under the curve analysis and attain a clearer picture of the HSPC kinetics throughout the entire session. However, if one desires to use the single-platform ISHAGE approach, careful consideration will need to be taken to ensure all blood samples can still be analysed within 1 hour of lysis as $CD34^+$ count is significantly reduced beyond this timepoint (Figure 4A). Another limitation is the inability to completely eradicate the effect of confounding variables on HSPC mobilisation. Psychological factors e.g. anxiety and unpleasant moods during exercise, lifestyle habits e.g. poor sleep quality and poor diet and environmental conditions e.g. extreme temperatures and humidity are associated with suppressed immune function [75], therefore, potentially hindering HSPC mobilisation. The present study partially accounted for these covariates thought to adversely affect immune function in order to mitigate their effect on the observed exercise-induced HSPC mobilisation response.

CONCLUSION

In summary, we demonstrate in the present study that HIIT evoked higher peak HSPC concentrations and sustained this elevation for a longer duration compared to MICT. Furthermore, LV-HIIT evoked a similar increase in HSPC concentration to HV-HIIT - despite a reduction in training volume, indicating that the intensity of exercise is the key driver for this exercise-induced HSPC mobilisation response. The study was unsuccessful in comparing the effect of each trial on the concentration of $CD34^{+}/CD38^{-}$ cells/μL, hence, more work is required investigating this most primitive HSC population. The present study also reports that the total leukocyte concentration was greater in response to HIIT compared to MICT, with the suggestion that HSPCs are recruited as part of a uniform mobilisation response alongside all leukocytes. Future studies should employ a more experimental approach to unravel the mechanisms underlying exercise-induced HSPC mobilisation, which is likely to be a combination of factors associated with increased blood flow and shear stress, increased adrenaline release and increased cytokine release. Our study suggests that HIIT serves promise as a time-saving, feasible exercise protocol that could possibly be used alongside pharmaceutical administration during the HSPC donation process. Considering the compromised efficacy of current HSPC collection protocols in the clinical donation setting, the possibility of using exercise as an adjuvant therapy to enhance HSPC mobilisation, improve the donor blood graft, and lessen the donor burden is certainly an area worth exploring in greater detail.

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APPENDICES

Appendix 1: PBMC isolation and cryopreservation

10mL of blood was collected from Pre-Ex and Post-Ex in separate vacutainer tubes containing EDTA (*BD Vacutainer, United Kingdom*) and was used to isolate PBMCs using density gradient centrifugation (*Thermo Fisher Scientific Multifuge X1R, USA*). Blood was diluted 1:1 with phosphate-buffered saline (PBS) (*Fisher Scientific, United Kingdom*), and then layered carefully on top of an equal volume of Histopaque 1077 (*Merck Life Science, United Kingdom*), before centrifuging at 400 × g for 40 minutes at 21°C. The PBMC layer was then removed to a fresh centrifuge tube and washed with PBS before being centrifuged at 300 × g for 10 minutes at room temperature. Finally, the supernatant was discarded, and the PBMC pellet was again washed with PBS and centrifuged at 300 × g for 10 minutes at room temperature before being resuspended in 1mL of Roswell Park Memorial Institute (RPMI) 1640 Medium (*Fisher Scientific, United Kingdom*). 20µL of the cell suspension was then stained with 20µL of Acridine Orange-Propidium Iodide (AOPI) (*VWR International, United Kingdom)* and then 20µL of this mixture was pipetted into a cell counting chamber to count the PBMCs using an automated cell viability counter (*Nexcelom Cellometer Auto 2000, USA*). Following this, ice-cold freeze media, made up of RPMI, fetal bovine serum (*Merck Life Science, United Kingdom)* and dimethyl sulfoxide (*Merck Life Science, United Kingdom*), was added dropwise to the PBMCs. The PBMCs were then distributed to cryogenic storage vials (*Fisher Scientific, United Kingdom),* placed in a CoolCell freezing box (*Fisher Scientific, United Kingdom*) and transferred to a -80^{o}C freezer in the University of Birmingham Human Biosciences Resource Centre (HBRC) for cryopreservation until analysis.

Appendix 2: Plasma isolation and storage

4mL of blood was collected from Pre-Ex and Post-Ex in separate vacutainer tubes containing EDTA (*BD Vacutainer, United Kingdom*) for isolation and storage of plasma. Following blood withdrawal, the samples were immediately placed on ice before being centrifuged at 1525 × g for 10 minutes at 4°C (*Thermo Fisher Scientific Multifuge X1R, USA*) to separate the blood components and isolate the plasma. Three 500µL aliquots of the plasma were then stored at -80°C for future enzyme-linked immunoassay (ELISA) analysis.