Acute high intensity interval exercise is less pro-oxidative/thrombotic compared to isovolumic moderate intensity steady state exercise

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ABSTRACT

While high intensity interval training (HIIT) has emerged as a more time-efficient alternative to moderate intensity steady state exercise (MISS) the impact on systemic free radical formation and link to activated coagulation remains unknown. We recruited sixteen healthy males aged 21 ± 3 y who performed incremental cycle ergometry to determine peak oxygen uptake ($\dot{V}O_{2PEAK}$). Participants were randomly assigned single-blind to two separate groups (MISS: n = 8; HIIT: n = 8) matched for $\dot{V}O_{2PEAK}$. HIIT participants completed five exercise cycles, each consisting of 3 min at 80 % $\dot{V}O_{2PEAK}$ alternating with 3 min at 40 % $\dot{V}O_{2PEAK}$ whereas MISS participants performed an isovolumic bout of 30 min at 60 % $\dot{V}O_{2PEAK}$. Cephalic venous blood was assayed for ascorbate free radical ($A^{\bullet-}$, electron paramagnetic resonance spectroscopy) and clot fractal dimension (d_f, rheometry) at rest every hour over a 6h period to determine critical difference (CD) and before/after submaximal/peak exercise. Submaximal MISS increased $A^{\bullet-}$ and d_f to a greater extent compared to HIIT (P = 0.039 to 0.057) though elevations generally fell within CD boundaries (54.2 % and 5.5 % respectively). No further elevations were observed during peak exercise (P = 0.508 to 0.827) and no relationships were observed between $A^{\bullet-}$ and d_f (r = 0.435 to -0.121, P = 0.092 to 0.655). Collectively, these findings suggest that HIIT is less pro-oxidative/thrombotic compared to more traditional MISS, advocating its prescription in patients given the potential for superior vascular adaptive benefit.

53 **Key Points**

54 • Safety of high intensity interval training (HIIT) has been questioned

55 • HIIT is less pro-oxidative/thrombotic versus moderate intensity steady state (MISS)

56 • No relationships between systemic free radical formation and activated coagulation

57 • HIIT is a potentially safer exercise intervention compared to MISS

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INTRODUCTION

It is well established that moderate-intensity steady-state exercise (MISS) can improve cardiorespiratory fitness (CRF), reducing the risk of cardiovascular disease and all-cause mortality across the human aging continuum [1]. Evidence further attests to its neuroprotective benefits with the capacity to reduce the risk of initial and recurrent stroke and improve cognition in older adults with healthy cognition, subjective memory complaints, mild cognitive impairment and dementia [2]. However, time demands are deemed a potential barrier to exercise participation [3] and as a consequence, attention has since turned to an alternative paradigm, high-intensity interval training (HIIT), given its capacity to further potentiate molecular, cardiopulmonary and cerebrovascular adaptation for a given training volume [4].

However, HIIT has the potential to further compound systemic oxidative-nitrosative stress (OXNOS) to a greater extent than MISS [5], confirmed by a free radical-mediated reduction in vascular nitric oxide (NO) bioavailability that when excessive, associates with structural tissue damage and vascular endothelial dysfunction. Indeed, elevations in local OXNOS and corresponding structural-vascular impairments have been observed in the systemic [6], pulmonary [7] and cerebral [8, 9] circulation. Despite emergent evidence indicating that activated coagulation in humans is subject to redox regulation [10, 11], the extent to which HIIT potentially predisposes to a more pro-thrombogenic profile subject to increased free radical formation remains unknown. This warrants careful consideration given its popular prescription in high-risk patients suffering from vascular arterial occlusive disease [4].

To explore this for the first time, we examined to what extent acute submaximal bouts of HIIT and isovolumic MISS independently impact systemic free radical formation and corresponding implications for hemostasis. We employed electron paramagnetic

resonance (EPR) spectroscopic detection of the ascorbate free radical ($A^{\cdot-}$) to directly assess global free radical formation [9] with hemorheological assessment of the fractal dimension (d_f) to directly assess insipient clot microstructure [12]. We also calculated the critical difference (CD) to determine whether the biomarker response was clinically meaningful and not simply statistically significant [13]. Given that exercise-induced free radical formation is both exercise-intensity dependent [9] and mechanistically linked to activated coagulation [11], we hypothesised that acute HIIT would be associated with more pronounced and clinically meaningful elevations in systemic $A^{\bullet-}$ and d_f, implying that this paradigm is more prothrombotic compared to its more traditionally prescribed MISS counterpart.

MATERIALS AND METHODS

Ethics

The study was approved by the University of South Wales Ethics Committee (#18LF0801HR) with all procedures carried out in accordance with the Declaration of Helsinki except for registration in a database. All participants were informed of the requirements of the study and provided written informed consent.

Participants

Based on a prospective power calculation on the primary outcome variable (d_f, see Statistical analysis), sixteen healthy males aged 21 (mean) ± 3 (SD) y, with a body mass index (BMI) of 27 ± 5 kg/m^2 were recruited into the study. All participants were free of cardiovascular, pulmonary, and cerebrovascular disease and were not taking any nutritional supplements including over-the-counter antioxidant or anti-inflammatory medications. They were advised to refrain from physical activity, caffeine and alcohol and follow a low nitrate/nitrite diet 24 h prior to formal experimentation with specific instructions to avoid fruits, salads and cured meats [8]. Participants attended the laboratory following a 12 h overnight fast.

Design

Primary study: The study adopted a randomised single-blind design where, following a baseline incremental (peak) cycling test to volitional exhaustion, participants were randomly assigned to undertake either an acute submaximal bout of HIIT or isovolumic MISS, with participants matched for body mass/BMI.

Secondary study: We also conducted a separate sub-study for specific determination of the critical difference (CD) of all blood-borne redox-rheometric metrics to

disassociate ‘authentic’ clinically meaningful changes attributable to exercise *per se* rather than simple background ‘noise’ associated with analytical imprecision and/or biological variation [13-15].

Primary study (exercise RCT)

Blood Sampling

Whole blood was collected without stasis into a sterile syringe for immediate analysis of hemorheological markers of blood clot microstructure. An 18-gauge cannula (Venflon IV cannula, Becton-Dickinson, Sweden) connected to a three-way sterile stopcock (Connecta plus 3, Ohmeda, Sweden) was inserted into a prominent antecubital vein. From this, we obtained a separate sample to calculate plasma volume (PV) shifts (see below) and for direct detection of $A^{\bullet-}$using the vacutainer method (Becton, Dickinson and Company, Oxford, UK). Vacutainers were centrifuged at 600 g (4 ºC) for 10 min and (K-EDTA) plasma supernatant was decanted into cryogenic vials (Nalgene Labware, Thermo Fisher Scientific Inc., Waltham, MA, USA) and immediately snap-frozen in liquid nitrogen. Plasma samples were thawed at 37 °C for 5 min prior to batch analysis (see later).

Peak exercise

Peak oxygen uptake ($\dot{V}O_{2MAX}$) was determined via a standardised incremental exercise test to volitional exhaustion using an upright two-legged cycle ergometer (Lode, Groningen, The Netherlands) and Ultima^{TM} CardiO$_2$® metabolic cart (MGC Diagnostics Corporation, MN, USA) [16]. Workload was set at 35 Watts (W) for 5 min (70 rpm) and increased by 35 W/min until participants could no longer meet the required power output. Breath-by-breath measurements of gas exchange (mid 5 of 7

149 breaths averaged) were obtained using a mouthpiece connected to a preVent® flow
150 sensor with a nose-clip to measure both inspired/expired oxygen/carbon dioxide
151 (O_2/CO_2) fractions and respiratory flow. Medgraphics BreezeTM software
152 automatically determined $\dot{V}O_{2PEAK}$, confirmed according to established criteria [15].
153 From this test, we determined the power output to $\dot{V}O_2$ relationship for each participant
154 to inform subsequent bouts of acute (HIIT/MISS) exercise.
155
156 ***Submaximal exercise***
157 ***HIIT:*** Following a standardised warm-up (3 min at 30 % $\dot{V}O_{2PEAK}$), each participant
158 completed five exercise cycles, each consisting of 3 min at 80 % $\dot{V}O_{2PEAK}$ alternating
159 with 3 min at 40 % $\dot{V}O_{2PEAK}$.
160 ***MISS:*** Following the same standardised warm-up, each participant cycled
161 continuously for 30 min at 60 % $\dot{V}O_{2PEAK}$.
162
163 ***PV shifts***
164 Changes in hemoglobin (Hb) and hematocrit (Hct) were determined to assess
165 exercise-induced shifts in PV (specifically hemoconcentration) since this can influence
166 data interpretation. Hb was measured photometrically according to established
167 procedures [17] (HaemoCue®, B-Haemoglobin, Sheffield, UK). Hct was prepared via
168 ultracentrifugation (Hawksley and Sons Ltd, Sussex, UK) and measured using a
169 Hawksley Micro Haematocrit Reader (Hawksley and Sons Ltd, Sussex, UK), corrected
170 for 1.5 % plasma trapped between erythrocytes [18]. Triplicate samples were obtained
171 for both Hb and Hct and the mean value used in overall analyses. Relative shifts in PV
172 were mathematically derived using the classical equation of Dill and Costill assuming

that the absolute mass of circulating red cells in the bloodstream remains unchanged given by [19]:

$$\Delta PV = \frac{PV_{Post} - PV_{Pre}}{PV_{Pre}} = \frac{Hb_{Pre} \times (1 - Hct_{Post})}{Hb_{Post} \times (1 - Hct_{Pre})} - 1$$

Secondary study (CD assessment)

Blood samples (see prior) were obtained from a random selection of 8 (4 per group) of the 16 participants. Samples were obtained in the seated position once every hour over a 6-h period. Immediately after blood sampling, participants were allowed to ambulate for 15 min while confined to the laboratory. Participants remained in a supine position for 45 min prior to each sample time-point and abstained from food throughout the day to control for hormone fluctuations. Water was permitted *ad-libitum* after each blood sample.

Biomarkers

Redox

Electron paramagnetic resonance (EPR) spectroscopy was used to directly measure $A^{\bullet -}$ as a global biomarker of free radical formation [9]. Exactly 1 mL of K-EDTA plasma was injected into a high-sensitivity multiple-bore sample cell (AquaX, Bruker Daltonics, Billerica, Massachusetts, USA) housed within a TM110 cavity of an EPR spectrometer operating at X-band (9.87 GHz). Samples were analysed using a modulation frequency of 100 kHz, modulation amplitude of 0.65 gauss (G), microwave power of 10 milliwatts (mW), receiver gain of 2×10^5 AU, time constant of 41 ms, magnetic field centre of 3477 G and scan width of ± 50 G for three incremental scans. After identical baseline correction and filtering, each of the spectra were subject to double integration using graphical analysis software (OriginPro V.8.5, OriginLab, Massachusetts, USA).

198 ***Rheology***

199 Exactly 7 mL of unadulterated whole blood was immediately injected into a double
200 walled concentric rheometer (Discovery Hybrid-2, TA Instruments, DE, USA), for
201 analysis of d_f at 37ºC according to established methods [16]. Briefly, blood was subject
202 to a constant torque of 10.5 μNm at 2 Hz, 0.93 Hz, 0.43 Hz, 0.2 Hz rotational
203 oscillation. The phase angle (δ) of the insipient clot was ascertained by Storage
204 Modulus (G')/Loss Modulus (G'') frequency harmony. Time for blood to gel (T_{GP}) and
205 dynamic viscosity (DV) were recorded from this point, and corresponding d_f of the
206 insipient clot calculated according to the established relationship:

207

208 $$\frac{(D + 2)(2\theta - D)}{2(\theta - D)}$$

209

210 where D is the space dimension (constant of 3 arbitrary units) and the exponent (δ)
211 calculated as $\delta = \theta\pi/2$. A compact (clot) network structure is reflected by a higher
212 value of d_f, whereas lower values correspond to more open/permeable networks [16].

213

214 **Statistical analysis**

215 **Primary study (exercise RCT)**

216 ***Power calculation.*** Power calculations were performed using G* Power 3.1 software.
217 Assuming comparable differences in d_f observed during pilot studies between (acute
218 submaximal) HIIT and MISS exercise and corresponding effect size of 1.31, the
219 present study required a (minimum) total sample size of 16 participants (1/1 allocation)
220 in order to achieve a power of 0.80 at $P < 0.05$.

221

Inferential statistics. Data were analyzed using IBM® SPSS® Statistics 28.0. (IBM, NY, USA). Following confirmation of distribution normality using repeated Shapiro–Wilk *W* tests, data were analysed using a three-factor mixed analysis of variance (ANOVA) incorporating one between (Group: HIIT vs. MISS) and two within (Intensity: Submaximal vs. Peak and State: Rest vs. Exercise) factors. Following a significant main effect and interaction, Bonferroni corrected paired samples *t*-tests were employed to make post hoc comparisons at each level of the within-subjects factor. Between-group comparisons were assessed using independent samples *t*-tests. Significance was established at $P < 0.05$ for all two-tailed tests and data presented as mean ± SD. Potential relationships between exercise-induced alterations in redox and rheological biomarkers were assessed using Pearson Product Moment correlations.

Secondary study (CD assessment)

The CD calculates the magnitude of random fluctuation around a homeostatic set point within which there is 95 % probability that repeated measures will fall. The 95 % probability is represented by a constant ($k = 2.77$ at $P < 0.05$) calculated from $\sqrt{2 \times 1.96\ (2\ \mathrm{SD})}$. The CD was calculated as [13]:

$$\mathrm{CD} = \mathrm{k}\ (2.77)\sqrt{\mathrm{CV_A^2} + \mathrm{CV_B^2}}$$

where CV_A is the coefficient of analytical variation and CV_B is the coefficient of biological variation.

The CV_A was conservatively assumed to be 1% since no standards allow for 'static' assessment of rheometric variables and repeated measurements of $A^{\bullet -}$ from the same

247 sample results in a progressive loss of EPR signal intensity due to air auto-oxidation
248 of ascorbate [11].
249
250 The CV_A was subtracted from the coefficient of total variation (CV_T) for derivation of
251 CV_B. The CV_T was calculated from the pooled mean and SD of each participant's
252 individual mean value of (7) repeated measurements given by:
253
254 $$CV_T = \frac{\bar{X}}{SD} \times 100\ (\%)$$
255

RESULTS

Baseline matching

By design, both groups were well-matched for all anthropometric and CRF variables (Table 1). Resting systemic assessments of all redox and rheometric variables were also comparable (see later, Figures 2 B-3 A-C).

CD assessment

Assuming a global CV_A of 1.0 %, basal $CV(s)_B$ were 19.5 %, 1.7 %, 13.2 % and 12.0 % for $A^{\bullet -}$, d_f, DV and T_{GP} (Figure 1 A) resulting in CD(s) of 54.2 %, 5.5 %, 36.8 % and 33.5 % respectively (Figure 1 B).

PV shifts

Exercise was generally associated with a hemoconcentration reflected by the combined elevation in Hb and Hct. The corresponding reduction in PV was comparable both as a function of group and exercise intensity (Submaximal HIIT: -13 ± 5 % vs. MISS: -10 ± 3 %; Peak HIIT: -15 ± 7 % vs. MISS: -13 ± 3 %, Group: P = 0.155, Intensity: P = 0.308, Group × Intensity, P = 0.698). Thus, we chose to present all redox and rheological parameters in absolute (uncorrected) terms.

Exercise RCT

Redox

The general reaction principles underlying $A^{\bullet -}$ formation and corresponding changes during submaximal HIIT/MISS and peak exercise are illustrated in Figure 2 A-B. Systemic $A^{\bullet -}$ formation was generally higher in the HIIT group due primarily to elevated basal values. Unlike submaximal HIIT, MISS increased $A^{\bullet -}$, whereas peak

exercise consistently increased $A^{\bullet -}$ in both groups. However, no between group differences were observed during either submaximal or peak exercise (P = 0.110-0.196) and all increases remained within the CD (upper) boundaries.

Rheology

Figure 3 illustrates the submaximal and peak exercise-induced changes in d_f (Figure 3A), DV (Figure 3B) and T_{GP} (Figure 3C). Exercise was generally associated with an increase in d_f and DV and corresponding tendency towards a reduction in T_{GP} with comparable differences observed between submaximal and peak intensity. The (submaximal) MISS-induced elevation in d_f tended to be greater compared to HIIT (+0.11 ± 0.07 vs. +0.06 ± 0.05 AU, P = 0.057) whereas changes in DV and T_{GP} were comparable and within (upper and lower) CD boundaries. In contrast, peak exercise-induced increases in d_f and DV exceeded (upper) CD boundaries and healthy reference range (1.85 AU) in the majority of participants.

Redox-hemorheological relationships

No relationships were observed between the (pooled) submaximal and peak exercise-induced increases in $A^{\bullet -}$ and alterations in d_f (Figure 4A), DV (Figure 4 B) or T_{GP} (Figure 4 C) for either group.

DISCUSSION

The present study highlights three important findings that are both mechanistically and clinically relevant. First, we demonstrate that an acute (submaximal) bout of MISS increased systemic free radical formation and d_f to a greater extent than HIIT implying that the latter was less pro-oxidative/thrombotic. Second, no relationships were observed between exercise-induced alterations in $A^{\bullet -}$ and any of the rheological biomarkers measured implying that activated coagulation may not be subject to redox-regulation. Third, while the exercise-induced biomarker response was generally considered statistically significant, submaximal changes generally fell within newly defined CD boundaries. Collectively, these 'proof-of-concept' findings suggest that HIIT is less pro-oxidative/thrombotic and a potentially safer exercise intervention compared to its more traditional MISS counterpart and emphasises the interpretive significance of natural variation.

Redox

We specifically chose to employ EPR spectroscopic detection of $A^{\bullet-}$ as our direct biomarker of 'global' free radical formation rather than constrain our focus to bespoke species best characterised by complex ex-vivo spin-trapping techniques that can be difficult to control and interpret [20]. Since the concentration of ascorbate in human plasma is orders of magnitude greater than any oxidising free radical, combined with the low one-electron reduction potential for the $A^{\bullet-}$/ascorbate monoanion (AH^-) couple ($E^{\circ\prime}$ = 282 mV) [21], any oxidising species ($R^{\bullet}$) generated within the systemic circulation will result in the one-electron oxidation of ascorbate. Since the unpaired electron is delocalised over a highly conjugated tri-carbonyl π-system, it is resonance

330 stabilised allowing for direct (EPR) detection of the distinctive $A^{\bullet-}$ doublet ($R^{\bullet} + AH^{-} \rightarrow$
331 $A^{\bullet-} + R\text{-}H$) as illustrated in Figure 2 [22].
332 The consistent elevation in $A^{\bullet-}$ thus provides convincing evidence that systemic free
333 radical formation increased during both acute submaximal and peak exercise.
334 However, contrary to original expectations, submaximal MISS resulted in a greater
335 elevation in $A^{\bullet-}$ compared to HIIT. This was unexpected since free radical formation,
336 reflected by ex-vivo detection of α-phenyl-*tert*-butylnitrone (PBN) spin trapped alkoxyl
337 radicals, has previously been shown to increase monoexponentially with exercise
338 intensity in proportion to the reduction in mitochondrial partial pressure of oxygen (O_2)
339 as opposed to a simple linear increase in O_2 flux per se [23]. That basal $A^{\bullet-}$ remained
340 consistently elevated in the HIIT group was intriguing, albeit difficult to reconcile, given
341 that both groups were meticulously matched at baseline for anthropometric and CRF
342 variables. This constrained 'bandwidth' may have limited their ability to further
343 increase free radical formation (ceiling effect), at least during submaximal exercise.
344 This finding challenges recent evidence for a greater elevation in thiobarbituric acid
345 reactive substances and protein carbonyl formation during acute low-volume HIIT (4
346 × 30s sprints alternating with 4 min recovery, intensity not specified) compared to
347 MISS (30 min at 70 % $\dot{V}O_{2PEAK}$) [5]. However, groups were not matched for volume
348 and the authors relied exclusively on 'indirect' and unreliable biological 'footprints' of
349 free radical formation, employing assays that purportedly reflect (radical-mediated)
350 oxidative damage to lipids, proteins and deoxyribonucleic acid. These analytical
351 techniques remain questionable given that the reactive intermediates, formed clearly
352 downstream of the primary production pathway, exhibit markedly different
353 thermodynamic and kinetic properties adding to the inconsistencies reported in the
354 exercise science literature (see Figure 1 of [24]). Our study is the first to apply EPR

spectroscopy to the HIIT/MISS setting, the most sensitive, specific, and direct molecular technique for the detection and subsequent identification of free radicals sine qua non [9], thus providing a more controlled examination of the underlying mechanisms and consequences associated with altered redox status.

Rheology

It is well established that acute exercise generally activates coagulation and that the response is potentially modulated by exercise intensity, duration and modality [25]. In support, we have previously demonstrated a consistent shortening of conventional coagulation times [10, 11, 26] and corresponding elevation in the novel biomarker d_f [16] during peak cycling exercise. The current findings extend these works by demonstrating consistent elevations in d_f during both HIIT and MISS, with greater increases observed in the latter, implying that incipient clot viscoelastic strength, polymerisation and crosslinking also increase during less strenuous (i.e., submaximal) exercise.

That (submaximal) MISS-induced elevations in $A^{\bullet-}$ and d_f were comparatively more marked tentatively suggests that free radical formation and activated coagulation are intrinsically linked, a concept we and others have proposed [10, 11, 27, 28]. This may be related to free radical-mediated bioactivation of coagulation factors involved in both the Tissue and Contact Factor Pathways. Factor VII and its interaction with Tissue Factor have been demonstrated to be redox sensitive in vitro [29]. It is suggested that thrombin formation up-regulates a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-dependent signalling cascade that leads to up-regulation of Tissue Factor (the principal coagulation factor in the initiation of the Tissue Factor Pathway) expression. Thus, NADPH oxidase has a crucial role in the regulation of Tissue Factor

380 which is the primary determinant of the thrombogenic response. With regards the
381 Contact Factor Pathway, it has long been suggested that Factor VIII may also be
382 subject to redox regulation, with the thioredoxin system as a possible mediator in-vivo
383 [30]. More recently, it has been suggested that the A2 domain of vWF, the domain that
384 unfurls to expose the protein's reaction sites, could potentially act as a novel 'redox
385 switch' for its activation [31]. However, the more comprehensive series of (repeated)
386 measurements and consistent lack of relationship(s) observed between $A^{\bullet-}$ and d_f in
387 the present study (for either group) fails to support this hypothesis, at least in the more
388 complex in-vivo setting.
389 An alternative explanation may relate to potential differences in the blood flow, strain
390 and shear stress phenotype imposed by the two different exercise regimens. Although
391 not measured in this study, vascular blood flow is more pulsatile and induces greater
392 peak oscillations in shear stress during HIIT compared to the less intense, albeit more
393 sustained increases induced by MISS [4]. The latter (i.e., duration favoured over
394 intensity) may result in more vWF multimers transiently bridging the platelet
395 glycoprotein Ib/IX/V receptor with either receptors or vessel wall constituents resulting
396 in increased platelet tethering and corresponding increases in primary hemostasis
397 i.e., mechanoreceptor activation of hemostasis distinct from myriad biochemical
398 pathways [32].
399 The safety aspects of HIIT, particularly its impact on the cerebrovasculature, are yet
400 to be systematically explored, raising concerns that continue to represent a major
401 barrier toward its more widespread clinical implementation especially for stroke
402 rehabilitation. However, the evidence to date, albeit in patients with coronary artery
403 disease or heart failure, challenges any such cause for concern [4]. In further support,
404 albeit in healthy participants, our 'proof-of-concept' findings confirm that for any given

volume of submaximal exercise, HIIT yields viscoelastically weaker clots that are of better quality and easier to fibrinolytically dissolve compared to those induced by its less intense MISS counterpart. Furthermore, that we failed to observe greater (Δ) elevations in d_f during peak exercise further suggests that intensity per se is not the primary stimulus underlying clot microstructure. This contrasts with our prior observations [16] although the experimental design was different involving serial measurements of d_f during a single bout of incremental exercise.

Natural variation

The molecular biomarkers measured in the present study inherently vary within the same individual (CV_B) and due to analytical error associated with their measurement (CV_A). These components collectively contribute to the CD defined as the change from baseline that must occur before a true difference of physiological/clinical significance can be claimed [13].

The present study extends our prior research documenting the CD of PBN-adducts and individual water/lipid soluble antioxidants within the (acute) exercise setting [14] by quantifying the CD of $A^{\bullet -}$, d_f, DV and T_{GP}. As anticipated, we observed variability in all measurements consistent with (mostly) CV_B, with $A^{\bullet -}$ and d_f exhibiting the highest and lowest variance respectively.

Application of this concept to the current setting indicated that while submaximal (HIIT and MISS) exercise-induced alterations in redox and rheological biomarkers were statistically significant, they fell comfortably within CD boundaries, arguing against physiological/clinical relevance. In contrast and despite comparable elevations during peak exercise (intensity main effects, $P > 0.05$), the combined elevations in d_f and DV exceeded the (upper) CD boundaries in the majority of participants implying that

changes could indeed be considered 'authentic', extending beyond background biological 'noise'. As previously mentioned here and extensively reviewed elsewhere by our group [4], there is a commonly held (mis)perception that HIIT is potentially more 'unsafe' compared to the more traditional MISS intervention despite clear evidence suggesting the contrary [33]. In support, the data presented herein tentatively suggest that HIIT is 'less' pro-oxidative/thrombotic compared to MISS, advocating its prescription in patients given its superior vascular protective potential mediated by enhanced integrated molecular, cardiopulmonary and cerebrovascular adaptive benefits combined with improved compliance given the reduced time constraints.

Experimental limitations

There are several limitations to the present study that warrant careful consideration. First, larger scale follow-up studies are encouraged in patients to confirm our preliminary findings given the limitations associated with the relatively small sample sizes employed and focus on healthy participants free of vascular pathology. Second, while we went to considerable lengths to match HIIT and MISS participants for body mass/BMI, this could have been further improved by matching participants for lean body mass. Third, we acknowledge the lack of complementary, more conventional kinetic biomarkers reflecting the equilibrium between coagulation and fibrinolysis. However, we specifically chose to focus *a priori* on d_f and associated metrics given that it is considered a more sensitive and specific downstream 'global' biomarker of clot microstructure and development [12].

Conclusion

These findings suggest that HIIT is a potentially safer exercise intervention compared to more traditional MISS given that it is less pro-oxidative/thrombotic. Furthermore, that exercise-induced biomarker changes fell within CD boundaries highlights the conceptual implications of viewing changes not simply as ‘single point’ estimates, but instead as a dynamic range of fluctuating values defined by natural variation. While further research is encouraged, these preliminary findings support the prescription of HIIT in patients given its superior vascular adaptative benefits.

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Competing interests

DMB is Chair of the Life Sciences Working Group and member of the Human Spaceflight and Exploration Science Advisory Committee to the European Space Agency and is a member of the Space Exploration Advisory Committee to the UK Space Agency. DMB is also affiliated to the companies FloTBI Inc., BrainEx Inc., and OrgEx Inc. focused on the technological development of novel biomarkers of brain injury in humans.

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Author contributions

LF and DMB conceived and designed the research. LF, BSS, TC, TO, KT, RG, RP and DMB contributed to data collection and/or analysis. LF and DMB interpreted the results of the experiments. DMB drafted the first draft manuscript and revisions thereof. LF, BSS, TC, TO, KT, RG, RP and DMB approved the final version submitted for publication. The authors declare that all data were generated in-house and that no paper mill was used.

603 **Legends**

604 **Table 1. Demographics**

605 Values are mean ± SD; BMI, body mass index; PPO, peak power output in Watts (W);

606 $\dot{V}O_{2PEAK}$, peak oxygen uptake.

607

608 **Figure 1. Composite coefficients of analytical/biological variation (A) and critical**

609 **differences (B) in redox-rheological biomarkers**

610 Values are mean based on n = 8 for each group. $A^{\bullet -}$, ascorbate radical; d_f, fractal

611 dimension; DV, dynamic viscosity; T_{GP}, time to gel point. CV, coefficient of variation;

612 CV_A/CV_B, coefficient of analytical/biological variation; CD, critical difference. CV_A

613 conservatively assumed to be a constant of 1 % for all variables (see Methods).

614

615 **Figure 2. Principle underlying ascorbate radical formation (A) and exercise-**

616 **induced responses (B)**

617 At the current settings, $A^{\bullet-}$ appears as a (filtered) doublet with a hydrogen hyperfine

618 coupling constant (a_H^{β}) of ~1.76 Gauss (see top right inset for simulated spectrum). B.

619 Values are mean ± SD based on n = 8 for each group. $A^{\bullet -}$, ascorbate radical; HIIT,

620 high-intensity interval training; MISS, moderate intensity steady state. Red shaded

621 bars highlight the boundary ranges whereby upper limits need to be exceeded for

622 exercise-induced increases in $A^{\bullet -}$to be considered clinically meaningful (i.e.,

623 surpassing the critical difference value illustrated bottom right inset). Statistical

624 outcomes for three-factor mixed ANOVA: Group: $F(1, 14) = 36.463$; $P = <0.001$;

625 Intensity: $F(1, 14) = 0.462$; $P = 0.508$; State: $F(1, 14) = 15.519$; $P = 0.001$; Group ×

626 Intensity: $F(1, 14) = 0.201$; $P = 0.661$; Group × State: $F(1, 14) = 1.535$; $P = 0.236$;

Intensity × State: $F(1, 14) = 4.725$; $P = 0.047$; Group × Intensity × State: $F(1, 14) = 6.624$; $P = 0.022$.

Figure 3. Exercise-induced changes in rheological biomarkers

Values are mean ± SD based on n = 8 for each group. d_f, fractal dimension; DV, dynamic viscosity; TGP, time to gel point; AU, arbitrary units. HIIT, high-intensity interval training; MISS, moderate intensity steady state. Red shaded bars highlight the boundary ranges whereby upper limits (lower limits for T_{GP}) need to be exceeded for exercise-induced increases (decreases for T_{GP}) in respective biomarkers to be considered clinically meaningful (i.e., surpassing the critical difference value illustrated bottom right inset). Red stippled line refers to upper limit (d_f = 1.85 AU) of healthy haemostatic reference range [16]. Statistical outcomes for three-factor mixed ANOVA: A. Group: $F(1, 14) = 0.012$; $P = 0.914$; Intensity: $F(1, 14) = 0.050$; $P = 0.827$; State: $F(1, 14) = 61.931$; $P = <0.001$; Group × Intensity: $F(1, 14) = 0.261$; $P = 0.618$; Group × State: $F(1, 14) = 1.428$; $P = 0.252$; Intensity × State: $F(1, 14) = 3.666$; $P = 0.076$; Group × Intensity × State: $F(1, 14) = 0.407$; $P = 0.534$. B. Group: $F(1, 14) = 0.059$; $P = 0.811$; Intensity: $F(1, 14) = 1.462$; $P = 0.247$; State: $F(1, 14) = 7.219$; $P = 0.018$; Group × Intensity: $F(1, 14) = 0.414$; $P = 0.531$; Group × State: $F(1, 14) = 0.006$; $P = 0.940$; Intensity × State: $F(1, 14) = 0.947$; $P = 0.347$; Group × Intensity × State: $F(1, 14) = 1.550$; $P = 0.234$. C. Group: $F(1, 14) = 7.667$; $P = 0.015$; Intensity: $F(1, 14) = 0.005$; $P = 0.945$; State: $F(1, 14) = 2.798$; $P = 0.117$; Group × Intensity: $F(1, 14) = 8.920$; $P = 0.010$; Group × State: $F(1, 14) = 0.034$; $P = 0.857$; Intensity × State: $F(1, 14) = 0.060$; $P = 0.810$; Group × Intensity × State: $F(1, 14) = 0.255$; $P = 0.622$.

652 **Figure 4. Relationships between redox and rheological biomarkers**

653 Values are mean ± SD based on n = 8 for each group. Δ, delta (exercise minus rest
654 value). $A^{\bullet -}$, ascorbate radical; d_f, fractal dimension; DV, dynamic viscosity; T_{GP}, time
655 to gel point. HIIT, high-intensity interval training; MISS, moderate intensity steady
656 state. Linear correlations fitted to (pooled) submaximal and maximal values (16 data-
657 points) for each group.

658
659
660

Table 1. Demographics

	HIIT (n = 8)	MISS (n = 8)	P value
Mass (kg)	82.9 ± 14.7	84.3 ± 13.4	0.420
Stature (m)	1.79 ± 0.05	1.80 ± 0.06	0.430
BMI (kg/m^2)	26 ± 4	26 ± 3	0.443
PPO (W)	306 ± 24	300 ± 53	0.383
$\dot{V}O_{2PEAK}$ (mL/kg/min)	40 ± 7	42 ± 4	0.311

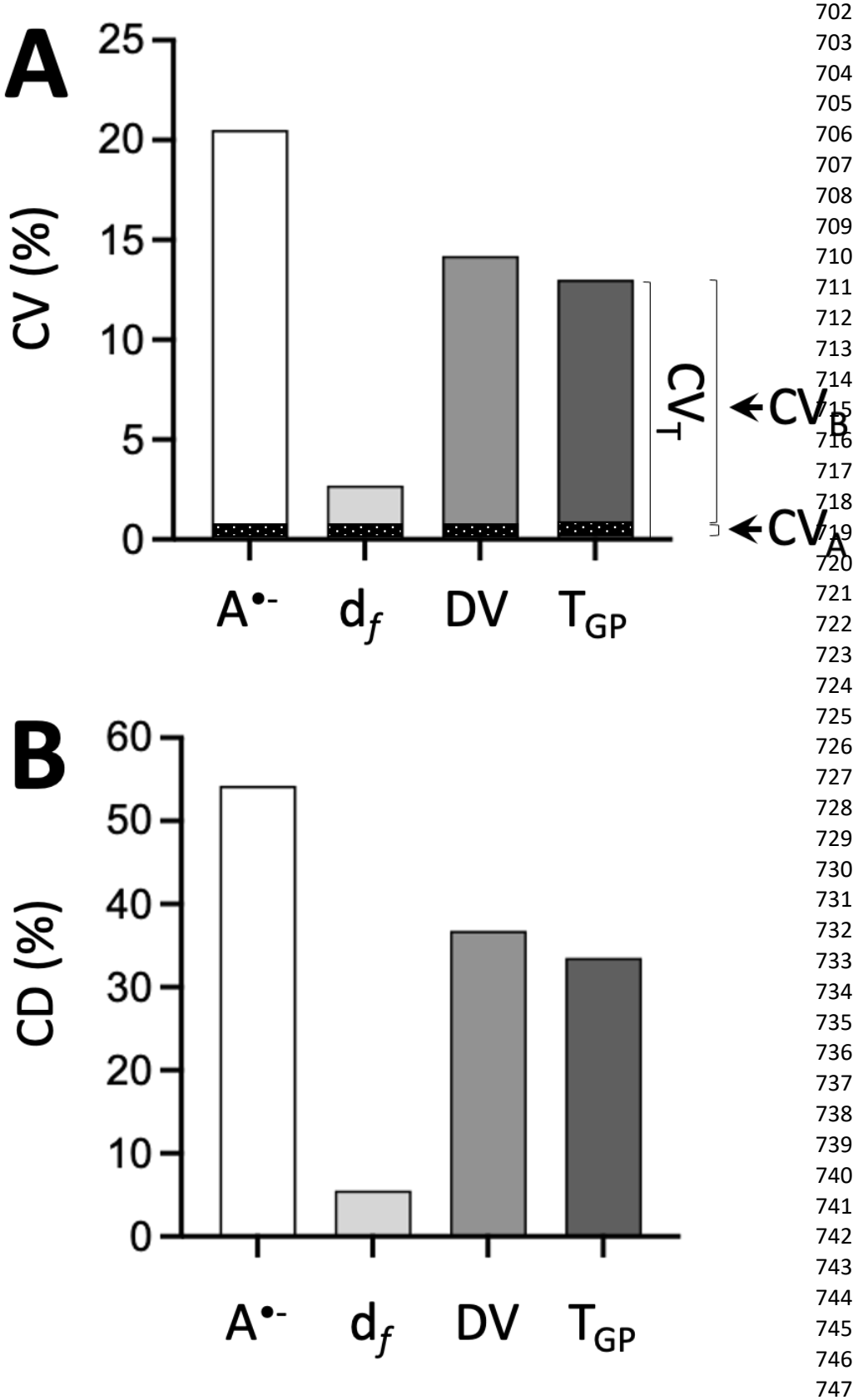

Figure 1. Composite coefficients of analytical/biological variation (A) and critical differences (B) in redox-rheological biomarkers

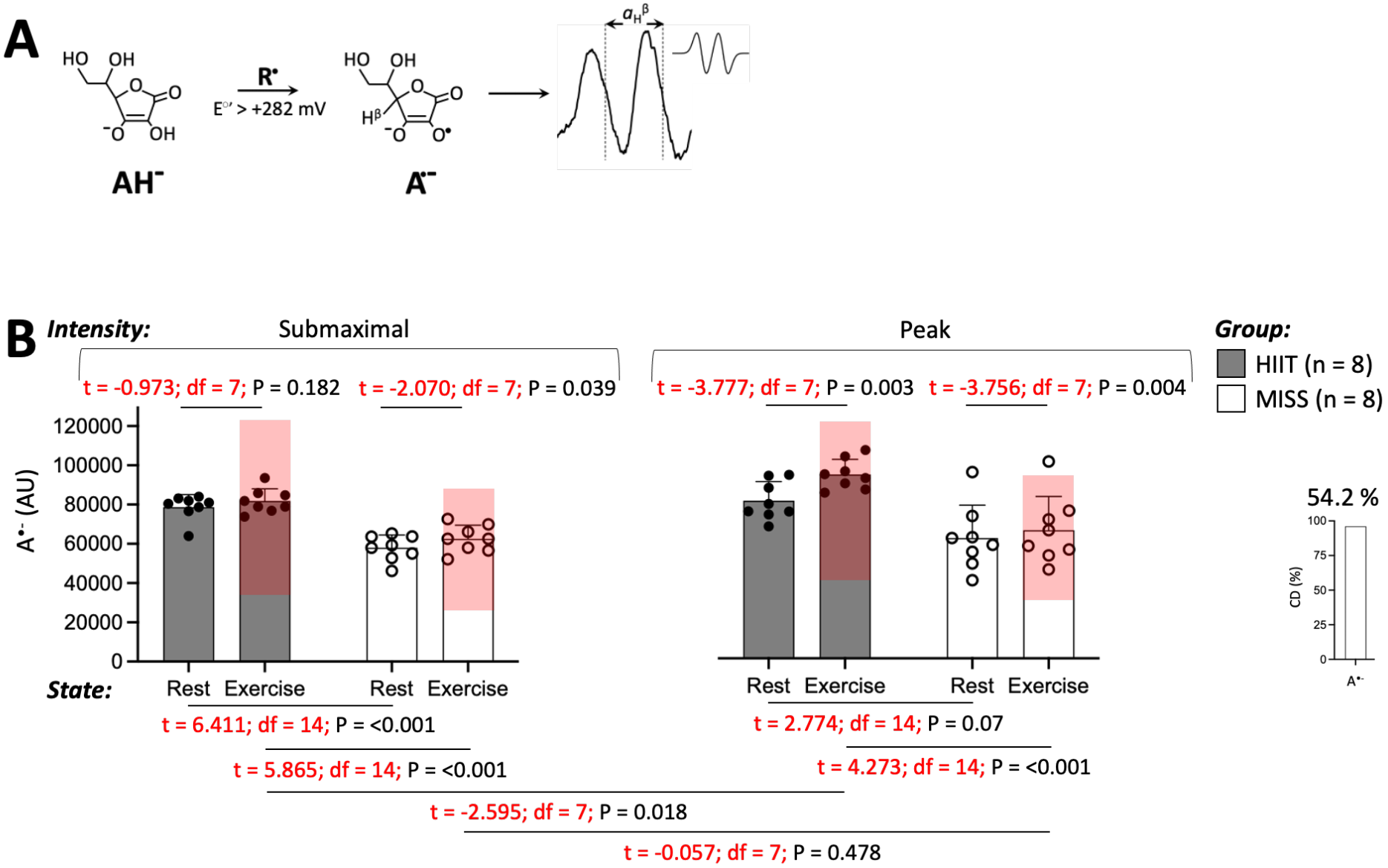

Figure 2. Principle underlying ascorbate radical formation (A) and exercise-induced responses (B)

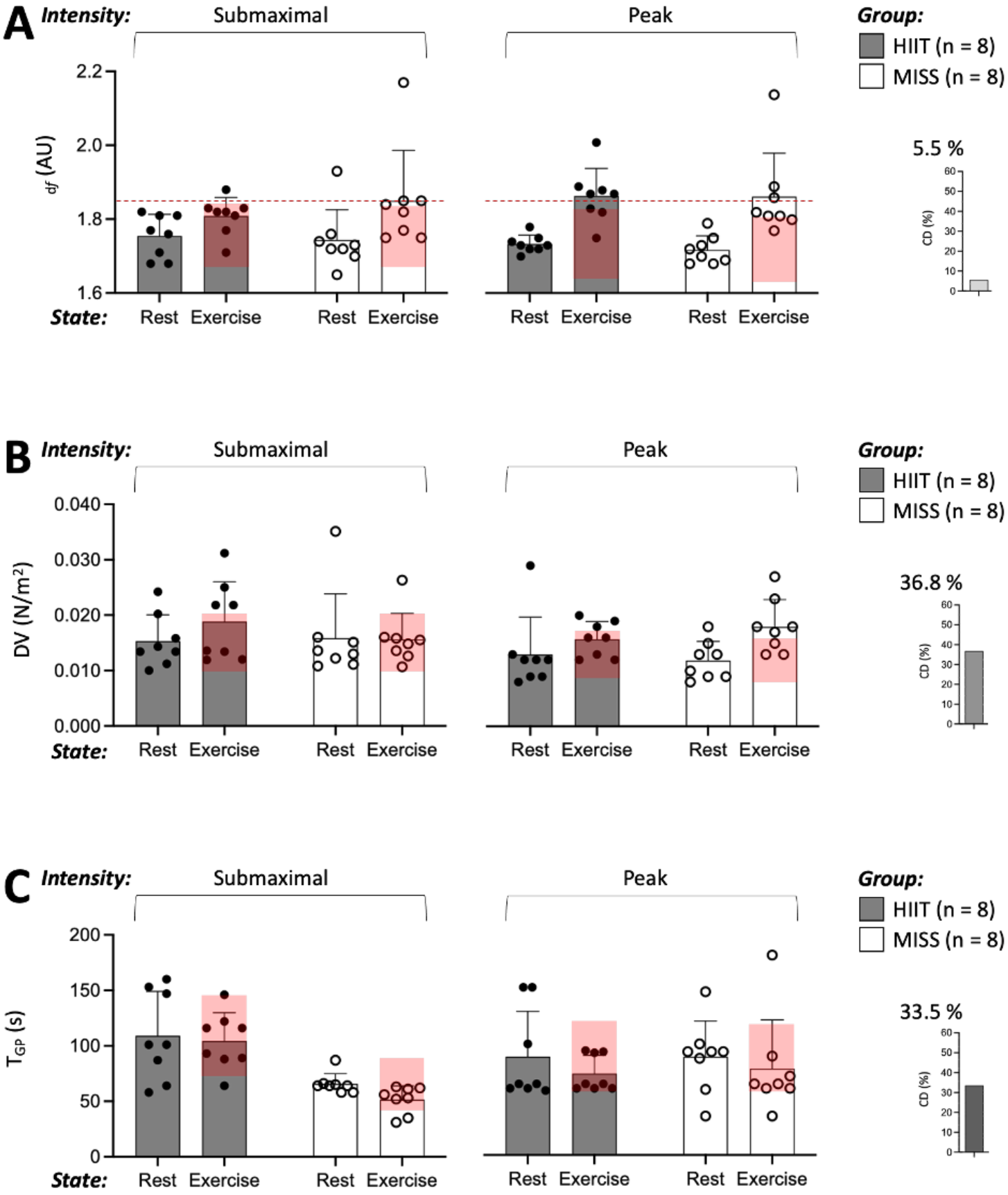

Figure 3. Exercise-induced responses in rheological biomarkers

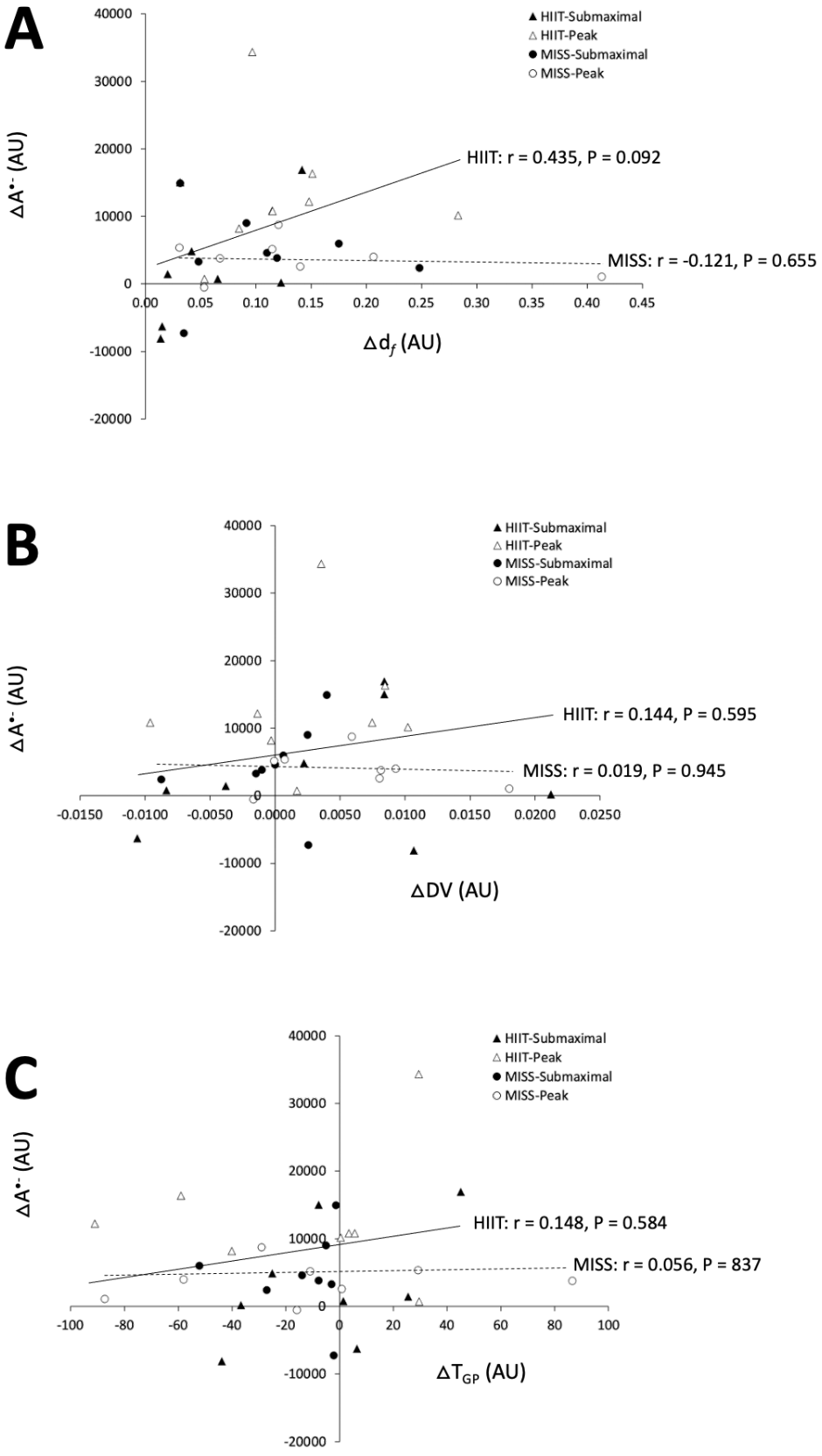

Figure 4. Lack of relationship between exercise-induced alterations in redox and rheological biomarkers