INTRODUCTION

There has been a steep rise in the interest regarding interval exercise regimes in recent years. Much of the attention stems from the intriguing effectiveness of interval training in improving maximal aerobic capacity ($\dot{V}O_2\text{max}$) and associated cardiovascular and peripheral physiological factors. The two most common forms of interval exercise are high intensity interval training (HIIT) and sprint interval training (SIT). As interval training can be modified in numerous ways by

Abstract

Intense interval exercise has proven to be as effective as traditional endurance exercise in improving maximal oxygen uptake. Shared by these two exercise regimes is an acute reduction in plasma volume, which is a suggested stimulus behind exercise-induced increases in blood volume and maximal oxygen uptake. This study aimed to link exercise-induced metabolic perturbation with volume shifts into skeletal muscle tissue. Ten healthy subjects (mean age 33 ± 8 years, 5 males and 5 females) performed three 30 s all-out sprints on a cycle ergometer. Upon cessation of exercise magnetic resonance imaging, $^{31}$Phosphorus magnetic resonance spectroscopy and blood samples were used to measure changes in muscle volume, intramuscular energy metabolites and plasma volume. Compared to pre-exercise, muscle volume increased from 1147.1 ± 35.6 ml to 1283.3 ± 11.0 ml 8 min post-exercise. At 30 min post-exercise, muscle volume was still higher than pre-exercise (1147.1 ± 35.6 vs. 1222.2 ± 6.8 ml). Plasma volume decreased by 16 ± 3% immediately post-exercise and recovered back to – 5 ± 6% after 30 min. Principal component analysis of exercise performance, muscle and plasma volume changes as well as changes in intramuscular energy metabolites showed generally strong correlations between metabolic and physiological variables. The strongest predictor for the volume shifts of muscle and plasma was the magnitude of glucose-6-phosphate accumulation post-exercise. Interval training leads to large metabolic and hemodynamic perturbations with accumulation of glucose-6-phosphate as a possible key event in the fluid flux between the vascular compartment and muscle tissue.

KEYWORDS

$^{31}$P-MRS, edema, HIIT, MRI, plasma volume, SIT
manipulating variables such as intensity, duration, and rest to work ratio, the terminology in the literature is rather inconsistent (Weston et al., 2014). In short, HIIT is interval training performed at an intensity eliciting 80–95% of maximal heart rate, whereas SIT is performed at an intensity corresponding to \( \dot{V}_\text{O}_\text{2,max} \) and above, including all-out efforts (MacInnis & Gibala, 2017).

When comparing interval training with more traditional endurance exercise (TEE) performed continuously and at lower intensities, it is evident that the nature of the two training modes is vastly different. Still, a large number of studies demonstrate that interval training induces similar metabolic adaptations as TEE (Burgomaster et al., 2005, 2008; Gist et al., 2014; Milanović et al., 2015; Rakobowchuk et al., 2008). At the molecular level, both HIIT and SIT stimulate skeletal muscle signaling pathways, suggested to play a key role in peripheral adaptations, to a comparable degree as TEE (Egan et al., 2010; Gibala et al., 2009; Little et al., 2014; Milanović et al., 2015; Rakobowchuk et al., 2008). Moreover, comparable changes in \( \dot{V}_\text{O}_\text{2,max} \) have generally been noted (Gist et al., 2014; Milanović et al., 2015; Phillips et al., 2017), which is intriguing since aerobic capacity is mainly dependent on central hemodynamic factors.

A tight relationship exists between total blood volume, cardiac output and \( \dot{V}_\text{O}_\text{2,max} \) (Celsing et al., 1987; Convertino, 2007; Convertino et al., 1980; Kjellberg et al., 1949). Plasma volume expansion accounts for nearly all the increase in blood volume during the first 2–3 weeks after onset of exercise (Fellmann, 1992; Gore et al., 1992). This is followed by a later increase in erythrocyte volume until reaching equilibrium, i.e., restored hematocrit value corresponding to pre-training levels (Sawka et al., 2000). Albumin is accountable for ~75% of the oncotic pressure in plasma and is therefore a crucial factor in the maintenance of plasma volume (Gillen et al., 1991). It has been shown that increased plasma albumin accounts for ~85% of the total plasma protein content during exercise-induced hypervolemia (Convertino et al., 1980). Thus, an increase in blood volume must be preceded by an increased synthesis of albumin (Tsutsumi et al., 1993). It has been hypothesized that reduced plasma volume acts to stimulate mechanisms leading to increased albumin synthesis and total plasma volume (Convertino et al., 1980, 1983; Röcker et al., 1976; Rothschild et al., 1972; Yang et al., 1998). Given that a reduction in plasma volume is seen with both HIIT, SIT (Lundvall et al., 1972; Metcalfe et al., 2015) and TEE (Convertino et al., 1983), it is possible that this comparable degree of plasma flux explains why increases in \( \dot{V}_\text{O}_\text{2,max} \) are possible with both interval training and TEE training.

Breakdown of muscle glycogen leads to a hypertonic intramyocellular environment through accumulation of metabolites such as hydrogen ions (H\(^+\)), inorganic phosphate (P\(_i\)), lactate, and glucose-6-phosphate (G6P). Interval training causes a 20–30% reduction in muscle glycogen content (Esbjörnsson-Liljedahl et al., 1999; Gibala et al., 2009; Parolin et al., 1999), and the muscle glycogen breakdown occurs predominantly during the first 15 s of a sprint (Metcalfe et al., 2015; Parolin et al., 1999). Accumulation of these metabolites has been demonstrated to lead to an influx of water in skeletal muscle (Raja et al., 2006; Ward et al., 1996; Watson et al., 1993), which provides a possible mechanism for plasma volume loss noted with interval exercise. Tissue water content can be assessed using magnetic resonance imaging (MRI) by measuring transverse relaxation time (T2), where increases in accumulated metabolites and tissue water content results in an increased signal intensity on T2-weighted MR images (Fisher et al., 1990; Fleckenstein et al., 1988; Price & Gore, 1998; Price et al., 1995). By combining MRI measurements with \(^{31}\text{P}\) magnetic resonance spectroscopy (\(^{31}\text{P}-\text{MRS}\)) it is possible to measure pH, inorganic phosphate (P\(_i\)), G6P and other intramuscular energy derivatives.

The aim with this study was to characterize sprint-induced plasma flux in relation to muscle metabolic perturbation where we hypothesize that 1) 3 × 30 s all-out sprints cause a robust increase in muscle volume and reduction in plasma volume, and that 2) the changes in metabolic perturbation are correlated with the changes in muscle volume.

2 | METHOD

2.1 | Exercise protocol

The exercise protocol was comprised of three 30 s all-out sprints against a breaking force equivalent to 7.5% of the subject's body weight. Each sprint was separated by 2 min of low intensity cycling. Subjects were given strong verbal encouragement during each interval. The sprints were performed on a mechanically braked cycle ergometer (Ergomedic 894E). The cycle setup was placed just outside the MRI-suite and subjects were moved from the cycle ergometer to the MRI and then positioned in a predefined supine position before the post-measurements started. This procedure was practiced with each subject prior to the experiment to minimize loss of time between exercise completion and the first measurement. The surface coil used for \(^{31}\text{P}-\text{MRS}\) was positioned at the vastus lateralis and the area of the coil was marked to ensure identical placement during both baseline and post-measurements. The transfer of the subjects from the cycle ergometer to the MRI took approximately 120 s, counting the time from exercise cessation until start of data acquisition. Phosphocreatine (PCr), pH, adenosine triphosphate (ATP), and G6P concentrations were acquired at baseline, and continuously from completion of exercise until 30 min post-exercise with interruptions of approximately 45 s every 5 min for collection of Dixon-sequences for assessment of muscle volumes. Exact time points can be found in Figure 1. Blood
was sampled before, immediately after exercise and thereafter in 5 min intervals up to 30 min post-exercise. Plasma volume drop was calculated using the Dill and Costill equation (Dill & Costill, 1974).

2.2 Dixon 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 2.3 ms, TR 3.8 ms, FOV 50 × 35 cm, and voxel size 1.2 × 1.2 × 4 mm with a scan time of about 1 min. 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 (Mandić et al., 2020). The pre-exercise baseline reading was acquired immediately after a 5-min 31P-MRS acquisition. All image acquisitions were performed on a Philips Ingenia 3T.

2.3 31P-MRS acquisition

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) with a diameter of 14 cm was used for spectroscopic measurements. The coil was manually tuned and matched before the 31P MRS acquisitions. A 42° block pulse was used for the spin excitation. The baseline measurements were performed using 15 s repetition time (TR), 153.4 μs echo time (TE), 3 kHz bandwidth, 2048 data points, 16 averages, 2 dummy scans, and a synthesizer frequency of 51.717 MHz. To increase the temporal resolution the recovery phase spectra were acquired with a 2 s TR and no spectral averaging, a total of 150 single spectra were acquired with no dummy scans. For post-processing of the 31P-MRS data, jMRUI (Naressi et al., 2001) was used with the AMARES algorithm (Vanhamme et al., 1997) 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 s to enhance SNR. Phosphocreatine (PCr) was used as a chemical-shift reference with assignments obtained from literature as previously described (Gerdle et al., 2013, 2020) and showed for a baseline dataset in Figure 2. In short, phosphomonoesters (PME) were assigned to phosphoethanolamine and...
phosphocholine, in the dynamic phase this resonance also includes glucose-6-phosphate (G6P; details below; Haan et al., 2003); 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 (Gerdle et al., 2013, 2020). 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 (De Graaf, 2007) 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 (Harris et al., 1974). 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 (Equation 1). While others have calculated the difference by subtracting baseline spectra from the dynamic sets (Haan et al., 2003), this was not feasible in the current setting since (1) the exercise bouts could not be performed within the MR bore and (2) the chemical shift differences due to the very high post-exercise intra-myocellular lactate concentrations.

\[
\Delta \text{G6P}(t) = \frac{\text{PME}(t)}{\text{PME}(t = \text{end})}.
\]  

(1)

The dynamics of PCr and P_i were estimated by relating each timepoint to the last (Equation 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 Equation 2.

\[
\text{Correction factor} = \frac{1}{1 - \exp\left(-\frac{\text{TR}}{T_1}\right) \exp\left(-\frac{\text{TE}}{T_2}\right)}.
\]  

(2)

TR and TE are specific to the 31P-MRS acquisition protocol and T_1 and T_2 are relaxation times of the resonances being corrected. The relaxation times were taken from the literature (Bogner et al., 2009) assuming β-NTP and PME to have similar T_2 characteristics as α-NTP and PDE, respectively.

### 2.4 | Statistics

All averages are given as geometrical mean and standard deviation if not stated otherwise; standard deviation is corrected for within-subjects effect (Morey, 2008). Principal Component Analysis was carried out with variables scaled to unit variance using FactoMineR version 2.0 on the statistical platform R version 3.5.3. Where appropriate, normality was tested using the Kolmogorov-Smirnov test, and 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. Subject-by-subject curve-fittings can be found in Figure S1.

### 2.5 | Ethical approval

All subjects were given oral and written information about the study before giving informed consent to participate. The study was approved by the Swedish Ethical Review Authority.

### 3 | RESULTS

#### 3.1 | Baseline characteristics

Ten healthy subjects participated in the study, the group consisted of 5 females and 5 males (age 33 ± 8 years, height 175.2 ± 7.4 cm, weight 74.1 ± 11.6 kg). At baseline, all subjects had normal hemoglobin and hematocrit values, 137.5 ± 6.5 g/L and 41 ± 2% respectively. The participants were moderately active but were not involved in any structured exercise programs.

#### 3.2 | Exercise and workload

For each individual and each exercise bout, power output was recorded and summarized into peak power output (PPO) and mean power output (MPO). Peak and mean power for bout 1 was 642 ± 113 and 472 ± 64 W which decreased to 465 ± 54 and 353 ± 27 W in bout number 2. (p < 0.010).
The peak and mean power output did not change significantly between bouts 2 and 3, 421 ± 63 and 312 ± 38 W, respectively (Figure 3).

3.3 | Plasma volume/Hemoconcentration

Hemoglobin concentration increased from 137.5 ± 6.5 g/L at baseline to 155.3 ± 9.2 g/L immediately after exercise ($p < 0.001$). Thereafter it decreased by an average of 0.5 g/min, reaching 140.9 ± 10.9 g/L 30 min post-exercise. Plasma volume dropped by 16 ± 3% immediately post-exercise and recovered back to −5 ± 2% at 30 min post-exercise (Figure 4a).

3.4 | Muscle volume

At baseline, muscle volume of the quadriceps was 1147.1 ± 35.6 ml, which increased by an average of 12% to 1283.3 ± 11.0 ml 8 min post-exercise. At 14 min post-exercise, the quadriceps volume had decreased to 1254.7 ± 5.8 ml; thereafter it followed a linear downward slope over the subsequent four measurements. At 30 min post-exercise, the muscle volume had decreased to 1222.2 ± 6.8 ml, but it was still significantly higher than pre-exercise (Figure 4b). Similarly, the muscle volume of the hamstring musculature increased from 1085.3 ± 29.3 ml to 1175.9 ± 7.5 ml 8 min post-exercise. Likewise, the hamstring volume decreased to 1147.3 ± 6.7 ml 30 min post-exercise, which also remained higher compared to the pre-exercise volume.

3.5 | $^{31}$P-MRS estimation of metabolites

Free phosphate (Pi) concentration was 6.6 ± 0.03 mM at pre-exercise and 6.7 ± 0.05 mM at 2 min post-exercise. The concentration of Pi decayed with a time-constant of 362 ± 198 s and with a rate of decay of 0.03 ± 0.08%/s (Figure 5a). Due to the low concentration, we were not able to obtain data on the baseline concentration of G6P. 2 min post-exercise G6P concentration was 3.85 ± 0.01 mM and recovered thereafter with a time-constant of 415 ± 70 s corresponding to a rate of 0.004 ± 0.002%/min (Figure 5b). At baseline, muscle pH was 6.94 ± 0.21. It dropped to 6.22 ± 0.06 at 2 min post-exercise and recovered to 6.79 ± 0.05 at 19 min post-exercise, with a recovery time constant of 960 ± 509 s corresponding to 0.10 units/min. Corresponding changes as H$^+$ is shown in Figure 5c. The baseline pre-exercise phosphocreatine concentration was 43.7 ± 1.6 mM. At 2 min post-exercise PCr was 34.9 ± 2.7 and it thereafter recovered with
a time-constant of $372 \pm 126$ s, which corresponded to a recovery rate of $5.4 \pm 0.8\%$/min (Figure 5d).

### 3.6 Workload, muscle volume and metabolite relationships

The association between the different intensity measures over the three exercise bouts was explored using PCA, which revealed that all intensity measures were highly correlated within each bout of exercise (i.e., a high peak power was associated with a higher mean power output). All intensity measures from bout numbers 2 and 3 were in turn highly correlated but the intensity measures of bout number 1 were largely independent from the other two. Thus, the power output of each individual can be largely described in terms of two components, PC1, mainly described by the power output from bout numbers 2 and 3, and PC2 described by the power output of bout number 1 (Figure 6). Subsequently a new principal component analysis was performed analyzing exercise performance, muscle and plasma volume, as well as $^{31}$P-MRS-data. This revealed a high degree of mutual correlation between the investigated variables and a substantial portion of the variance could be summarized into the first (34.3%) and second component (25%; Figure 7).
Variables with a significant correlation to the first two components are shown in Figure 7, loadings of 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 bouts 2 and 3 are also mutually correlated but cluster along PC2 indicating no significant correlations with the other variables.

FIGURE 7 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 bouts 2 and 3 are also mutually correlated but cluster along PC2 indicating no significant correlations with the other variables.

FIGURE 8 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 positive 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.

4 | DISCUSSION

This study used a novel approach to estimate the exercise-induced fluid shift into skeletal muscle tissue and to relate this muscle volume change to the metabolic perturbation induced by exercise. Following three bouts of intense interval exercise, a drop in plasma volume and an increase in thigh muscle volume was observed. Coupled with these changes, measurements using \(^{31}\)P-MRS revealed an increase in PI and G6P together with a simultaneous decrease in pH and PCr. Accumulation of G6P and the increase in muscle volume was highly correlated. Exercise power output during the second and third exercise bout was consistent whereas the first
bouts was largely independent from the other two. Principal component analysis revealed that changes in muscle and plasma volume co-varied with PCr depletion, pH drop and G6P-accumulation whereas power-output during the second and third exercise bout co-varied with post-exercise PCr-recovery.

Previously it has been suggested that the increase in tissue fluid with exercise is due to an accumulation of fluid in the intra-cellular compartments (Raja et al., 2006). This has been explained by contraction-induced increases in intra-muscular osmolality and capillary filtration pressure (Lundvall et al., 1972; Stick et al., 1990). Using the Du Bois formula to calculate body surface area and the normative tables of plasma volume from Hurley, we can estimate a baseline plasma volume of 3027 ± 428 ml in the current study (Du Bois & Du Bois, 1989; Hurley, 1975). The drop in plasma volume in the present study amounted to 16 ± 3%, which corresponded to ~484 ml of plasma loss. This change is of similar magnitude as earlier reports exploring exercise of a longer duration (Greenleaf et al., 1979; Lundvall et al., 1972; Sjøgaard & Saltin, 1982). Furthermore, the loss matches the increase in muscle tissue water we observed in the presumably most active muscles when performing interval exercise on a bike (Silva et al., 2016). The plasma volume recovery was faster than what was observed for the muscle tissue. While plasma volume was close to be completely restored 30 min post-exercise muscle volume was still substantially higher compared to baseline measurements. This discrepancy has been reported earlier and has been suggested to be due to extra-vascular fluid exchange between inactive tissues and contracting skeletal muscle tissue (Lundvall et al., 1972; Stick et al., 1990). Plasma fluid loss into the contracting muscle increases the plasma osmolality, which is one of the driving forces behind fluid outflow from inactive tissue. Based on the vasoconstriction in inactive tissue during exercise, a reduced filtration pressure has also been suggested to contribute (Lundvall et al., 1972; Stick et al., 1990). Thus, it can be assumed that plasma volume can be replenished from sources other than the active muscle post-exercise and thereby attenuate the drop in plasma volume, thus protecting venous return and cardiac stroke volume (Wingo et al., 2012). Regardless of these effects, our data clearly show a substantial plasma volume drop and corresponding muscle fluid infiltration after intense interval exercise.

During more conventional type of endurance exercise, the exercise-induced loss of plasma volume leads, in an intensity-dependent fashion, to hypertonic hypovolemia, which is highly correlated to increased releases of aldosterone and renin-angiotensin (Convertino et al., 1981). These hormones are assumed to drive the development of hypotonic hypovolemia occurring post-exercise (Convertino et al., 1980; Green et al., 1984). Importantly, oncotic pressure in plasma is the major regulator of albumin synthesis (Rothschild et al., 1972), which has also been reported to increase following short term high intensity exercise (Yang et al., 1998). Altogether, the current data support our hypothesis that three very brief bouts of high intensity interval training induce similar reduction in plasma volume as has been previously reported with more conventional types of endurance training. We therefore suggest that increased blood volume and VO2max in response to traditional endurance training as well as interval training plausibly occurs through the same mechanism.

As stated above, exercise-induced increases in muscle fluid and volume are assumed to be at least partly due to osmosis, mainly because of the increased intramuscular osmolality driven by exercise-induced intramuscular metabolic perturbations (Lundvall et al., 1972; Stick et al., 1990). Thus, metabolic perturbation is assumed to be one of the key processes behind exercise-induced fluid inflow to the exercising skeletal muscle. In the present study, the G6P concentration measured post-exercise explained muscle volume changes to the greatest extent: A positive correlation was observed between changes in PME-intensity corresponding to an accumulation of G6P and muscle volume expansion and a negative correlation was seen between G6P and plasma volume drop. This is an indication that during sprint exercise the accumulation of lactate and shift in NADH/NAD+ ratio leads to a decrease in the flux-rate of metabolite though the glycolysis leading to an accumulation of G6P due to a mismatch in the flux though glycolysis and the rapid breakdown of glycogen. Based on the mechanisms underlying the accumulation of G6P, it may serve as a proxy-measure for accumulation of several osmotically important metabolites and metabolic stress on the exercising muscle.

The absolute concentration of PCr immediately post-exercise could not be established. However, based on earlier studies using the same type of exercise (Bogdanis et al., 1996; Parolin et al., 1999), it can be assumed that PCr is close to depletion directly after the third interval. As the PCr-recovery relies on oxidative phosphorylation of ATP, the PCr-recovery time follows that of citrate-synthase; the rate-limiting step for oxidative phosphorylation in the muscle, and the oxidative capacity of the muscle (Bogdanis et al., 1996; McCully et al., 1993). Interestingly, PCr-recovery was highly correlated with the performance measurements from exercise bouts 2 and 3 but unrelated to perturbation or recovery of pH, Pi or G6P. The strong link between PCr-recovery and the power output variables as well as the lack of relationship between power output and other muscle metabolites such as pH and Pi has previously been reported from studies using muscle biopsies to assess metabolite content (Bogdanis et al., 1996, 1998). Results showed a strong correlation between power output during the second and third exercise bouts but that was unrelated to the power output during the first bout. It could be argued that power output during bouts 2 and 3...
reflects endurance capacity, i.e., the ability to sustain a higher power output over time. This is supported by the identified correlation between power output of the later bouts and PCr-recovery time post-exercise. Further support is provided by earlier work showing that resynthesis of PCr after HIIT relates to endurance fitness (Bogdanis et al., 1996). This supports the concept that PCr-recovery time and power output in exercise bouts 2 and 3 mainly reflects skeletal muscle oxidative capacity. Importantly, there is no relationship between these variables and plasma volume drop or muscle volume expansion was observed. This indicates that exercise at relatively high workloads for longer duration has less influence on muscle volume expansion or plasma volume drop compared to very high workloads for a shorter duration, which is supported by earlier reports (Convertino et al., 1981). This is corroborated by findings showing that glycogenolysis (and hence accumulation of metabolites) is maximally activated during the first 15 s of the initial exercise bout (Parolin et al., 1999). Also, recent data indicate that additional interval bouts do not result in any additive effects on \( \dot{V}O_{2}\max \) (Metcalf et al., 2012, 2015). Instead, it seems that adding bouts at the expense of the exercise intensity reduces the training effect; this is presumably related to a decrease in maximal work load during each interval (Phillips et al., 2017). The present data provide mechanistic insight into these observations and indicate that metabolic perturbation by decreased glycolytical flux-rate and accumulation of muscle G6P is a key event in fluid flux between the vascular compartment and muscle tissue. This could also be a plausible stimulus behind the hemodynamic adaption to exercise including the increase in plasma volume and subsequent expansion of the blood volume leading to improvements in \( \dot{V}O_{2}\max \).

In the current study, we aimed to estimate the exercise-induced volume shift into skeletal muscle tissue and to link this fluid shift to exercise-induced metabolic stress in the tissue. While simultaneous assessments are only possible using MRI, it is necessary to perform the exercise outside the MRI suite. Thus, we acknowledge that the time (90–120 s after completed exercise) to the first measurements includes the resynthesis of numerous metabolites such as high energy substrates, i.e., PCr and ATP. It may be suggested that this limits the capacity to characterize the post-exercise metabolic condition and to relate changes in the metabolic condition within the skeletal muscle with the volume shifts. Nevertheless, the consecutive measurements over time of both volume and metabolites allow for accurate curve-fitting and estimation of changes over time and the integrated analysis is not dependent on \( t_0 \) or fluxes during the actual exercise. Importantly, the estimations that were generated commensurate with what has been reported in invasive studies (Kemp et al., 2007). For future studies, a valuable addition to the literature would be the inclusion of interval protocols of varying intensities and number of sprints in an effort to investigate dose responses.

In summary, this is the first study, to our knowledge, showing that three very brief supramaximal intervals lead to similar acute effects on plasma volume and muscle swelling as more prolonged types of exercise. Our results further identify a relationship between muscle metabolism and muscle volume, with post-exercise G6P concentration being a good marker for the changes in muscle volume. The current findings support the plausible idea that exercise-induced reduction of the plasma volume serves as the common primary stimulus underpinning the increase in \( \dot{V}O_{2}\max \) seen with both endurance and high-intensive interval training.

ACKNOWLEDGMENTS
This study was funded by grant to MM, ER and TG from the Swedish Council for Research in Sport Science (CIF).

CONFLICT OF INTEREST
The authors declare no competing interests.

AUTHOR CONTRIBUTIONS
MM, ER, TG and PS designed the study. MM, PW, TR, MF and ER conducted the experiments and analyzed the data. MM, TG and ER drafted the manuscript. All authors read, revised and approved on text and figures.

ORCID
Mirko Mandić https://orcid.org/0000-0002-7414-482X
Eric Rullman https://orcid.org/0000-0003-2854-7262

REFERENCES
Bogdanis, G. C., Nevill, M. E., Boobis, L. H., & Lakomy, H. K. (1996). Contribution of phosphocreatine and aerobic metabolism to energy supply during repeated sprint exercise. Journal of Applied Physiology, 80, 876–884. https://doi.org/10.1152/jappl.1996.80.3.876
Bogdanis, G. C., Nevill, M. E., Lakomy, H. K. A., & Boobis, L. H. (1998). Power output and muscle metabolism during and following recovery from 10 and 20 s of maximal sprint exercise in humans. Acta Physiologica Scandinavica, 163, 261–272. https://doi.org/10.1046/j.1365-201x.1998.00378.x
Bogner, W., Chmelik, M., Schmid, A. I., Moser, E., Trattnig, S., & Gruber, S. (2009). Assessment of (31)P relaxation times in the human calf muscle: A comparison between 3 T and 7 T in vivo. Magnetic Resonance in Medicine, 62, 574–582. https://doi.org/10.1002/mrm.22057
Burgomaster, K. A., Howarth, K. R., Phillips, S. M., Rakobowchuk, M., Macdonald, M. I., McGee, S. L., & Gibala, M. J. (2008). Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. The Journal of Physiology, 586, 151–160. https://doi.org/10.1113/jphysiol.2007.142109
Burgomaster, K. A., Hughes, S. C., Heigenhauser, G. J. F., Bradwell, S. N., & Gibala, M. J. (2005). Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity...
in humans. *Journal of Applied Physiology*, 98, 1985–1990. https://doi.org/10.1152/japplphysiol.1985.98.6.1152

Celsing, F., Svedenhag, J., Pihlstedt, P., & Ekblom, B. (1987). Effects of anaemia and stepwise-induced polycythemia on maximal aerobic power in individuals with high and low haemoglobin concentrations. *Acta Physiologica Scandinavica*, 129, 47–54. https://doi.org/10.1111/j.1748-1716.1987.tb08038.x

Convertino, V. A. (2007). Blood volume response to physical activity and inactivity. *The American Journal of the Medical Sciences*, 334, 72–79. https://doi.org/10.1097/MAJ.0b013e318063c6e4

Convertino, V. A., Brock, P. J., Keil, L. C., Bernauer, E. M., & Greenleaf, J. E. (1983). Exercise training-induced hypervolemia: Role of plasma albumin, renin, and vasopressin. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, 48, 665–669. https://doi.org/10.1152/jappl.1980.48.4.665

Convertino, V. A., Keil, L. C., Bernauer, E. M., & Greenleaf, J. E. (1981). Plasma volume, osmolality, vasopressin, and renin activity during graded exercise in man. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, 50, 123–128. https://doi.org/10.1152/jappl.1981.50.1.123

Convertino, V. A., Keil, L. C., & Greenleaf, J. E. (1983). Plasma volume, renin, and vasopressin responses to graded exercise after training. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, 54, 508–514. https://doi.org/10.1152/jappl.1983.54.2.508

da Silva, J. C. L., Tarassova, O., Ekblom, M. M., Andersson, E., Rönquist, G., & Arndt, A. (2016). Quadriceps and hamstring muscle activity during cycling as measured with intramuscular electromyography. *European Journal of Applied Physiology*, 116, 1807. https://doi.org/10.1007/s00424-016-3428-5

De Graaf, R. A. (2007). *In vivo NMR spectroscopy: Principles and techniques* (2nd ed.). John Wiley & Sons.

de Haan, J. H., Klomp, D. W. J., Tack, C. J., & Heerschap, A. (2003). Optimized detection of changes in glucose-6-phosphate levels in human skeletal muscle by 31P MR spectroscopy. *Magnetic Resonance in Medicine*, 50, 1302–1306. https://doi.org/10.1002/mrm.10630

Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology*, 37, 247–248. https://doi.org/10.1152/jappl.1974.37.2.247

Du Bois, D., & Du Bois, E. F. (1989). A formula to estimate the approximate surface area if height and weight be known. 1916. *Nutrition*, 5, 303–311; discussion 312–313.

Egan, B., Carson, B. P., Garcia-Roves, P. M., Chibalin, A. V., Sarsfield, F. M., Barron, N., McCaffrey, N., Moya, N. M., Zierath, J. R., & O’Gorman, D. J. (2010). Exercise intensity-dependent regulation of peroxisome proliferator-activated receptor coactivator-1 mRNA abundance is associated with differential activation of upstream signalling kinases in human skeletal muscle. *The Journal of Physiology (London)*, 588, 1779–1790. https://doi.org/10.1113/jphysiol.2010.188011

Esbjörnsson-Liljedahl, M., Sundberg, C. J., Norman, B., & Jansson, E. (1999). Metabolic response in type I and type II muscle fibers during a 30-s cycle sprint in men and women. *Journal of Applied Physiology*, 87, 1326–1332. https://doi.org/10.1152/jappl.1999.87.4.1326

Fellmann, N. (1992). Hormonal and plasma volume alterations following endurance exercise. A brief review. *Sports Medicine*, 13, 37–49. https://doi.org/10.2165/00007256-19921301-00004

Fisher, M. J., Meyer, R. A., Adams, G. R., Foley, J. M., & Potchen, E. J. (1990). Direct relationship between proton T2 and exercise intensity in skeletal muscle MR images. *Investigative Radiology*, 25, 480–485. https://doi.org/10.1097/00004424-199005000-00003

Fleckenstein, J. L., Canby, R. C., Parkey, R. W., & Peshock, R. M. (1988). Acute effects of exercise on MR imaging of skeletal muscle in normal volunteers. *American Journal of Roentgenology*, 151, 231–237. https://doi.org/10.2214/ajr.151.2.231

Gerdle, B., Forsgren, M. F., Bengtsson, A., Leinhard, O. D., Sören, B., Karlsson, A., Brandjesky, V., Lund, E., & Lundberg, P. (2013). Decreased muscle concentrations of ATP and PCR in the quadriceps muscle of fibromyalgia patients – A 31P-MRS study. *European Journal of Pain*, 17, 1205–1215. https://doi.org/10.1002/ej.1532-2149.2013.00284.x

Gerdle, B., Ghafouri, B., Lund, E., Bengtsson, A., Lundberg, P., van Ettinger-Veenstra, H., Leinhard, O. D., & Forsgren, M. F. (2020). Evidence of mitochondrial dysfunction in fibromyalgia: Deviating muscle energy metabolism detected using microdialysis and magnetic resonance. *Journal of Clinical Medicine*, 9, 3527. https://doi.org/10.3390/jcm9113527

Gibala, M. J., McGee, S. L., Garnham, A. P., Howlett, K. F., Snow, R. J., & Hargreaves, M. (2009). Brief intense interval exercise activates AMPK and p38 MAPK signaling in human skeletal muscle. *Journal of Applied Physiology*, 106, 929–934. https://doi.org/10.1152/japplphysiol.09880.2008

Gillen, C. M., Lee, R., Mack, G. W., Tomaselli, C. M., Nishiyasu, T., & Nadel, E. R. (1991). Plasma volume expansion in humans after a single intense exercise protocol. *Journal of Applied Physiology*, 71, 1914–1920. https://doi.org/10.1152/jappl.1991.71.5.1914

Gist, N. H., Fedewa, M. V., Dishman, R. K., & Cureton, K. J. (2014). Sprint interval training effects on aerobic capacity: A systematic review and meta-analysis. *Sports Medicine*, 44, 269–279. https://doi.org/10.1007/s40279-013-0115-0

Gore, C. J., Scroop, G. C., Marker, J. D., & Catcheside, P. G. (1992). Plasma volume, osmolality, total protein and electrolytes during treadmill running and cycle ergometer exercise. *European Journal of Applied Physiology and Occupational Physiology*, 65, 302–310. https://doi.org/10.1007/BF00868132

Green, H. J., Thomson, J. A., Ball, M. E., Hughson, R. L., Houston, M. E., & Sharratt, M. T. (1984). Alterations in blood volume following short-term supramaximal exercise. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, 56, 145–149. https://doi.org/10.1152/jappl.1984.56.1.145

Greenleaf, J. E., Van Beaumont, W., Brock, P. J., Morse, J. T., & Mangseth, G. R. (1979). Plasma volume and electrolyte shifts with heavy exercise in sitting and supine positions. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, 236, R206–R214. https://doi.org/10.1152/ajpregu.1979.236.3.R206

Harris, R. C., Hultman, E., & Glycogen, N. L. O. (1974). glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. *Scandinavian Journal of Clinical and Laboratory Investigation*, 33, 109–120. https://doi.org/10.3109/0036517409082477

Hurley, P. J. (1975). Red cell and plasma volumes in normal adults. *Journal of Nuclear Medicine*, 16, 46–52.

Kemp, G. J., Meyerspeer, M., & Moser, E. (2007). Absolute quantification of phosphorus metabolite concentrations in human muscle in
vivo by 31P MRS: A quantitative review. *NMR in Biomedicine*, 20, 555–565. https://doi.org/10.1002/nbm.1192

Kjellberg, S. R., Rudhe, U., & Sjöstrand, T. (1949). Increase of the amount of hemoglobin and blood volume in connection with physical amount. *Acta Physiologica Scandinavica*, 19, 146–151. https://doi.org/10.1111/j.1748-1716.1949.tb00146.x

Little, J. P., Saldar, A., Bishop, D., Tarnopolsky, M. A., & Gibala, M. J. (2011). An acute bout of high-intensity interval training increases the nuclear abundance of PGC1α and activates mitochondrial biogenesis in human skeletal muscle. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 300, R1303–R1310. https://doi.org/10.1152/ajpregu.00538.2010

Lundvall, J., Mellander, S., Westling, H., & White, T. (1972). Increase of the muscle. *Physiological Reports*, 1, 2329. https://doi.org/10.1038/s41598-020-59267-x

McCully, K. K., Fielding, R. A., Evans, W. J., Leigh, J. S., & Posner, J. D. (1993). Relationships between in vivo and in vitro measurements of metabolism in young and old human calf muscles. *Journal of Applied Physiology*, 75, 813–819. https://doi.org/10.1152/japplphysiol.1993.75.2.813

Metcalfe, R. S., Babraj, J. A., Fawkner, S. G., & Vollaard, N. B. J. (2012). Towards the minimal amount of exercise for improving metabolic health: Beneficial effects of reduced-exertion high-intensity interval training. *European Journal of Applied Physiology*, 112, 2767–2775. https://doi.org/10.1007/s00421-011-2254-z

Metcalfe, R. S., Koumanov, F., Ruffino, J. S., Stokes, K. A., Holman, G. D., Thompson, D., & Vollaard, N. B. J. (2015). Physiological and molecular responses to an acute bout of reduced-exertion high-intensity interval training (REHIT). *European Journal of Applied Physiology*, 115, 2321–2334. https://doi.org/10.1007/s00421-015-3217-6

Milanović, Z., Sporiš, G., & Weston, M. (2015). Effectiveness of high-intensity interval training (HIT) and continuous endurance training for VO2max Improvements: A systematic review and meta-analysis of controlled trials. *Sports Medicine*, 45, 1469–1481. https://doi.org/10.1007/s40279-015-0365-0

Morey, R. D. (2008). Confidence intervals from normalized data: A correction to Cousineau (2005). *Tutorials in Quantitative Methods for Psychology*, 4, 61–64. https://doi.org/10.20982/tqmp.04.2.p061

Naessi, A., Couturier, C., Devos, J. M., Janssen, M., Mangeat, C., de Beer, R., & Graveron-Demilly, D. (2001). Java-based graphical user interface for the MRUI quantitation package. *Magna: Magnetic Resonance Materials in Physics, Biology, and Medicine*, 12, 141–152. https://doi.org/10.1007/BF02668096

Norrbom, J., Sundberg, C. J., Ameln, H., Kraus, W. E., Jansson, E., & Gustafsson, T. (2004). PGC1-alpha mRNA expression is influenced by metabolic perturbation in exercising human skeletal muscle. *Journal of Applied Physiology*, 96, 189–194. https://doi.org/10.1152/japplphysiol.00765.2003

Parolin, M. L., Chesley, A., Matsos, M. P., Spriet, L. L., Jones, N. L., & Heigenhauser, G. J. (1999). Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise. *American Journal of Physiology: Endocrinology and Metabolism*, 277, E890–E900.

Phillips, B. E., Kelly, B. M., Lilja, M., Ponce-González, J. G., Brogan, R. J., Morris, D. L., Gustafsson, T., Kraus, W. E., Atherton, P. J., Vollaard, N. B. J., Rooyackers, O., & Timmons, J. A. (2017). A practical and time-efficient high-intensity interval training program modifies cardio-metabolic risk factors in adults with risk factors for type II diabetes. *Frontiers in Endocrinology*, 8. https://doi.org/10.3389/fendo.2017.00229

Price, T. B., & Gore, J. C. (1998). Effect of muscle glycogen content on exercise-induced changes in muscle T2 times. *Journal of Applied Physiology*, 84, 1178–1184. https://doi.org/10.1152/japplphysiol.1998.84.4.1178

Price, T. B., McCaughey, T. R., Daleba, A. J., Wilkins, K. L., & Gore, J. C. (1995). Changes in magnetic resonance transverse relaxation times of two muscles following standardized exercise. *Medicine & Science in Sports & Exercise*, 27, 1421–1429.

Raja, M. K., Raymer, G. H., Moran, G. R., Marsh, G., & Thompson, R. T. (2006). Changes in tissue water content measured with multiple-frequency bioimpedance and metabolism measured with 31P-MRS during progressive forearm exercise. *Journal of Applied Physiology*, 101, 1070–1075. https://doi.org/10.1152/japplphysiol.01322.2005

Rakowchuk, M., Tanguay, S., Burgomaster, K. A., Howarth, K. R., Gibala, M. J., & MacDonald, M. J. (2008). Sprint interval and traditional endurance training induce similar improvements in peripheral arterial stiffness and flow-mediated dilation in healthy humans. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, 295, R236–R242. https://doi.org/10.1152/ajpregu.00069.2008

Röcker, L., Kirsch, K. A., & Stoboy, H. (1976). Plasma volume, albumin and globulin concentrations and their intravascular masses. A comparative study in endurance athletes and sedentary subjects. *European Journal of Applied Physiology and Occupational Physiology*, 36, 57–64. https://doi.org/10.1007/BF00421634

Rothschild, M. A., Oratz, M., & Schreiber, S. S. (1972). Albumin synthesis. 1. *New England Journal of Medicine*, 286, 748–757. https://doi.org/10.1056/NEJM197204062861404

Sawka, M. N., Convertino, V. A., Eichner, E. R., Schneider, S. M., & Young, A. J. (2000). Blood volume: Importance and adaptations to exercise training, environmental stresses, and trauma/sickness. *Medicine and Science in Sports and Exercise*, 32, 332–348. https://doi.org/10.1097/00005768-20000200-00012

Sjogaard, G., & Saltin, B. (1982). Extra- and intracellular water spaces in muscles of man at rest and with dynamic exercise. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, 243, R271–R280. https://doi.org/10.1152/ajpregu.1982.243.3.R271

Stick, C., Heinemann, W., & Witzel, E. (1990). Slow volume changes in calf and thigh during cycle ergometer exercise. *European Journal of Applied Physiology and Occupational Physiology*, 61, 428–432. https://doi.org/10.1007/BF00236063

Tsutsumi, T., Nakao, K., Mitsuoka, S., Hamasaki, K., Tsuruta, S., Shima, M., Nakata, K., Tamaoki, T., & Nagataki, S. (1993). Regulation of albumin and alpha-fetoprotein gene expression by colloid osmotic pressure in human hepatoma cells. *Gastroenterology*, 104, 256–262.
Vanhamme, L., van den Boogaart, A., & Van Huffel, S. (1997). Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *Journal of Magnetic Resonance, 129*, 35–43. https://doi.org/10.1006/jmre.1997.1244

Ward, D. S., Hamilton, M. T., & Watson, P. D. (1996). Measurement of tissue volume during non-steady state high-intensity muscle contraction. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology, 271*, R1682–1690. https://doi.org/10.1152/ajpregu.1996.271.6.R1682

Watson, P. D., Garner, R. P., & Ward, D. S. (1993). Water uptake in stimulated cat skeletal muscle. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology, 264*, R790–R796. https://doi.org/10.1152/ajpregu.1993.264.4.R790

Weston, K. S., Wisløff, U., & Coombes, J. S. (2014). High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: A systematic review and meta-analysis. *British Journal of Sports Medicine, 48*, 1227–1234. https://doi.org/10.1136/bjsports-2013-092576

Wingo, J. E., Ganio, M. S., & Cureton, K. J. (2012). Cardiovascular drift during heat stress: Implications for exercise prescription. *Exercise and Sport Sciences Reviews, 40*, 88–94. https://doi.org/10.1097/JES.0b013e31824e43af

Yang, R. C., Mack, G. W., Wolfe, R. R., & Nadel, E. R. (1998). Albumin synthesis after intense intermittent exercise in human subjects. *Journal of Applied Physiology, 84*, 584–592. https://doi.org/10.1152/jappl.1998.84.2.584

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Mandić M, Forsgren MF, Romu T, et al. Interval-induced metabolic perturbation determines tissue fluid shifts into skeletal muscle. *Physiol Rep*. 2021;9:e14841. [https://doi.org/10.14814/phy2.14841](https://doi.org/10.14814/phy2.14841)