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BLOOD VOLUME EXPANSION FOLLOWING SUPRAMAXIMAL EXERCISE

–OCCURRENCE AND CONTRIBUTION TO MAXIMAL OXYGEN UPTAKE

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Thesis for Doctoral Degree (Ph.D.)

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Popular science summary of the thesis

Sprint-interval training is a form of exercise characterized by short bursts of maximal intensity or "all-out" effort, i.e., each interval is performed with the intention of being as hard/fast as possible. The intervals are separated by a few minutes of rest before a new interval is performed. Sprint-interval training has proven to be an effective form of training when it comes to increasing maximal oxygen consumption. Despite the low time expenditure (~10 minutes), the exercise-induced effects after sprint-interval training are comparable to those after training programs involving traditional endurance exercises with a 3-4-fold higher time expenditure per session. Previous studies have shown that sprint-interval training has a pronounced effect on adaptations within the skeletal muscle. These adaptations include muscle capillarization, mitochondrial content and function, and a number of genes involved in the ability of skeletal muscle to obtain and utilize oxygen during exercise respond positively to sprint-interval training. However, less is known about the central hemodynamic effects after sprint-interval training. Central adaptations include factors responsible for oxygen delivery to the working muscle. Cardiac size and structure, total blood volume, and hemoglobin mass are all part of central hemodynamics.

This thesis shows that sprint-interval training leads to numerous changes in the body that occur acutely after sprint-interval training. Some of these changes can be used to model and predict how much maximal oxygen uptake will increase after 6 weeks of sprint-interval training. Most improvements in maximal oxygen uptake after 6 weeks of sprint-interval training are due to an increase in total blood volume. This leads to an improved ability to transport oxygen from the atmosphere to the working muscles and allows for improved maximal oxygen uptake. In addition to improving central adaptations, sprint-interval training also leads to adaptations that improve the body's ability to extract oxygen from the blood and transport it to the muscles. All of these adaptations are important factors in increasing maximal oxygen uptake.

Abstract

Previously published research using various types of exercise has shown that central hemodynamic factors such as blood volume (BV) and maximal cardiac output (Q_{max}) are of large importance in the mediation of improvements in VO_{2max}. Whether this is true for adaptations induced by sprint-interval training (SIT) is unclear. Three experimental studies were carried out investigating the occurrence and contribution of hypervolemia to SIT-induced improvements in VO_{2max}. Forty-eight study participants performed the interventions. Significant increases in BV and Q_{max} were observed after the 6-week training interventions in conjunction with the expected increases in VO_{2max} (Paper I and II). The hypervolemic response was shown not only to be associated with the increase in VO_{2max} but also to be the primary mediator of it, as demonstrated by the elimination of the exercise-induced increases in VO_{2max} when BV was normalized to pre-intervention levels by phlebotomy. This demonstrates that central adaptations are paramount for the SIT-induced increase in VO_{2max}. In addition, systemic oxygen extraction increased as a consequence of decreased venous oxygen content during maximal exercise (Paper II). This suggests that both peripheral and central factors are responsible for the adaptations in VO_{2max} observed with SIT and refutes previous theories proposing that the increase in VO_{2max} was mediated primarily by peripheral adaptations. Metabolic and intravascular perturbation has been proposed as an important stimuli for exercise adaptation. Since SIT is one of the most intense forms of exercise available the immediate effects after SIT are interesting in order to understand how such small amounts of exercise can lead to cardiovascular adaptations usually associated with more prolonged types of exercise. Acute effects of one session of SIT caused pronounced disturbance of the intravascular milieu and perturbations of the muscle metabolism. The variable that correlated best with changes in plasma and muscle volume was glucose-6-phosphate (Paper III). Similarly, plasma osmolality and plasma concentration of arginine and citrulline was shown to be the best predictors of improvements in VO_{2max} after a training intervention of 6-weeks (Paper IV).

Overall, the present work demonstrates that brief supramaximal exercise leads to improvements in VO_{2max} and that these improvements are mediated mainly by central adaptations. Peripheral adaptations occur concurrently but cannot alone explain the improvements in VO_{2max}, as previously hypothesized. The data presented show that both central and peripheral adaptations are involved in the improvement of VO_{2max} after an SIT intervention.

List of scientific papers

I. **Mandić, M.**, Hansson, B., Lovrić, A., Sundblad, P., Vollaard, N. B. J., Lundberg, T. R., Gustafsson, T., & Rullman, E. (2022). Improvements in Maximal Oxygen Uptake After Sprint–Interval Training Coincide with Increases in Central Hemodynamic Factors. *Medicine & Science in Sports & Exercise*, *54*(6), 944–952.

II. **Mandić, M.**, Eriksson L. M. J., Melin, M., Skott, V., Sundblad, P., Gustafsson, T., & Rullman, E. (2022). Increased maximal oxygen uptake after sprint–interval training is mediated by central hemodynamic factors as determined by right heart catheterization – *Manuscript in review*

III. **Mandić, M.**, Forsgren, M. F., Romu, T., Widholm, P., Sundblad, P., Gustafsson, T., & Rullman, E. (2021). Interval-induced metabolic perturbation determines tissue fluid shifts into skeletal muscle. *Physiological Reports*, *9*(7).

IV. **Mandić, M.**, Gustafsson, T., Sundblad, P., Rooyackers, O., & Rullman, E. (2022). Changes in metabolite concentrations after supramaximal exercise – *Manuscript*

Contents

1 Background

1.1 Oxygen uptake

In the last decade of the 18th century, Antoine-Laurent Lavoisier showed in a series of experiments that the consumption of oxygen (VO_2) was dependent on a number of factors such as food intake, environmental temperature and muscular work (1). Since the days of Lavoisier we have learned a great deal regarding the importance of oxygen (O_2) and confirmed some of the early theories proposed by Lavoisier more than 200 years ago.

Under resting conditions, VO_2 is 2.5-4.0 mL kg^{-1} min^{-1}. These values are approximations and depend on a number of factors, the most important being body composition, i.e. the amount of metabolically active tissue (2–5). As a result of the increased metabolic rate and adenosine triphosphate (ATP) turnover during muscular work, VO_2 can increase many-fold in order to meet the demands set out by the intensity of the activity.

Maximal oxygen uptake (VO_{2max}) is defined as the maximal rate at which O_2 can be taken up and used by the body during exercise (6). For healthy adults, VO_{2max} ranges between 35-60 mL kg^{-1} min^{-1} but will vary depending on sex, age, training status, genetic predisposition and exercise mode, just to name a few (7–9).

Since the human body is a multicellular organism in which most cells are located far from atmospheric O_2, we rely on a transport and gas exchange system to move O_2 from the air to tissues and to support the continuous resynthesis of ATP by oxidative metabolism in mitochondria. This is accomplished by gradient-dependent diffusion, in which O_2 passively moves down a concentration gradient, and by convective transport through the cardiovascular system.

The importance of the cardiovascular system is conceptualized by the Fick principle (VO_2 = Q x a-vO_2diff) which states that VO_2 is the product of the cardiac output (Q) and the arteriovenous oxygen difference (a-vO_2diff). In other words, any change in VO_{2max} must be explained by a change in either maximal cardiac output (Q_{max}) or a-vO_2diff, or both of the variables concomitantly.

VO_{2max} is not only an important determinant of endurance performance but also a strong predictor of mortality (10). Therefore, there is continued interest in this variable and its underlying mediators. The most effective way to increase VO_{2max} is through endurance training, and historically in the general population, prolonged submaximal training sessions have been the preferred method to improve VO_{2max}. This type of exercise, hereafter referred to as traditional endurance training (TET), typically consist of hour-long activities such as running, cross-country skiing, or rowing at an intensity of 50-75% of VO_{2max} with little or no variation in intensity. In the last decade, more and more attention has been

paid to different types of interval training regimes, characterized by short, strenuous efforts just below, at, or above VO$_{2max}$, interspersed with recovery periods. Although there is no standard nomenclature for these different types of interval regimes, they can be broadly divided into two basic types. High-intensity interval training (HIIT) and sprint-interval training (SIT). HIIT is defined by interval intensities that are close to maximal effort (≥80% of maximal heart rate), whereas SIT is characterized by interval efforts performed at or above the intensity that elicits VO$_{2max}$ [11] (Figure 1).

HIIT and SIT have been shown to be efficient in increasing VO$_{2max}$ [12,13] in both younger [14] and older individuals [15]. Moreover, this type of training has been shown to be well tolerated by different groups of patients [16,17].
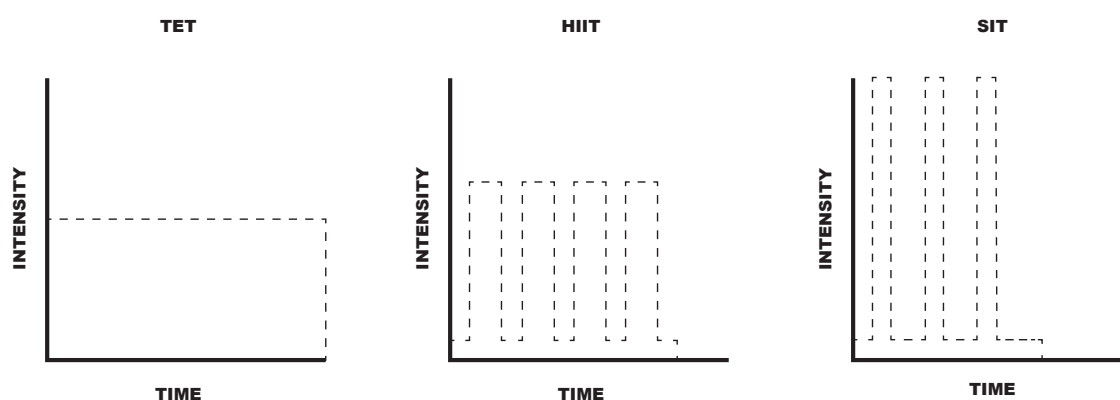

Figure 1. Intensity profile for traditional endurance training (TET), High-intensity interval training (HIIT) and sprint-interval training (SIT).

1.2 Underlying adaptations mediating increases in maximal oxygen uptake following traditional endurance exercise

When discussing the underlying mediators of improvement or the limiting factors of VO$_{2max}$, an overarching concept is utilized that divides the adaptations that occur into peripheral and central adaptations. Incorporating the Fick equation into this concept, central adaptations are represented by Q and peripheral adaptations by a-vO$_2$diff. The same framework will be utilized in the current thesis, although it should be recognized that these factors are not completely independent of each other. This is exemplified by the possibility of increased systemic O$_2$ extraction as a consequence of an increase in Q$_{max}$ through augmented blood flow to the active tissue and/or improved capillary recruitment, shortening the diffusion distance [18].

1.2.1 Central adaptations

During exercise, heart rate (HR) and stroke volume (SV) increase, regulating Q to meet the metabolic demand dictated by the intensity of the exercise [19,20]. At rest, Q is ~5 L min^{-1} and can increase to ~15-25 L min^{-1} in healthy adults [21–23] and to ~30-40 L min^{-1} in athletes [21,24] during maximal whole-body exercise. It is primarily the linear increase in

HR (~2.5 fold increase) that facilitates the higher Q value as intensity increases whereas SV increases (~1 fold increase) non-linearly (25). However, after a training intervention, maximal HR (HR_{max}) does not change, if anything it decreases which has been reported in both longitudinal studies and cross-sectional data when age-matched subjects with different aerobic training status were compared (26,27). As HR_{max} does not increase with chronic exposure to endurance exercise, the augmentation in Q_{max} is the result of an increased SV.

Part of the increase in SV with long-term exercise is suggested to be due to cardiac remodeling. As early as 1899, Salomon Eberhard Henschen reported that the hearts of cross-country skiers were enlarged as a result of exercise (28). Modern diagnostic tools have allowed these exercise-induced morphologic changes to be characterized, and the term "athlete's heart" has been coined to describe these structural changes, which include balanced enlargement of the end-diastolic dimensions of the left (LV) and right (RV) ventricles, increased LV mass and increased right atrial volume (LA) (29–31). These changes are, however, dependent on a continuous exposure to exercise over months or years (32–34).

In addition to cardiac morphology, SV is also strongly influenced by the volume of circulating blood (BV). Plasma volume (PV) may increase by ~10% above pretraining values within 4 days of exercise exposure. In the first 1-3 weeks, PV expansion accounts for almost all of the increase in BV (35,36). Later, red blood cell volume (RBCV) also increases until equilibrium with PV is reached, resulting in a restored hematocrit value (Hct) equal to pretraining values but with an increased total BV (37). The absolute increase in RBCV correlates strongly with the initial PV expansion (38), and the addition of thermal factors that further enhance PV expansion also results in a greater increase in RBCV (39). Acute lowering of central venous pressure (CVP) by whole-body tilting, similar to that after exercise, results in an increase in plasma erythropoietin (EPO) concentration, an effect possibly mediated by the concomitant increase in vasopressin (VPN) (40,41). Thus, it appears that exercise-induced erythropoiesis may be regulated independently of the endocrine feedback loops that regulate PV and interstitial fluid homeostasis. In addition, exercise alters the secretion patterns of catecholamines, peptides (growth hormone, insulin-like growth factor), and steroid hormones (testosterone, cortisol), all of which can affect the production and/or release of red blood cells from bone marrow (42).

Cross-sectional studies have shown that BV is higher in endurance-trained individuals compared to untrained individuals, a relationship that is independent of sex or body size (43). The importance of BV has been demonstrated by the reversal of exercise-induced improvements in VO_{2max} and Q_{max} when BV is normalized to pre-exercise levels by phlebotomy (44,45). A negative effect on VO_{2max} was also found when athletes donated blood. A single blood donation (450 ml) results in an immediate decrease in VO_{2max}, from

which it takes more than a week to recover (46,47). RBCV can reach more than 3500 mL during long-term endurance training, ~40% higher than in untrained individuals (48–50).

The effect of RBCV expansion on VO_{2max} can be explained by two underlying factors that impacts convective O_2 supply: improved O_2 transport capacity and increased circulating volume. These factors facilitate venous return, resulting in increased cardiac preload, SV, and Q_{max} (51–54).

1.2.2   Peripheral adaptations

In healthy individuals exercising at sea level, peripheral factors do not appear to be major determinants of VO_{2max} (55). This is evident from the 2-3 fold higher muscle VO_2 when exercise is performed under conditions where a greater fraction of Q can be directed towards the working muscle, i.e. one-legged exercise models (56,57). That does however not mean that peripheral adaptations are unimportant for the increase in VO_{2max}.

Peripheral adaptations include a myriad of physiological variables that undoubtedly respond to exercise. With TET, increases in capillary density (58,59), mitochondrial content (60–65) and respiration (66,67) are regularly seen. However, these adaptations do not always translate to measurable increases in a–vO_2diff (45,68–70). A meta-analysis synthesizing data from 9 studies (114 subjects) lasting 5-13 weeks could not show any linearity in the increase of VO_{2max} and a–vO_2diff or a significant increase in a–vO_2diff after the training interventions (71). More recently, a curvilinear relationship between a–vO_2diff and VO_{2max} was reported, with a–vO_2diff decreasing in the subjects with the highest VO_{2max} (18). This is in line with early observations showing that there is no major difference in a–vO_2diff between elite athletes and trained subjects nor between trained and untrained individuals (24,70).

The interpretation of the data on a–vO_2diff is complicated by the wide range of durations used for the different interventions, exemplified by the increases seen in a–vO_2diff when interventions last more than 12 weeks (72,73). In addition, most of the studies assessing a–vO_2diff during VO_{2max} are doing so by calculating the variable from the Fick equation (a–vO_2diff = VO_{2max}/Q_{max}). This will inevitably introduce unwanted variance in the data from the measurement errors of the VO_{2max} and Q_{max} assessments. This in combination with small sample sizes leads to insufficient statistical power to detect changes and associations connected to a–vO_2diff.

1.3   Underlying adaptations mediating increases in maximal oxygen uptake following interval exercise

In contrast to the literature concerning TET and its effect on mediators governing the improvements in $VO_{2max,}$ less is known about HIIT and SIT.

1.3.1 Central adaptations

Studies investigating the effects of HIIT on BV and PV have showed contradictory results. In the only study that has assessed total hemoglobin mass (tHb), BV and PV using carbon monoxide rebreathing, no changes in the aforementioned factors were seen following 2 weeks of HIIT (74). The absent effect on RBCV is not surprising since it is well established that the expansion of RBCV takes approximately 4-6 weeks (75). The lack of change in PV, on the other hand, stands in contrast to earlier studies demonstrating HIIT-induced expansion in PV after interventions of similar duration and intensity when PV was estimated using changes in Hct (76,77). Similarly, 12 weeks of HIIT elicited clear increases in PV and BV measured using Evan's blue (78). Besides changes in intravascular volumes, it has also been reported that HIIT leads to cardiac remodeling reflected by increases in LV mass (79–81). In contrast, only improved LA mechanics but not LV mass has been reported for SIT (82). The improvements in Q_{max} mediated by increased SV following 8-12 weeks of HIIT further strengthens the importance of central adaptations for improvements in VO_{2max} (78,83–85).

Investigations of Q_{max} and its response to SIT are scarce. Two studies where the duration of the interventions lasted for 4 and 6 weeks showed no increases in Q_{max} despite large improvements in VO_{2max}, 9.3% and 11.5% respectively (86,87). As stated earlier, any change in VO_{2max} should be reflected by either changes in Q or a-vO$_2$diff. Despite the fact that neither of the two studies could show an increase in a-vO$_2$diff that was statistically significant following the training interventions, they concluded that the increase in VO_{2max} was mediated by peripheral adaptations. Similarly a recent investigation could not detect any changes in Q_{max} in women after 12 weeks of SIT (88) however the male participants displayed an significant increase at both 6 and 12 weeks of SIT.

1.3.2 Peripheral adaptations

As with TET, both HIIT and SIT are efficient in promoting local changes in the skeletal muscle. Molecular signaling pathways supporting mitochondrial biogenesis and capillarization, such as phosphorylation of AMP-activated protein kinase (AMPK), p38 mitogen activated protein kinase (p38 MAPK), the expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1a) and vascular endothelial growth factor A (VEGFA) have been reported to be activated after a single session of HIIT or SIT (89–93). This is consistent with the findings of increased mitochondrial content and capillary density after prolonged exposure to interval exercise (74,86,93–98). It has been suggested that the rapid reduction in muscle glycogen content associated with HIIT and SIT acts as an effective "switch" for signaling pathways involved in the adaptive process after training (91). As mentioned earlier, despite the pronounced and clear acute and long-term effects of interval training on peripheral adaptations, there is insufficient data to demonstrate that these adaptations actually lead to an increase in a-vO$_2$diff. To my

knowledge only one study has showed an increased a–vO$_2$diff, derived from the Fick equation, following 6 and 12 weeks of SIT (88).

1.4 Acute exercise–induced events mediating the expansion of blood volume

During exercise, PV decreases as a function of exercise intensity. This reduction in PV is accompanied by increased electrolyte concentration and osmolality, as well as activation of the renin–angiotensin–aldosterone system (RAAS) and an increase in VPN levels, leading to renal water retention (99,100). Through the mechanisms of baroreflex reset (101,102), increased albumin synthesis (103), redistribution of lymphoid proteins (104), and plasma protein augmentation (105), PV increases as early as 24 hours after a single exercise session (105–107). It is suggested that the decrease in CVP, which is also often observed after prolonged TET (101), is part of the explanation for this cascade of events, ultimately leading to sodium and water retention that increases PV and normalizes CVP (108–111). The influence of the endocrine response to exercise and its role in regulating PV is illustrated by an experiment in which spironolactone, an aldosterone antagonist, reduced exercise-induced PV expansion by two-thirds (112). In contrast to the well-described events involved in the increase in PV after TET, these events are not addressed in as much detail in the context of SIT.

1.5 Thesis rationale

Data gathered from training studies using TET as training stimuli have clearly showed that most of the improvements in VO$_{2max}$ are explained by central adaptations such as Q$_{max}$ and BV whereas peripheral factors play an important, but considerably smaller role. It is well established that SIT promotes peripheral adaptations involved in oxygen extraction. However, the data are not clear whether these adaptations in fact explain the improvements in VO$_{2max}$, and to what degree. Despite this, there are suggestion implying that with SIT central adaptations are less important and that the increases in VO$_{2max}$ could be explained by peripheral adaptations. Physiologically, this is counterintuitive considering what we know from earlier published literature. An investigation of the mediators governing the improvements in VO$_{2max}$ following short, supramaximal exercise is therefore warranted. In addition, if SIT leads to expansion of BV as has been shown for TET, the acute responses following SIT needs to be investigated with focus on factors important for the control of PV expansion as this is a prerequisite for a later increase in RBCV. Therefore, studies investigating the acute responses to SIT in relation to metabolic perturbation and intravascular environment are needed in order to better describe these events.

2 Thesis aims

The overreaching aim of this thesis was to study how brief supramaximal exercise affects total blood volume, maximal cardiac output and arteriovenous oxygen difference and how these factors are involved in the mediation of increases in VO_{2max}.

Specific study aims:

Paper Ⅰ Examine the effects of a 6-week SIT intervention on VO_{2max}, tHb, BV and Q_{max}

Paper Ⅱ Investigate the relative importance of the hypervolemic response after 6 weeks of SIT on VO_{2max} and Q_{max}.

Paper Ⅲ Characterize SIT-induced plasma volume flux in relation to metabolic perturbation

Paper Ⅳ Investigate if acute changes in plasma amino acids and markers of intravascular environment have any predictive value for VO_{2max} improvements after 6 weeks of SIT

3 Materials and methods

3.1 Experimental design

Three experimental studies were performed (Study Ⅰ-Ⅲ; Table 1). Study participants completed 6 weeks of SIT, three times per week (Study Ⅰ and Ⅱ). One acute experiment was performed consisting of one session of SIT (Study Ⅲ). Study Ⅰ resulted in two separate manuscripts (Paper Ⅰ and Ⅳ).

Table 1: General overview of experimental design.

	Study Ⅰ		Study Ⅱ	Study Ⅲ
Design	Exercise intervention (6 weeks) Acute		Exercise intervention (6 weeks)	Acute
Training protocol	3 x 30s all-out sprints 2 min rest between sprints 3 training sessions per week		3 x 30s all-out sprints 2 min rest between sprints 3 training sessions per week	3 x 30s all-out sprints 2 min rest between sprints
Subjects	N=29 (13♀/16♂) Age 27±5 yr Height 175±8 cm Weight 73±12 kg	N=15 (7♀/8♂) Age 28±5 yr Height 175±7 cm Weight 74±9 kg	N=9 (5♀/4♂) Age 27±5 yr Height 175±14 cm Weight 72±16 kg	N=10 (5♀/5♂) Age 33±8 yr Height 175±7 cm Weight 74±12 kg
Baseline VO_{2max} (mL x kg^{-1} x min^{-1})	41.0±7.8	39.5±7.7	34.8±6.1	N/A
Publication	Paper Ⅰ	Paper Ⅳ	Paper Ⅱ	Paper Ⅲ

3.1.1 Study participants

Study participants were recruited through adds on campus, social media and word-of-mouth. All the subjects were informed about the experimental design of the studies and all of the measurements included in each study. Subjects were given information both orally and in writing before informed consent was obtained. All studies were approved by the Swedish Ethical Review Authority.

3.2 Exercise protocols

The same training protocol was used for all experimental studies (Figure 2). This protocol elicits venous lactate values of 13±3 mmol x L^{-1} and a mean VO_2 corresponding to 98±7% of post-intervention VO_{2max} (Figure 3) All training sessions, in all of the studies were performed on a mechanically braked cycle ergometer (Monark 894E, Varberg, Sweden). Each training session consisted of 10 minutes of unloaded cycling interspersed with three 30-second all-out intervals. The intervals were performed against a braking force equivalent to 7.5% of the subject's body weight. The subjects were instructed to pedal as fast as possible against the inherent resistance of the cycle ergometer. Braking force was

applied manually when the maximum cadence was reached. Before the first sprint-interval, the subjects completed a short, unloaded warm-up (2.5 minutes). The subjects were strongly encouraged to exert maximum effort during the intervals. The sprints were separated by a 2-minute rest period with unloaded cycling. After the last interval, the subjects cycled for 2 minutes unloaded, intended as a cool-down.
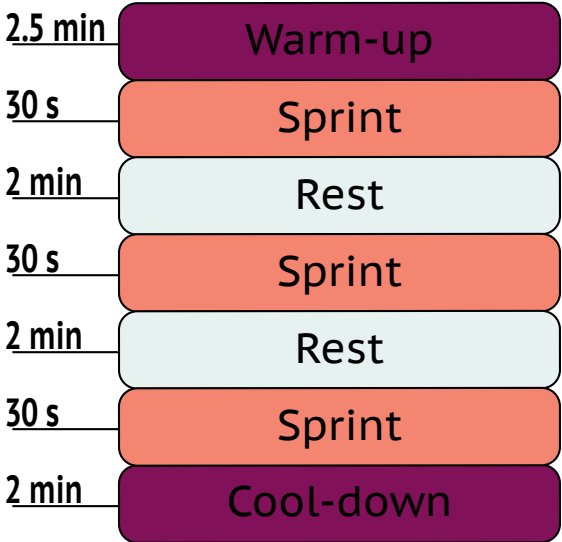
2.5 min	Warm-up
30 s	Sprint
2 min	Rest
30 s	Sprint
2 min	Rest
30 s	Sprint
2 min	Cool-down

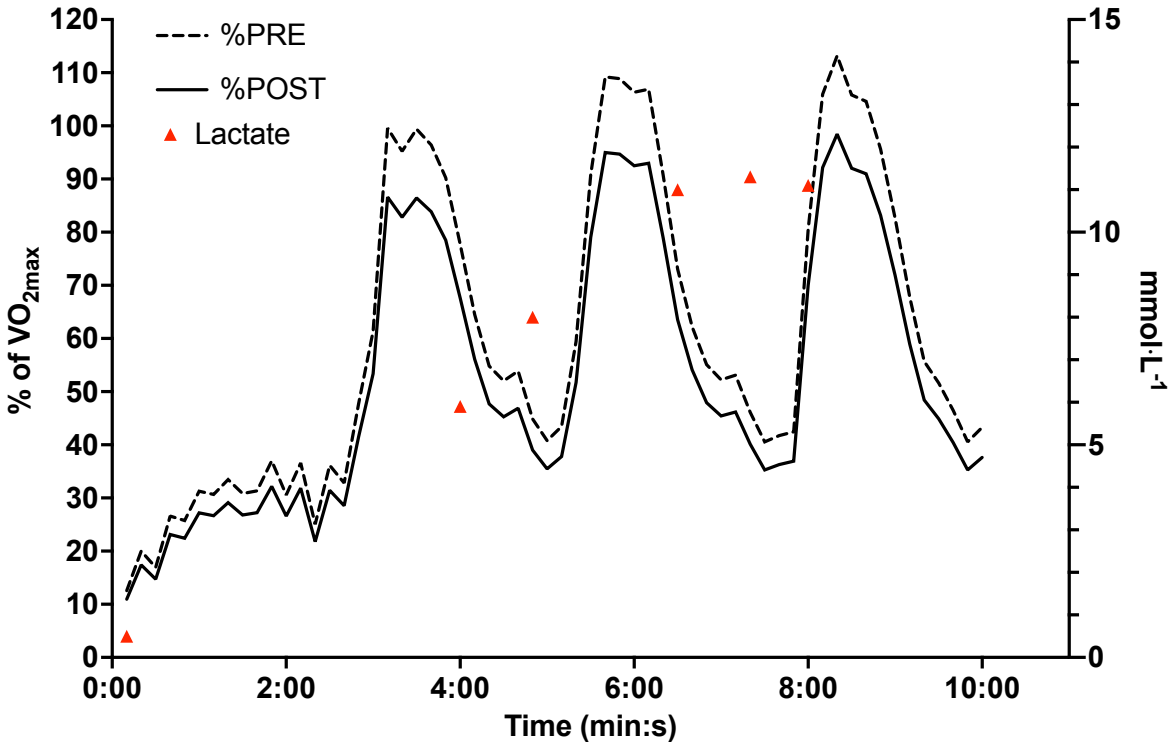Figure 2: Training protocol used in all experiments.

Figure 3: Intensity during one SIT session in week 5 of study II depicted as percent of VO_{2max} pre- and post-intervention. The graph is based on breath-by-breath data from one subject that is representative for the mean values of included participants. Unpublished data from study II.

3.3 Experimental procedures

3.3.1 Maximal oxygen uptake

To determine VO_{2max} and maximal workload (W_{max}), subjects performed an incremental cycling test to volitional fatigue on an electronically braked ergometer (Study Ⅰ: Lode, Groningen, Netherlands; Study Ⅱ: Monark LC6, Vansbro, Sweden) with identical pre- and post-intervention protocols. Using an online gas collection system (Study Ⅰ: Vmax Encore, Illinois, USA; Study Ⅱ: COSMED Quark CPET, Rome, Italy), fractions of inspired and expired O_2 and CO_2 were measured continuously and recorded as breath-by-breath values. The subjects started the test with 50 or 100 W for 5 min as a warm-up. Subsequently, the resistance was increased by 1 W every 3 s, corresponding to an increase of 20 W per minute, until the subjects reached volitional fatigue. VO_{2max} was considered to be the highest 20-second average reached during the test. Criteria for the test were either a plateau in VO_2 or RER > 1.15, a heart rate within 10 beats of the age-related maximum and perceived exertion ≥18 RPE.

3.3.2 Maximal cardiac output

For the determination of Q_{max} two different approaches were used, one non-invasive (Study Ⅰ) and one invasive (Study Ⅱ).

Non-invasive determination of Q_{max} was performed using inert gas rebreathing (Innocor, Odense, Denmark). This method assumes that pulmonary uptake of blood-soluble gas, nitrous oxide (N_2O) is proportional to pulmonary blood flow. During a maximal incremental test, subjects inhaled a gas mixture containing 5% blood-soluble N_2O, 1% insoluble sulfur hexafluoride (SF_6), and 94% O_2. The gas mixture was mixed with ambient air before the start of the rebreathing protocol. When subjects reached maximal workload (as determined by the VO_{2max} assessment), they were switched from ambient air breathing to a closed-circuit system in which they inhaled the gas mixture while photoacoustic gas analyzers continuously quantified closed-circuit gas concentrations. Pulmonary N_2O uptake was determined by the decrease in N_2O concentration over three consecutive exhalations after complete mixing between the remaining pulmonary air and the gas in the rebreathing bag, as determined by a stable gas fraction of the blood insoluble gas (SF_6). Subjects were instructed on how to perform rebreathing, and each subject had two trial runs of the rebreathing protocol before the actual measurement.

The invasive determination of Q_{max} was performed using the direct Fick technique with pulmonary artery catheterization and radial artery cannulation. Subjects were placed in a supine position on the examination table, and the two access sites were sterilely cleaned and covered. Under continuous electrocardiogram and blood pressure monitoring, a flow-guided pulmonary artery catheter was placed percutaneously by ultrasound under local anesthesia. Using a double Seldinger technique, access to the internal jugular vein was

created via an 18-gauge needle, followed by insertion of a 0.038-inch guidewire. After vein placement was confirmed, an introducer sheath was slid over the wire, which was then removed. Finally, a Swan-Ganz balloon-tipped catheter was inserted and advanced through the right atrium, right ventricle, and pulmonary artery in a wedge position before being returned to the pulmonary artery. Combined fluoroscopy and pressure wave morphology was used to confirm correct catheter placement (Figure 4). After subcutaneous local anesthesia at the right wrist, the radial pulse was palpated and the catheter was placed using a guide wire after intra-arterial cannulation. The needle was removed, and the arterial catheter was inserted into the radial artery via the wire. The guidewire was then removed, the arterial catheter was secured, and the placement of the catheter was checked by flushing with saline. Insertion of the arterial catheter was performed under ultrasound guidance when palpation was considered difficult. At baseline, a blood sample was obtained from the pulmonary and radial arteries to determine the subject's oxygen saturation and hemoglobin concentration [Hb].
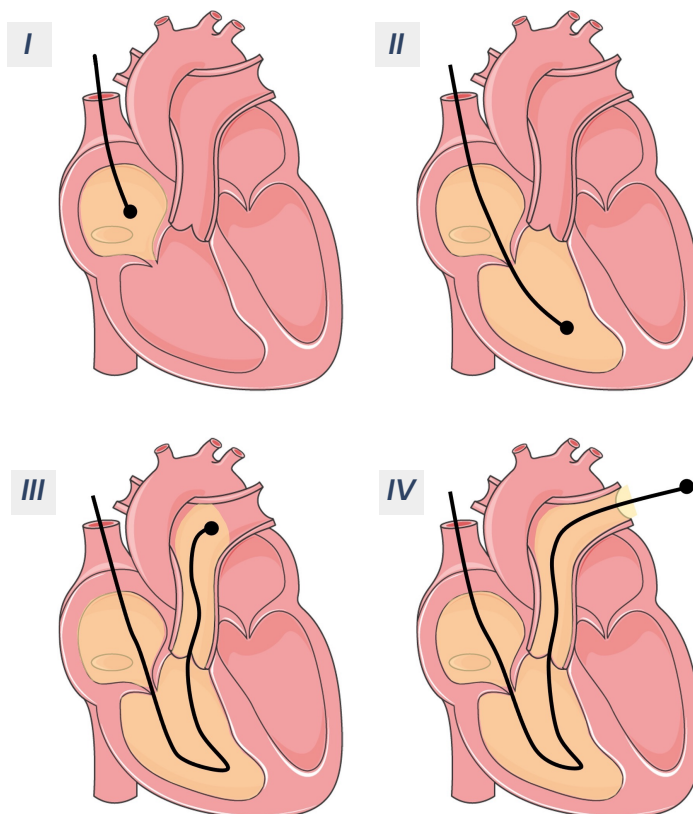

Figure 4: Placement of pulmonary artery catheter.

Following placement of catheters, the subjects performed a VO_{2max} test as described earlier. Q_{max} was determined according to Fick's equation based on the measured VO_{2max} and from the a–vO_2diff derived from pulmonary and radial artery samples. Q_{max} was calculated as $VO_2 \times (CaO_2 - CvO_2)^{-1}$ and $cO_2 = (1.36 \times [Hb] \times SO_2) + (0.003 \times PO_2)$ where SO_2 is hemoglobin O_2 saturation and PO_2 partial pressure of O_2. Arterial and venous blood samples were collected simultaneously in 2 mL heparinized syringes at maximal effort during the VO_{2max} test and were immediately analyzed in duplicates (ABL 800, Radiometer A/S, Copenhagen).

3.3.3 Carbon monoxide rebreathing

Carbon monoxide (CO) rebreathing was used to determine tHb, from which BV and PV later were calculated using [Hb] and Hct (113–115). Subjects rested for 15 minutes before a venous blood sample was taken from the median cubital vein. The sample was immediately analyzed for baseline carboxyhemoglobin (%HbCO), [Hb] and Hct (microcentrifugation, 4 min at 13500 rpm). End–tidal carbon monoxide (CO) was measured at baseline and after rebreathing using a CO gas analyzer (Dräger, PAC 700, Lübeck, Germany). Throughout rebreathing, the same gas analyzer was used to check for CO leaks. Subjects breathed a gas mixture of chemically pure (99.97%) CO (0.8 mL x kg–1) and medical oxygen (AGA, Stockholm, Sweden) for 2 minutes before disconnecting from the spirometer (Blood tec GmbH, Bayreuth, Germany). Two blood samples (1 mL) were taken, one before rebreathing and one 7 minutes after administration of CO. The samples were then analyzed for %HbCO (ABL 800, Radiometer A/S, Copenhagen).

Calculation of tHb:

$$tHb = K \; x \; MCO \; x \; 100 \; x \; (\Delta HbCO\% \; x \; 1.39)^{-1}$$

K = (barometric pressure in mmHg x 273) x 760^{-1} x (273 x temperature in °C)

$$MCO = CO_{adm} - (CO_{system + lung} + CO_{exhaled})$$

CO_{adm} = CO volume administered into the system

$CO_{system + lung}$ = CO concentration in spirometer x (spirometer volume + lung residual volume)

$CO_{exhaled}$ = end-tidal CO concentration x estimated alveolar ventilation x time

Calculation of intravascular volumes:

$$RBCV = (tHb \; x \; Hct) \; x \; [Hb]^{-1}$$

$$BV = (RBCV \; x \; 100) \; x \; (Hct \; x \; 0.91)^{-1}$$

$$PV = BV - RBCV$$

3.3.4 Magnetic resonance imaging

Using the built-in transmit/receive body coil a standard T1-weighted gradient echo sequence with a 2-point Dixon reconstruction (Dixon VIBE) was acquired in the axial plane centred around the phosphorus surface coil. Acquisition parameters were TE1 1.15 ms, TE2 2.3ms, TR 3.8 ms, FOV 50 × 35 cm, voxel size 1.2 × 1.2 × 4 mm with a scan time of about 1 minute. Fat, water, in-phase and out-of-phase images were reconstructed on the scanner console. The images were then imported into AMRA Researcher where semi-automated volume analysis were performed as described earlier (116). The pre-exercise baseline reading was acquired immediately after a 5 minute ^{31}P-MRS acquisition. All image acquisitions were performed on a Philips Ingenia 3T (Best, Netherlands).

3.3.5 ^{31}P magnetic resonance spectroscopy

Two different spectroscopic acquisition protocols were used; one for baseline acquisition, with high signal-to-noise ratio (SNR), and one for rapid dynamic measurements during the recovery phase following the exercise. For both protocols, a 31P transmit-receive surface loop coil provided by the scanner manufacturer ('P-140'; Philips, Best, Netherlands) with a diameter of 14 cm was used for spectroscopic measurements. For post-processing of the ^{31}P-MRS data, jMRUI (117) was used with the AMARES algorithm (118) for relative quantification of the resonances. Prior to quantifying the dynamic data, the first two FIDs of each sequence were dropped (i.e. dummy scans) thereafter the data was averaged in blocks of ten, reducing the temporal resolution to 20 seconds to enhance SNR. Phosphocreatine (PCr) was used as a chemical-shift reference with assignments obtained from literature as previously described (119,120). In short, phosphomonoesters (PME) were assigned to phosphoethanolamine and phosphocholine, in the dynamic phase this resonance also includes glucose-6-phosphate (G6P; details below) (121); inorganic phosphate (P_i) and PCr were defined as singlets. The phosphodiester (PDE) resonance was assigned to glycerophosphoethanolamine and glycerophosphocholine. In addition, the resonance corresponding to NAD(H) was also observed. Finally, the nucleotide triphosphate (NTP-Mg, mainly adenosine triphosphate [ATP]) resonances were assigned and interpreted as α-,β-, and γ-NTP as previously reported (119,120). pH was estimated in the spectra using the modified Henderson-Hasselbach equation to assess the chemical shift difference between P_i and PCr (with pK_A = 6.77, δ_{HA} = 3.23 ppm, and δ_A = 5.70 ppm) as described previously (122) using the built in functionality in jMRUI.

The baseline acquisition served to establish the steady-state resting concentrations of the metabolites of interest where absolute concentrations of metabolites were estimated using ATP as reference, assuming an intramuscular concentration of 8.2 mM or 5.5 mmol/kg wet weight muscle (123). During the recovery phase after exercise, it was assumed that all metabolites, including ATP/NTP, were perturbed and therefore the rate of recovery over time for each metabolite was calculated, rather than their respective ratio to ATP. G6P was estimated by calculating the differences between the quantified

PME resonances and the last acquisition (Eq. 1). While others have calculated the difference by subtracting baseline spectra from the dynamic sets (121), this was not feasible in the current setting since i) the exercise bouts could not be performed within the MR bore and ii) the chemical shift differences due to the very high post–exercise intra–myocellular lactate concentrations.

$$\Delta G6P(t) = \frac{PME(t)}{PME(t=end)} \quad Eq.1$$

The dynamics of PCr and P_i were estimated by relating each timepoint to the last (Eq.1; assuming that the last time point was acquired in pseudo–steady state). The increase of G6P was also assessed in relation to NTP ('G6P:NTP'). Correction for relaxation differences of the resonances due to the not fully–relaxed spectra in the dynamic acquisition was performed by correcting each quantised resonance with Eq. 2.

$$correction\ factor = \frac{1}{1 - Exp\left(\frac{-TR}{T_1}\right)} \frac{1}{Exp\left(\frac{-TE}{T_2}\right)} \quad Eq.2$$

The relaxation times were taken from the literature (124) assuming β–NTP and PME to have similar T_2 characteristics as α–NTP and PDE, respectively.

3.3.6 High–performance liquid chromatography

Plasma samples were deproteinized with 3% sulfosalicylic acid (SSA), including 0.2 mM L–norvaline as an internal standard. After incubation on ice for 1 hour, they were centrifuged at 600 g for 20 minutes at 41°C. The supernatant was filtered through a 0.22 mm membrane filter and stored at –80°C until HPLC analysis. Free amino acids in plasma were analyzed as previously described (125). Analysis was performed with an automated online HPLC with precolumn derivatization using ortho–phtalaldialdehyde/3–mercaptopropionic acid (OPA /3–MPA). The HPLC instrument consisted of the Waters 600 E system and the Waters 717 plus auto–sampler (Waters Sweden AB). With a cooling system to optimize the sampler to 5°C. Amino acids were separated on a Ymc–pack ODS–AQ, 150 x 4.6mm column. Fluorometric detection of amino acids was performed at excitation 340mm and emission 450mm using an 821 FP Jasco fluorescence detector. The derivatization reagent was prepared as a stock solution consisting of 1% (w/v) OPA in borate buffer (1M, pH 10.4)/methanol (1:9) with 1%(v/v) 3–MPA. The stock solution was stored in the dark (4°C) for up to 2 months. On the day of analysis, the sample was diluted 100–300 times with borate buffer/water (1:4). The OPA stock solution was diluted 10–fold with borate buffer and mixed with the diluted sample (1:1) 1.5 minutes before injection. The mobile phase used consisted of solvent A: 12.5mM phosphate buffer adjusted to pH 6.93 with NaOH

and tetrahydrofuran (98.9:1.1) and solvent B: methanol, acetonitrile and distilled water (35:15:50). The flow rate was 1 ml/min throughout the chromatography, except for the first minute when it increased linearly from 0.75 to 1 ml/min. The linear gradient profile was as follows: 0–1min, 10% B; 1–15 min, 50% B; 15–18 min, 56% B; 18–26 min, 65% B; 26–44 min, 100% B; and 44–45min, 0% B.

3.3.7 Blood sampling

Venous blood was collected in pre-coated tubes for each specific analysis. All analysis were carried out by the Karolinska University Hospital, department of Clinical Chemistry (study Ⅲ–Ⅳ).

Phlebotomy (study Ⅱ) was performed through the central venous introducer catheter placed during right heart catheterization. The total amount of blood drawn during phlebotomy was based on each subject's individual increase in tHb with the goal of returning tHb to pretraining levels.

3.3.8 Statistics

In study Ⅰ, comparison of pre- and post-intervention data, were derived using mixed linear models. The Linear and Nonlinear Mixed Effects Models (nlme and LME) library in R version 3.5.5 was used for this purpose. Principal component analysis (PCA) was used to explore correlations between change-scores (FactoMineR, version 2.0).

In study Ⅱ, training and phlebotomy effects were derived by mixed linear models. Analysis was performed using the lme4 (126) and lmerTest (127) packages in R version 4.1.1. Effects of training on tHb, BV and PV were analyzed with paired t-tests. Pearson's correlation was used for all correlations.

In study Ⅲ, PCA was carried out with variables scaled to unit variance using FactoMineR version 2.0, R version 3.5.3. Pairwise t-tests were used to contrast pre- and post-exercise measures. One-way repeated measures ANOVA was used with Tukey HSD as post-hoc tests. The parameters describing rate of recovery post-exercise for the different 31P-MRS spectra (i.e metabolites) were calculated by exponential curve-fitting on an individual basis and the time-constant, rate of recovery at t_0 and concentration at t_0 (as fraction of the final concentration) was retained.

In study Ⅳ, repeated measure (ANOVA) was used to investigate all variables, including tests for possible interaction between training sessions. Variables with significant changes over time were tested for association with VO_{2max} using a linear model of the change-score. A multivariate orthogonal projections to latent structures (OPLS) regression model was constructed using the concentration change-scores as independent variables and the VO_{2max} change-score as the outcome. All statistical models were built using R version 4.1.0. The OPLS regression models were run with ropls (128).

3.4 Ethical considerations

All procedures used in this thesis are well-established clinical methods with little or no overall risk to participants. All measurements were performed in a hospital with contingency plans in case of a serious adverse event. All subjects were well informed about what to expect from the study, in which they volunteered to participate. This included meetings with research staff to explain and discuss the procedures and general design of the studies, and written information given to participants so they could read it at their leisure without feeling pressured from outside sources. In all studies, we ensured that participants had control over the decision and that we left the initiative to participate to them. Initial contact was always initiated by participants, who emailed research staff asking for more information about the study. We responded to their request by providing them with written information about the study, including detailed information about all procedures included in the experimental design. In the same email, we instructed participants to contact us if they were still interested in participating. At the second contact, research staff met with participants either in person or via Zoom to answer final questions. Participants were then instructed to sign the consent form if they were still interested in participating in the study and to contact the research team again if they were ready to take the next step in their participation.

The right heart catheterization performed in study Ⅱ was the most invasive method used in this thesis. First, it should be noted that although the procedure is invasive, it is routinely performed in our hospital and worldwide by experienced personnel specialized in this method, and very few complications occur, and those that do occur are mild to moderate and are resolved spontaneously or with direct treatment (129). To ensure that participants fully understood the procedure, it was explained to them step by step by one of the physicians involved in the catheterization. They were also invited to visit the laboratory where the catheterization would be performed and to meet some of the staff members.

I argue that the benefits of a structured training program, the ability to plan a sustainable training program after the study was completed with the help of the researchers involved in the studies, and the knowledge that resulted from the subjects' participation outweighed the risks associated with participating in the studies.

4 Results

In the interventional studies, VO_{2max} increased by 10.3% and 11.0% respectively (Paper I and II) after 6 weeks of SIT. Similarly, W_{max} increased by 11.3% and 8.9% respectively (Figure 5AB and Figure 6AB). In both studies, tHB (5.7% and 4.2%), PV (8.1% and 6.0%) and BV (6.8% and 5.4%) increased from pre-intervention to post-intervention (Figure 7). Q_{max} also showed an clear increase in both experimental studies, 8.5% and 8.8%, respectively (Figure 5C and Figure 6C).
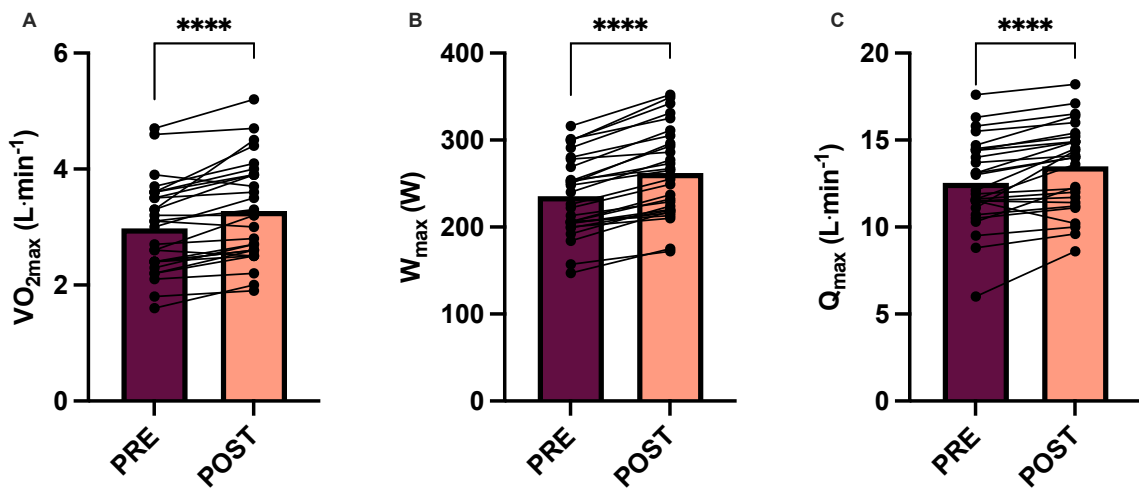

Figure 5: Main outcomes from paper I, measurements obtained at baseline (pre) and after the training intervention (post), VO_{2max} and W_{max} n=28, Q_{max} n=27, data presented as group mean and individual data with directional changes, ****P<0.0001.
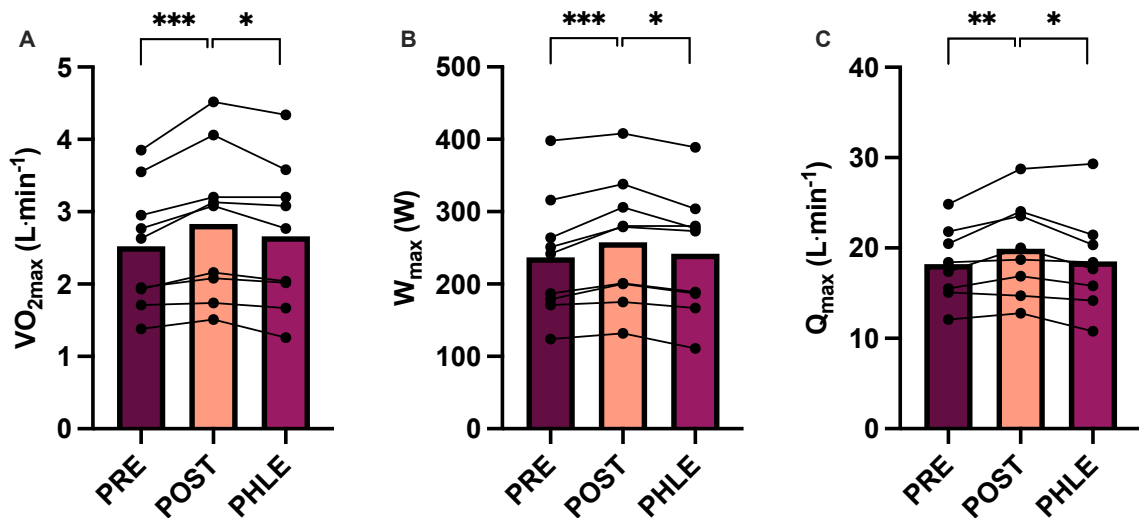

Figure 6: Main outcomes from paper II, measurements obtained at baseline (pre), after the training intervention (post) and after phlebotomy (phle), VO_{2max} and W_{max} n=9, Q_{max} n=8, data presented as group mean and individual data with directional changes, ***P<0.001, **P<0.01, *P<0.05.
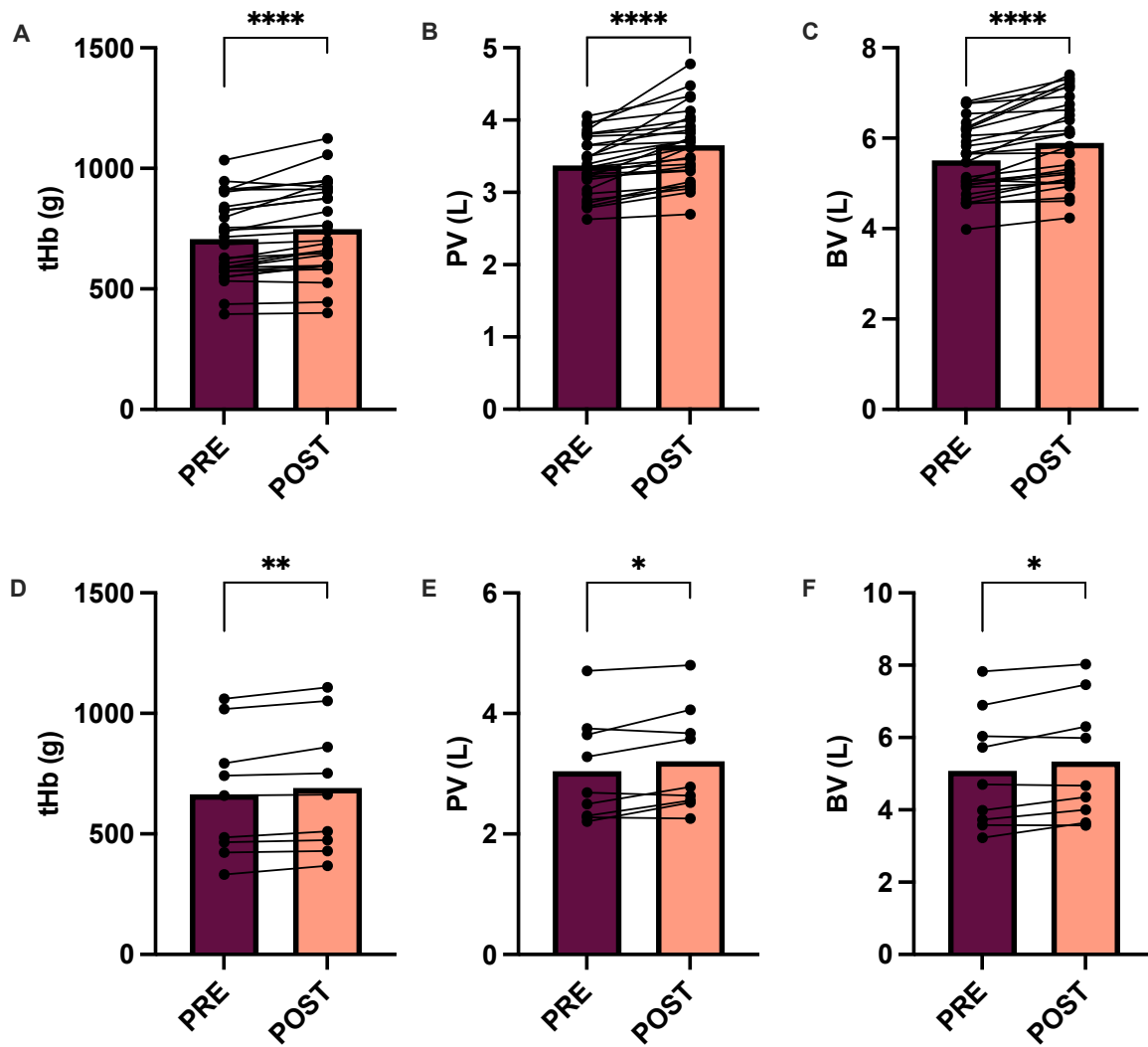
Figure 7: ABC are results from paper I, n=28, DEF are results from paper II, n=9, baseline (pre) and after the training intervention (post), data presented as group mean and individual data with directional changes, ****P<0.0001, **P<0.01, *P<0.05.

Normalization of BV after 6 weeks of training by the means of phlebotomy (study II) returned VO_{2max}, W_{max} and Q_{max} back to baseline levels. VO_{2max} decreased 6.3%, W_{max} decreased 6.6% and Q_{max} decreased 7.7%. All of the mentioned variables were significantly lower after phlebotomy when compared to post-intervention and there was no significant difference between values after phlebotomy and pre-intervention (Figure 6). Percent changes in VO_{2max} and Q_{max} were strongly correlated both for the training effect and the effect from phlebotomy (Figure 8).
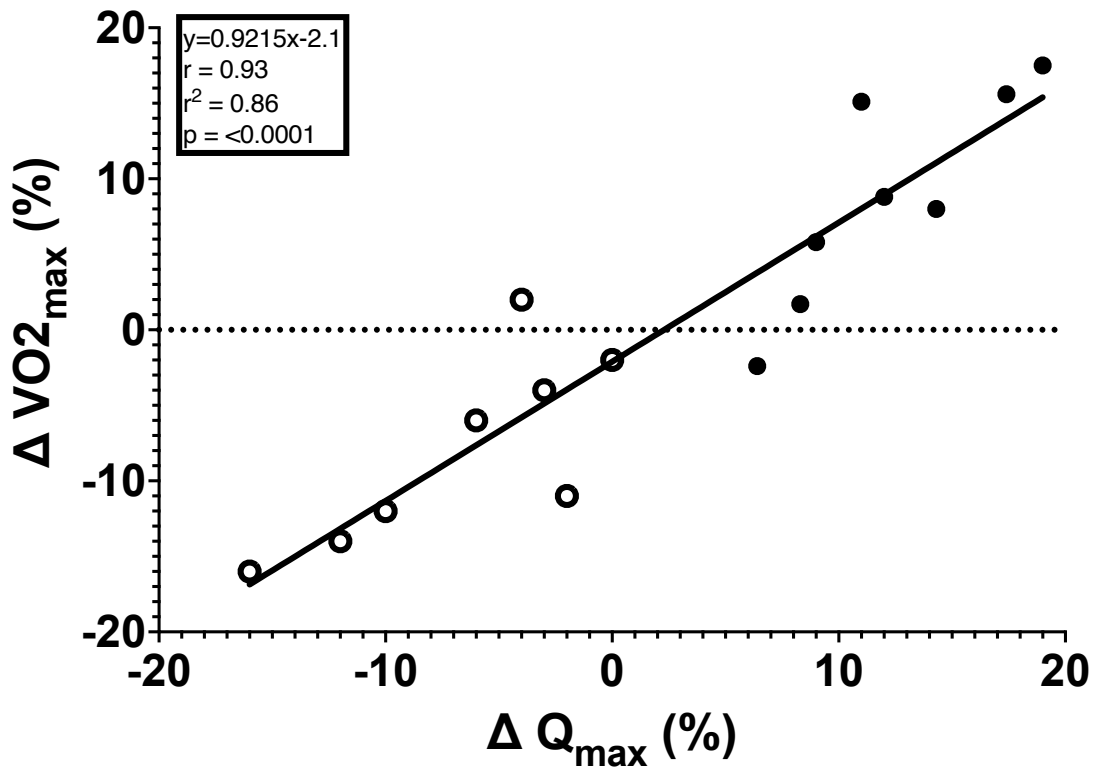
Figure 8: Correlation between percent change in VO_{2max} (%) and Q_{max} (%). Filled circles denote training effect and open circles denote effect of phlebotomy, n=8.

The decline in VO_{2max} was proportional to the amount of blood phlebotomized (Figure 9) and falls in line with previous reports measuring VO_{2max} after phlebotomy (Figure 10).

Figure 9: The relationship between amount of blood phlebotomized and the associated decrease in VO_{2max}, n=9.

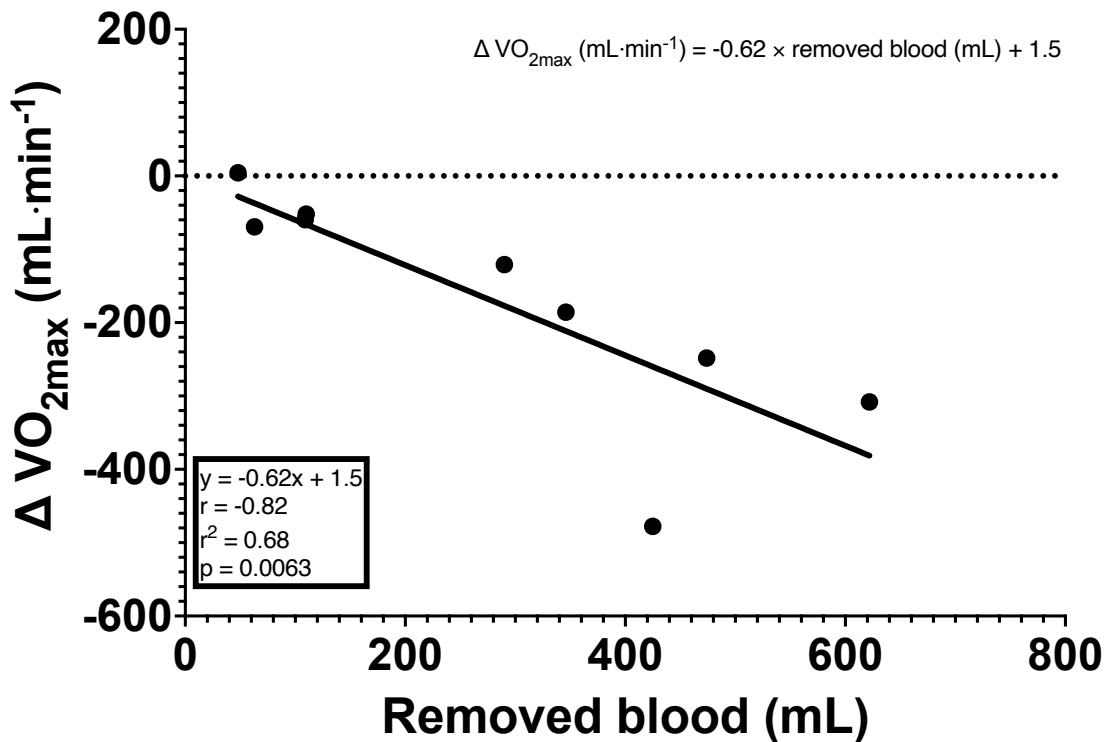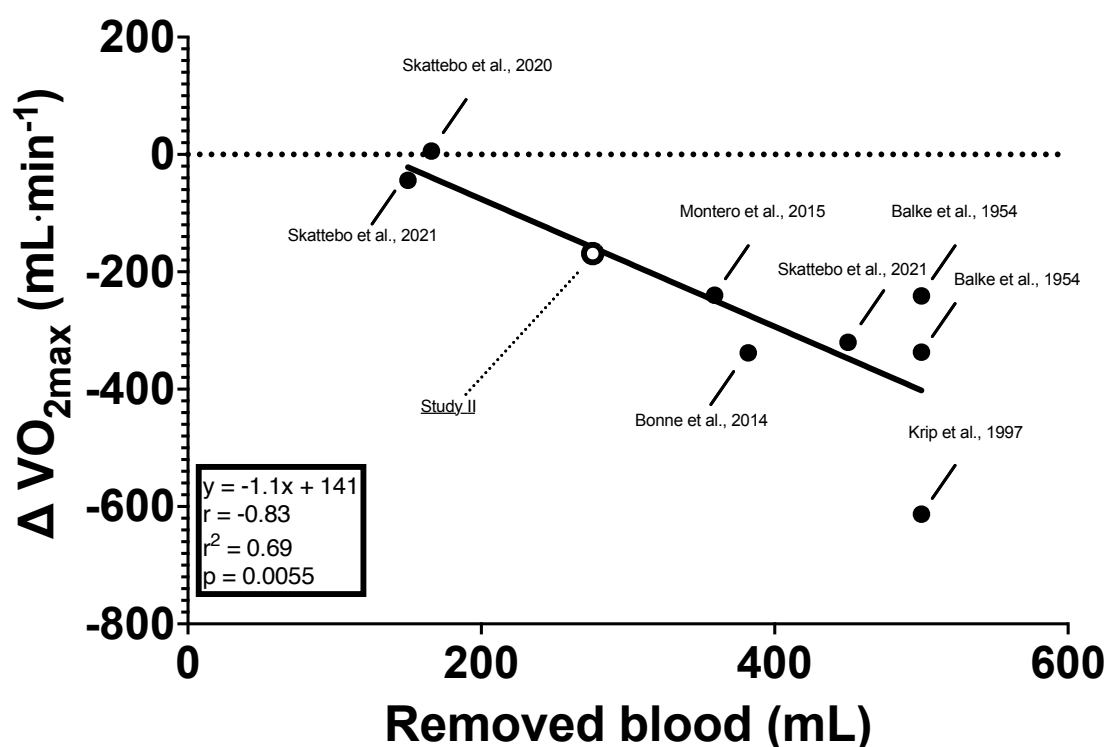*Figure 10: The relationship between amount of blood phlebotomized and the associated decrease in VO_{2max}. Figure adapted from Skattebo et al., 2021 (130). Studies (44,45,130–133) included in the analysis, in addition to the data presented in paper II (study II).*

CaO_2 did not change at any time point during study II. CvO_2 decreased by 12.4% during the training intervention with a concomitant non-significant increase in a–vO_2diff. Systemic oxygen extraction increased by 4.0% following the training intervention. Phlebotomy did not change any of the mentioned variables when compared to post-intervention. However, a–vO_2diff was significantly higher after phlebotomy when compared to pre-intervention (Table 2).

Table 2: Measurements obtained during maximal exercise at baseline (pre), after the training intervention (post) and after phlebotomy (phle), n=8.

	PRE	POST	PHLE
a–vO_2diff (mL $\cdot$ dL^{-1})	14.18±1.94	14.64±2.03	14.82±2.04*
c_aO_2 (mL $\cdot$ dL^{-1})	18.83±2.03	18.70±1.95	18.68±2.10
c_vO_2 (mL $\cdot$ dL^{-1})	4.64±1.25	4.06±1.18*	3.86±0.95***
Systemic O_2 extraction (%)	75.3±6.3	78.2±6.2**	79.3±4.9***

Arteriovenous oxygen difference, a–vO_2diff; arterial oxygen content, c_aO_2; mixed venous oxygen content, c_vO_2

*P<0.05 significantly different from PRE; **P<0.01 significantly different from PRE; ***P<0.001 significantly different from PRE

During one acute session of SIT (Paper Ⅲ) the muscle volume of the quadriceps increased by 12% with a simultaneous decrease in PV of 16% (Figure 11).
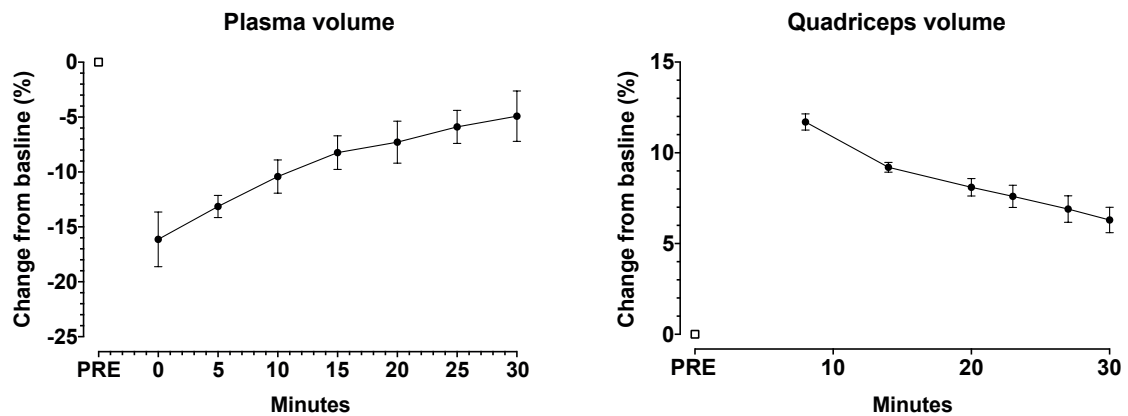
Figure 11: Changes in plasma volume compared to baseline, samples taken at baseline (pre), immediately after cessation of exercise (O) and at, 5, 10, 15, 20, 25 and 30 minutes after exercise. Measurements of quadriceps muscle volume were taken at 8, 14, 20, 23, 27 and 30 minutes after exercise. Values expressed as mean±SD.

PCA-analysis showed a high degree of mutual correlation between the measured variables. The first (34.3%) and second (25.0%) component in the PCA summarized a substantial part of the variance in the measures (Figure 12).
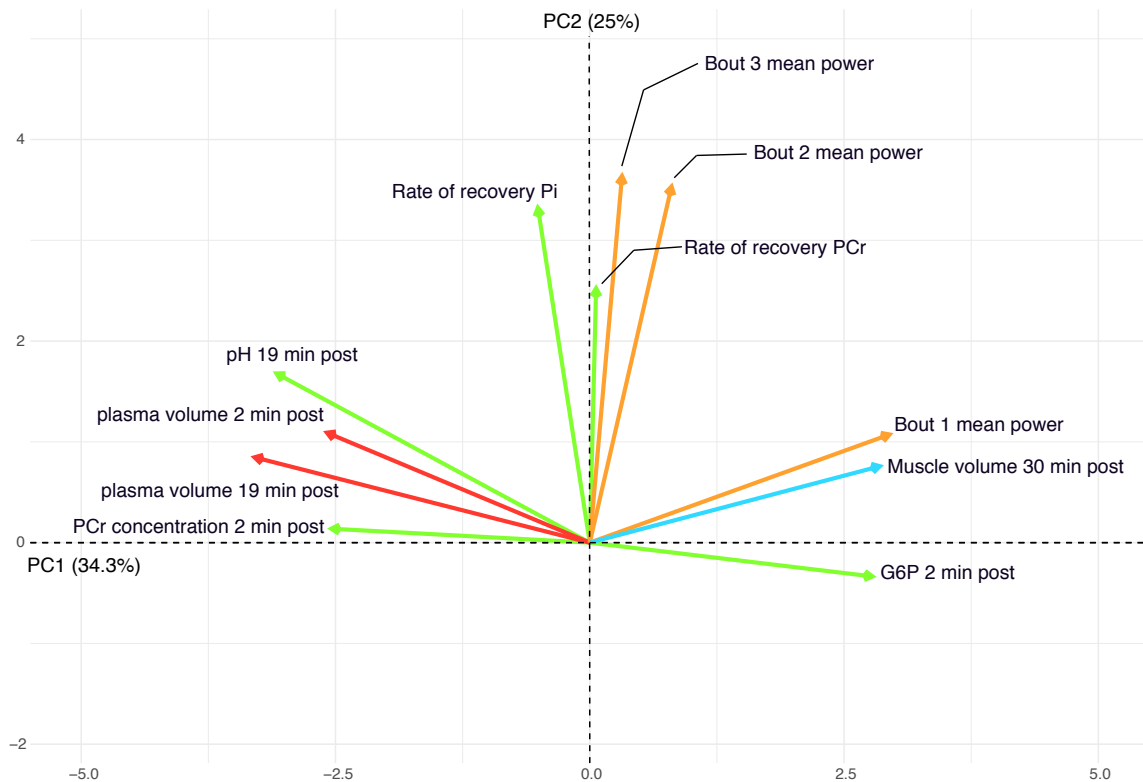
Figure 12: Principal component analysis (PCA) including both intra-muscular metabolites and power output outcomes where all variables with significant correlations are showed. PCr depletion, pH drop and G6P accumulation are together with changes in muscle and plasma volume mutually correlated and cluster along PC1. PCr recovery and power output variables from bout 2 and 3 are also mutually correlated but cluster along PC2 indicating no significant correlations with the other variables.

The metabolite most significantly correlated with the first component was G6P together with changes in muscle volume and PV. This finding was further strengthened when the mutual correlation between these variables were investigated (Figure 13).

Figure 13: To confirm the relationship between muscle and plasma fluid shift and G6P accumulation post-exercise as indicated by the principal component analysis a linear regression model was fitted: There was a negative correlation between G6P and plasma volume drop (R = -0.72) and a corresponding p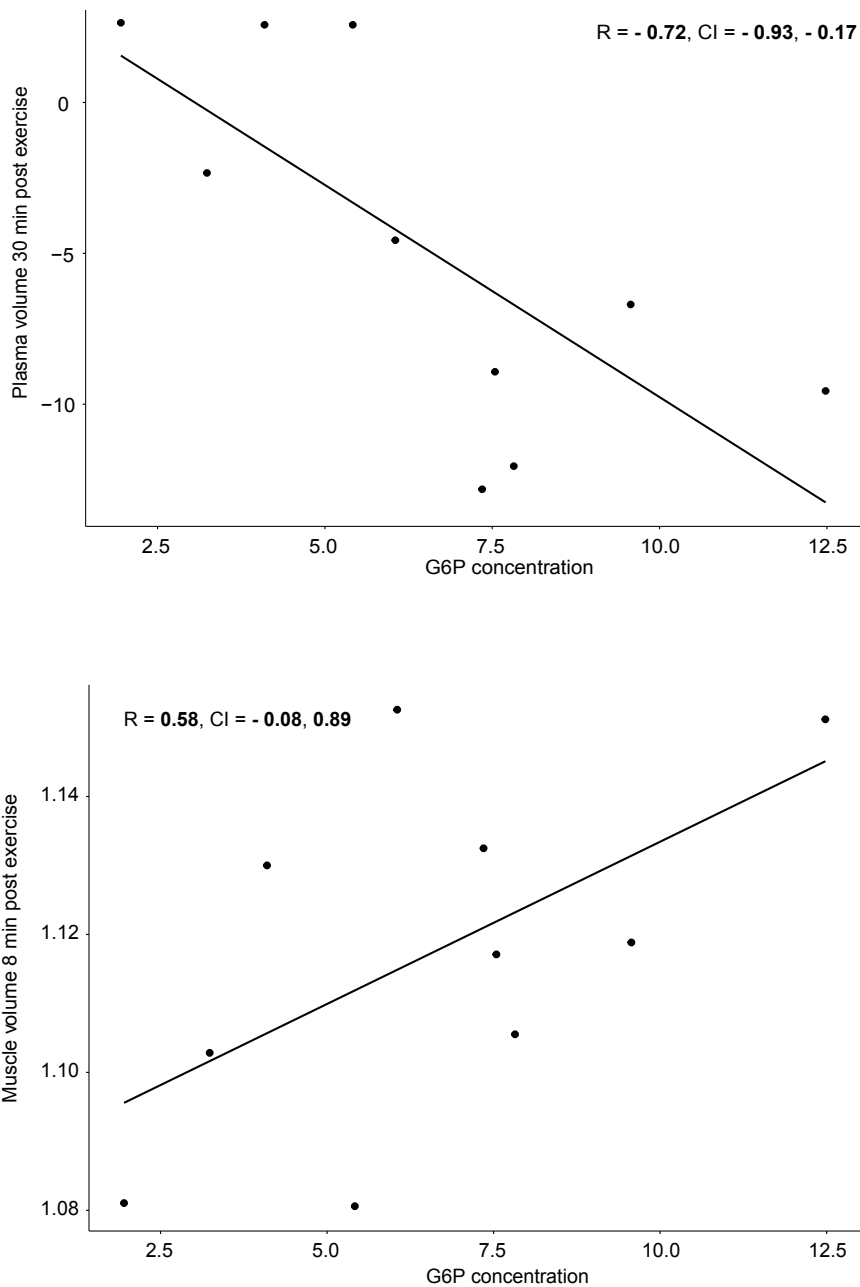ositive correlation between G6P accumulation and muscle volume increase following exercise (R = 0.58). Changes in PME-intensity corresponding to an accumulation of G6P. Plasma and muscle volumes are relative delta changes compared to pre-exercise.

In paper IV, numerous markers of intravascular environment and amino acids were changed acutely after a SIT session. However, there was no training effect on these acute responses (Figure 14 and Figure 15). Three of the measured variables were associated with the changes in VO_{2max}, these were citrulline, plasma osmolality and arginine. The associations were confirmed using multi variate OPLS regression and showed a global fdr-value of 0.01, an R^2-value of 0.73 and a root mean squared error of 0.042 for VO_{2max} prediction. This was further strengthened by the fitted linear regression model which had a R^2 of 0.71 for the VO_{2max} change-score (Figure 16).
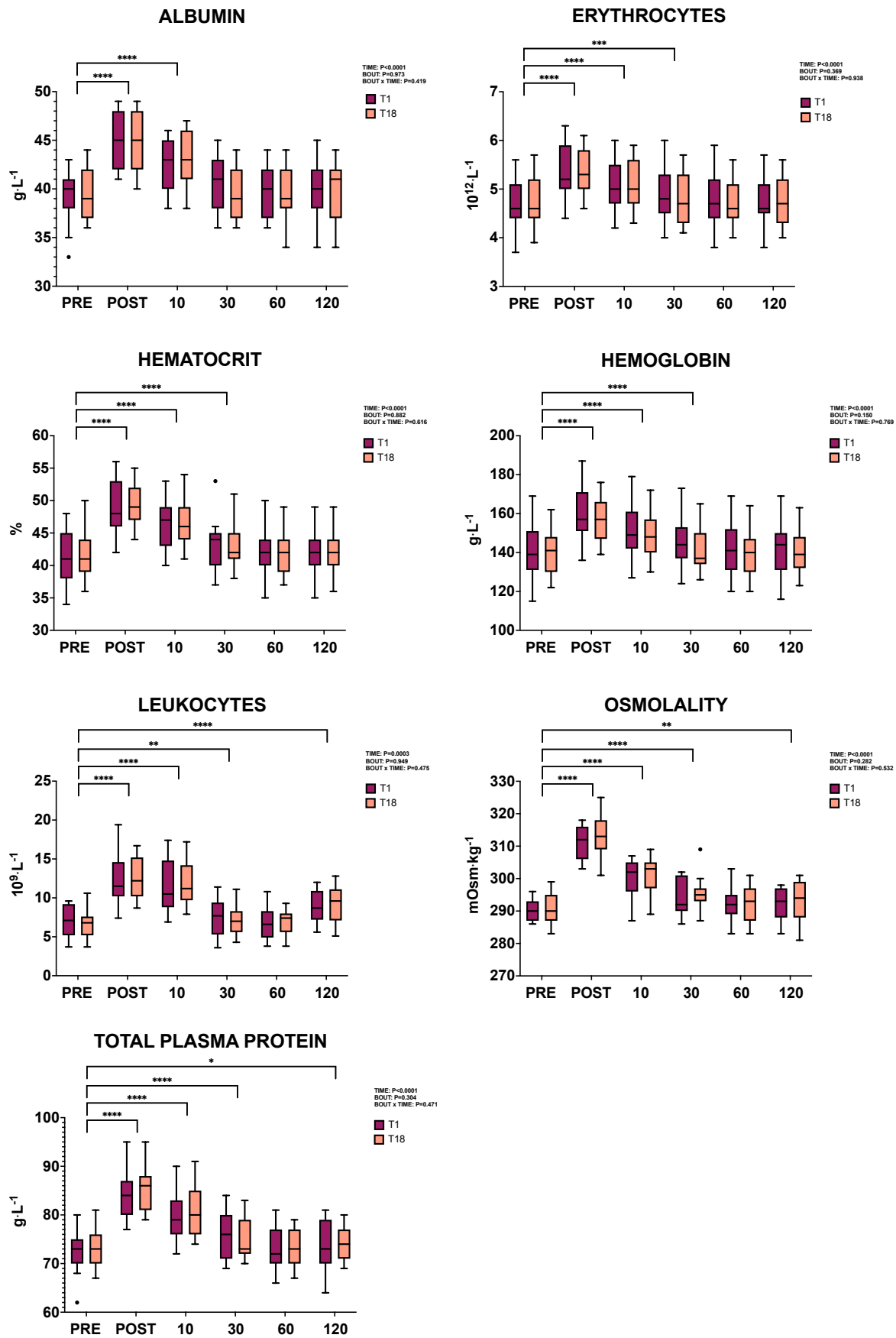
Figure 14: Blood parameters with significant effects of time (fdr<0.05) analyzed through ANOVA, Bonferroni-corrected paired t-test were used as post-hoc tests. T1 denotes the first training session and T18 the last training session of the 6-week intervention. *P<0.05, **P<0.01. ***P<0.001, ****P<0.0001.
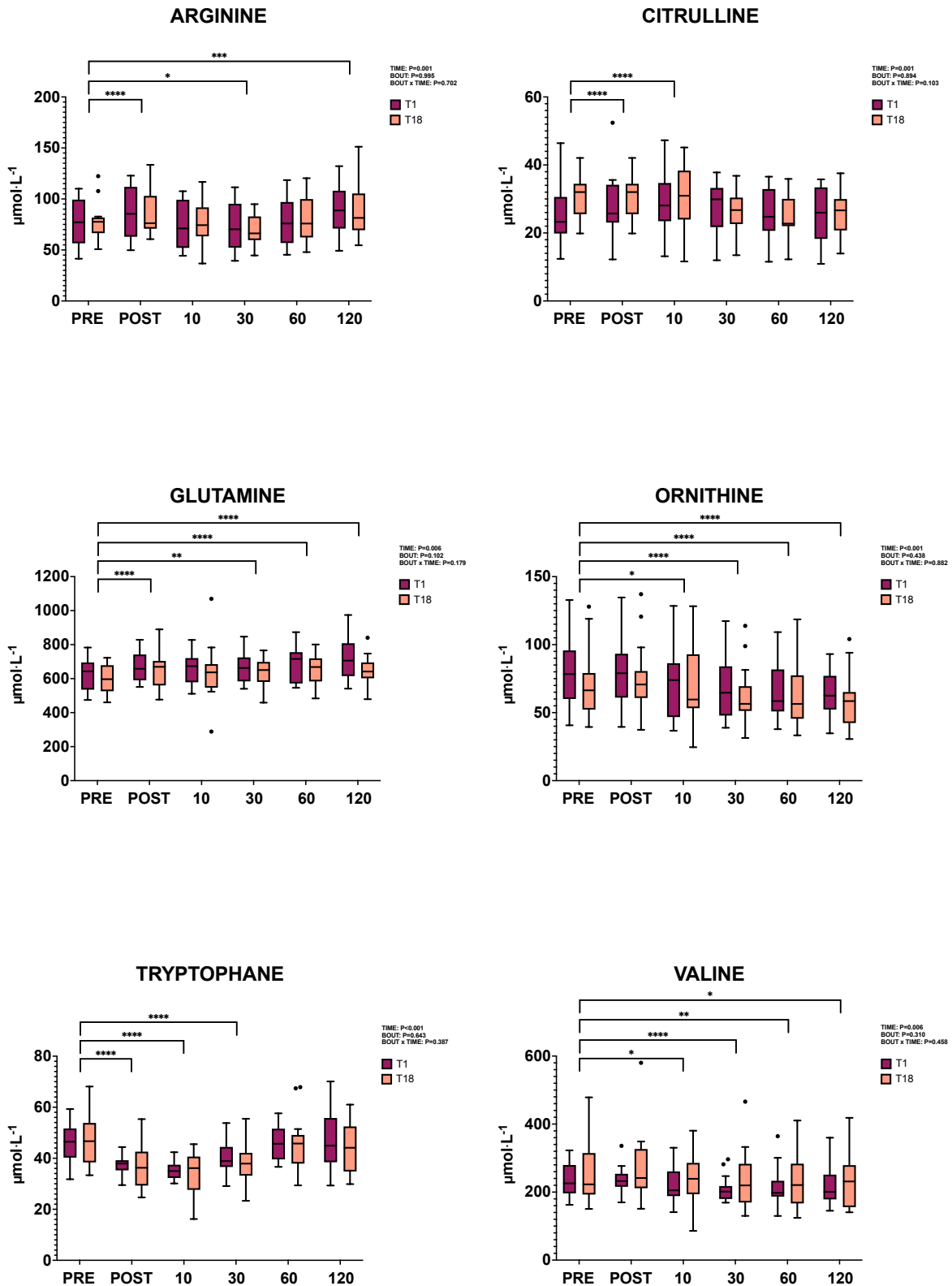
*Figure 15: Amino acids with significant effects of time (fdr<0.05) analyzed through ANOVA, Bonferroni-corrected paired t-test were used as post-hoc tests. T1 denotes the first training session and T18 the last training session of the 6-week intervention. *P<0.05, **P<0.01. ***P<0.001, ****P<0.0001.*

$y = 0.491 + 0.545x$
p-value < 0.001
R^2 0.71
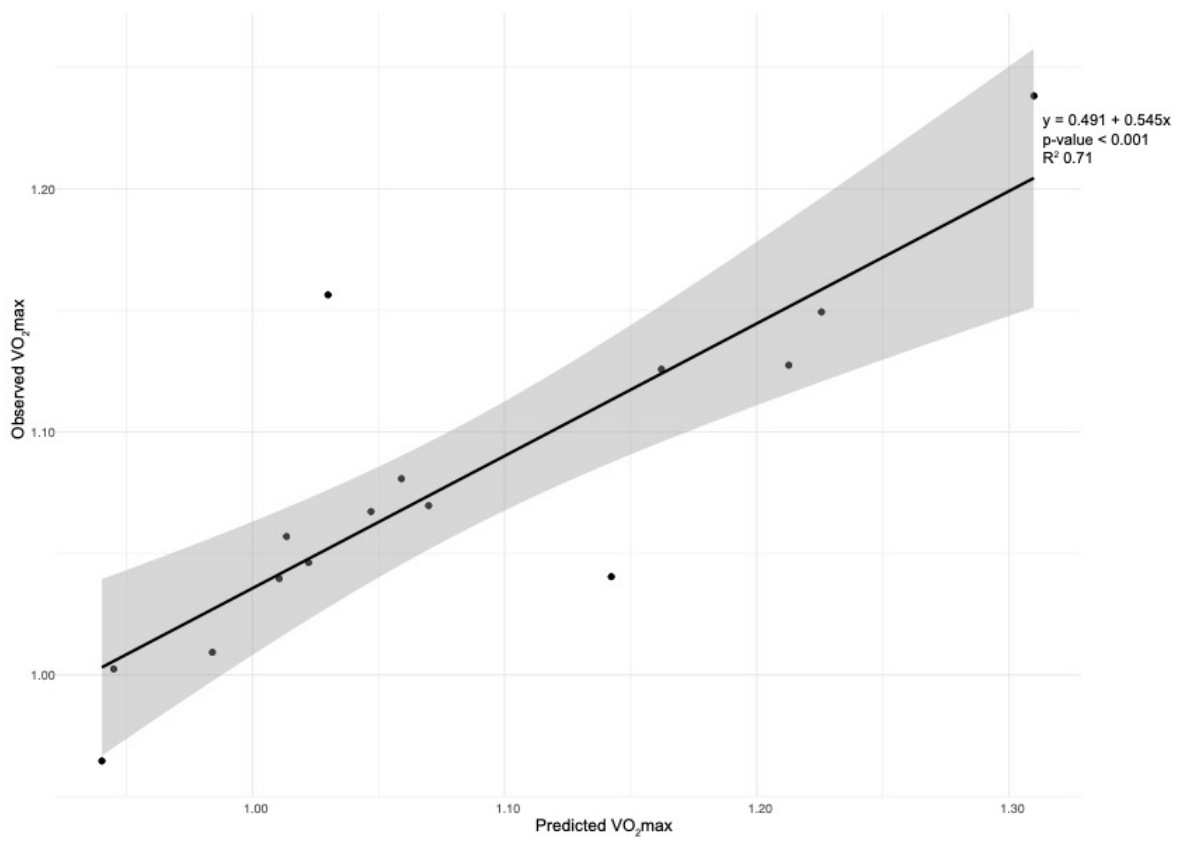
Figure 16: Predicted versus observed change–scores in VO_{2max} for model using arginine, citrulline and plasma osmolality from the first training session (T1) as independent variable.

5 Discussion

In numerous published studies (13,14), SIT interventions consisting of short supramaximal exercise-bouts have been shown to be effective in increasing VO_{2max}. Paper Ⅰ and Ⅱ corroborates these findings. In addition, the increase in VO_{2max} was not only concomitant with an hypervolemic response (Paper Ⅰ), but also mainly mediated by the same (Paper Ⅱ). The changes in Q_{max} and VO_{2max} in relation to training and phlebotomy were both highly correlated and causally linked to the changes in BV (Paper Ⅱ). Each session of SIT was associated with substantial fluid efflux from the intravascular compartments with a concomitant increase in volume of the active muscles. Processes involved in the accumulation of G6P seemed to be of importance for these changes (Paper Ⅲ). Furthermore, a single bout of SIT induced a rapid and profound shift in plasma amino acid concentrations and increases in blood cell counts, plasma protein concentrations and plasma osmolality. From these variables, plasma concentrations of citrulline and arginine together with plasma osmolality showed the strongest relationship with the improvements in VO_{2max} (Paper Ⅳ).

The observed increases in VO_{2max} (Paper Ⅰ and Ⅱ) are consistent with previous studies in which comparable interventions were performed. Similar increases have been demonstrated after 4 weeks of repeated 15-30-second bouts of SIT (134), after 6 weeks of combined HIIT and SIT (135), and after a variety of other SIT protocols (14,88). The data presented in this thesis shows that 6 weeks of SIT leads to robust improvements in both BV and Q_{max}. These findings are corroborated by two recent study showing that 8 and 12 weeks of SIT leads to improvements in BV and Q_{max} (88,136) but stands in contrast to two earlier studies that failed to detect any changes in Q_{max} despite robust increases in VO_{2max} after 4 and 6 weeks of SIT (86,87). In one of the studies (86), neither improved Q_{max} nor a-vO_2diff were associated with the increased VO_{2max} until the study participants were stratified into "high and low responders" and changes in a-vO_2diff could be detected. However, it should be noted that the change could only be detected when comparing a-vO_2diff pre- to post-intervention within the stratified groups and not between the groups. Similarly, the other study reporting that Q_{max} is unaffected by SIT performed the assessment of Q_{max} on intensities below VO_{2max} where the HR_{max} was lower than what was observed during the assessment of VO_{2max}. This challenges the validity of the results in question (87).

Although numerous studies have shown that skeletal muscle adaptations that are associated with the ability to extract O_2 increase following SIT (13) there is still no evidence that these adaptations translate to increases in a-vO_2diff of a magnitude necessary in order to explain improvements of ~10-15% in VO_{2max}. To my knowledge, paper Ⅱ is the first study to assess a-vO_2diff directly by catheterization after SIT and as evident from the results, mixed venous oxygen saturation (SvO_2) at VO_{2max} prior to the exercise

intervention was on average 23.9±6.2% and decreased to 21.0±6.1% following the training-intervention. Simultaneously, we observed an increase in systemic O_2 extraction, driven by the decreased CvO_2 and in contrast to SV and Q_{max}, it was unaffected by phlebotomy. While studies in athletes, sampling blood from the right atrium, have shown that SvO_2 and CvO_2 can reach values as low as 10% and 2.01 mL $\cdot$ dL^{-1} respectively, it seems unlikely, and yet to be proven, that SIT interventions lasting 2–12 weeks would elicit peripheral improvements matching the data from elite cross-country skiers (137) or one-leg exercise models (138). In line with the findings presented in this thesis, increases of similar magnitude in O_2 extraction have also been observed following other types of exercise interventions (72,138–141). Taken together, this would imply that the peripheral adaptations commonly reported after SIT lead to improvements reflected by increased O_2 extraction. However, without a substantial increase in O_2 delivery in the form of increased Q_{max}, the magnitude of these adaptations cannot explain all of the increases in VO_{2max} observed with SIT.

The data from paper II shows a causal relationship between the hypervolemic response after SIT and the increases in Q_{max} and VO_{2max}. This is in line with what has been showed after 6 weeks of TET where normalization of BV to pre-intervention levels also lead to elimination of the training-induced increases in VO_{2max} and Q_{max} (44,45). The importance of BV in relation to VO_{2max} and Q_{max} has been demonstrated in multiple earlier experiments and is well known (131,132,142,143). The addition of the data from this thesis expands our knowledge and suggest that central adaptations such as BV and Q_{max} are of paramount importance for increased VO_{2max} independently of the training mode used to accomplish the improvements in VO_{2max}. Interestingly, a recent 10-week TET intervention resulted in an increase in VO_{2max} and Q_{max} of 11% and 10%, respectively, concomitant with a BV expansion of 3.7%. Following phlebotomy, however, VO_{2max} and Q_{max} were unaltered (133). In agreement with the authors, I believe that the explanation for these discrepancies are multifactorial, with the difference in the amount of blood phlebotomized and the difference in the time between phlebotomy and start of the exercise having important roles in the explanation. Montero et al (45), Bonne et al (44) and we (Paper II) started the post-phlebotomy assessment of VO_{2max} within 10 minutes after normalization of BV whereas in Skattebo et al (133), participants started the test 45 minutes after phlebotomy. The time delay and the smaller amount of blood phlebotomized increases the possibility that the subjects in Skattebo et al. managed to partially re-establish their BV through redistribution of extracellular fluid from the interstitial space to the intravascular space, and hence mitigate the decremental effects associated with venesection. The same group of investigators later demonstrated that phlebotomy of 450 mL but not 150 mL impaired VO_{2max} and exercise capacity, suggesting the existence of compensatory mechanisms able to counteract the effects of small BV losses or that the changes induced from a 150 mL phlebotomy are under the detection limit (144).

One acute session of SIT has been shown to reduce skeletal muscle glycogen by ~20% and leads to a marked disruption of the physiological homeostasis exemplified by acute decreases in PV and large increases in plasma lactate concentration reported in paper Ⅲ and by others (91). Although the absolute levels of glycogen utilization is similar between 30 minutes of TET at 50% of VO_{2max} and SIT (91,145), there is a large difference in the speed at which glycogenolysis occurs. As seen in paper Ⅲ, the accumulation of G6P was correlated with both the plasma volume decrease from the intravascular compartments and the increase in muscle volume after one session of SIT. This indicates that during SIT, the accumulation of lactate and the shift in the ratio of NADH/NAD+ impairs the flux-rate of metabolites through the glycolysis resulting in an accumulation of G6P. This is in line with the notion that exercise-induced increases in muscle fluid infiltration and volume is due to the accumulation of intramuscular metabolites (146,147). Exercise-induced efflux of PV from the intravascular compartments leads to hypertonic hypovolemia which is correlated to increased activity of aldosterone and renin-angiotensin (99). It is assumed that these hormones together with plasma proteins are responsible for the hypervolemia occurring in the hours and days after exercise (148,149). In line with this, albumin synthesis increase following HIIT, facilitating the increased albumin content and PV expansion. As many of the acute features following SIT, HIIT and TET are similar, albeit of different magnitudes, it is tempting to speculate that underlying mediators of these changes, that result in the increased BV as shown in paper Ⅰ and Ⅱ, are the same for all three of the mentioned training modalities.

As exercise intensity has been proposed to be a key variable in the explanatory model for the variance in VO_{2max} with different exercise regimes (38,150), and acute PV efflux is intensity dependent (99), we investigated if any of the acute perturbations in the metabolic and intravascular environment had any predictive value for the increases in VO_{2max} after 6 weeks of SIT (Paper Ⅳ). A number of the amino acids investigated showed profound shifts in plasma concentration. Blood cell counts, osmolality and plasma protein concentration were greatly perturbed showing that SIT introduces great levels of "disturbance" in whole body homeostasis. Interestingly, there was no difference in this response between the first week of training and the last week of the 6-week intervention, suggesting that the patterns seen are repeatable despite the training adaptations that have occurred during the intervention. In paper Ⅲ, we reported that the immediate decrease in PV caused by extravasation of fluid into active muscle was closely associated with metabolic disturbances such as a decrease in pH and G6P accumulation.

In paper Ⅳ, it was confirmed that a single bout of SIT leads to an immediate and robust hemoconcentration and an increase in plasma osmolality, but none of the variables directly involved in this process was associated with long-term improvement in VO_{2max}. In contrast, the increase in arginine and its derivative citrulline immediately after exercise, together with increased plasma osmolality 2 hours after exercise, correlated significantly

with an improvement in VO_{2max}. The timing as well as the time course of arginine and osmolality suggest that immediate fluid extravasation after exercise is not the underlying process but a secondary, presumably compensatory, mechanism leading to an "overshoot" in plasma osmolality as fluid returns to the circulation in the hours after exercise. Although the mechanism causing the changes in plasma arginine and citrulline concentrations after exercise remains enigmatic, our data clearly suggest that amino acids, which play a more complex role in exercise physiology than those directly associated with substrate turnover and energy production during acute exercise, contribute in some way to the adaptations that mediate the changes in VO_{2max}. This is consistent with previous studies that have shown that another marker of immediate disturbances in exercise metabolism, lactate, is not associated with long-term adaptations or improvements in VO_{2max} (151,152).

The lack of a training group performing TET warrants cautious interpretation of the data from paper Ⅲ and Ⅳ as it makes it more difficult to directly compare the current results with those previously published using TET as a training stimulus. Especially since the literature is not as unanimous as it is in the context of central adaptations and their importance for VO_{2max}. I believe that the evidence for the importance of BV and Q_{max} for changes in VO_{2max} is sufficiently solid and strong and that the results in paper Ⅰ and Ⅱ represent a true causal relationship.

One of the main strengths of the present work is that it not only presents data showing robust central hemodynamic adaptations occurring concomitantly with the increase in VO_{2max} after SIT (Paper Ⅰ), but also demonstrates a causal relationship between BV and VO_{2max} (Paper Ⅱ) by using the gold standard method for measuring Q_{max} (153). Furthermore, the data presented for a–vO_2diff were obtained as accurately as possible by using right heart catheterization and did not rely on indirect measurements of Q_{max} as do most other studies examining these factors. The limitations of these indirect methods are illustrated by the Q_{max} data in paper Ⅰ, where the absolute values are underestimated and, if used to derive a–vO_2diff from the Fick equation, would lead to large and unrealistic overestimates of a–vO_2diff. In the case of the underestimation of Q_{max} in paper Ⅰ, the explanation is most likely the recirculation of N_2O into the pulmonary system during rebreathing. Although the method has been shown to be reproducible and reliable (154,155), physiological parameters derived from indirect measurement of Q_{max} should be interpreted with caution.

6 Conclusions

The data presented in this thesis highlight the importance of central hemodynamic factors such as BV and Q_{max} in SIT-induced increases in VO_{2max}. In addition, the data shows that SIT leads to significant metabolic and intravascular perturbations and that processes related to amino acid metabolism and altered osmolality are associated with the training response after 6 weeks of SIT. Many of the acute, SIT-induced effects resemble those previously reported after TET. Finally, the data suggest that the contribution of central and peripheral factors to the improvement in VO_{2max} after SIT is similar to that previously reported for HIIT and TET.

7 Points of perspective

Based on the results presented in this thesis it appears that SIT, although very different in terms of duration and intensity, leads to similar adaptations as those reported for TET. Earlier studies have shown that SIT is at least equally effective as TET in improving VO_{2max} but the data presented in the current thesis also suggests that the underlying mediators of these improvements are the same. This gives rise to a new question, how can SIT and TET lead to similar adaptations when so many of the training related variables are completely different?

An interesting aspect of the adaptations seen with exercise that leads to hypervolemia is that the expansion of RBCV is always preceded by an expansion of PV. Future studies should investigate what in the acute response to exercise allows for an accumulation of plasma albumin that is known to facilitate PV expansion. Also, investigations looking at central and peripheral factors and how they contribute to the increase in VO_{2max} should focus on direct measurements of a–vO_2diff as the indirect methods available are not sensitive enough to detect the adaptations occurring peripherally partly due to the variability introduced in the data when deriving a–vO_2diff from indirect measures of Q_{max}.

In light of the data presented in this thesis, both SIT and TET must trigger a stimulus and processes leading to increased albumin synthesis in order to facilitate the expansion of PV preceding the increase in RBCV. Albumin synthesis is driven by decreased plasma albumin concentration in the hepatocyte environment but it is unclear how this occurs during exercise, as albumin concentration often increases due to loss of PV to peripheral tissues. There are several theories associated with the acute loss of PV, including osmolality–related increases in various antidiuretic hormones and increased water retention leading to a small decrease in albumin concentration, the major stimulator of albumin synthesis. In this regard, the data in this thesis shows that PV decreases acutely after SIT to a similar extent as after TET. However, the same acute decrease in PV can also be observed after whole–body resistance training, which we know has marginal effects on VO_{2max} and BV expansion. This calls into question the importance of PV efflux, but does not rule out the possible importance of changes in osmolality, as other training–related mechanisms are known to have an effect on osmolality. Future investigations should measure PV, osmolality, hormonal response, and albumin concentration and synthesis under different conditions in order to better understand these processes and how the relate to the hypervolemic response seen after SIT and TET.

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