

Supplementary information

Supplementary Notes

Exclusion criteria

All participants

- Body mass index ≥ 35 kg/m²
- Diagnosed with severe asthma or daily use of inhaler, and/or treatment with oral steroids
- Pregnancy/breastfeeding women
- Ongoing requirement of oxygen therapy
- Taking antihypertensive, nitrate, steroid or immunosuppressant medication or medication
- Major illness e.g., cancer, inflammatory disease (including vasculitis) or receiving palliative care
- History of organ transplantation or are candidates for organ transplantation at the time of screening
- History of Chronic Fatigue Syndrome prior to COVID-19 infection
- Diagnosed cardiovascular disease (including current non-benign arrhythmia, chronic heart failure, hypertension)
- History of major psychiatric disorder including bipolar disorders, schizophrenia, schizoaffective disorder, major depression.
- Diagnosis of structural lung disease (such as COPD or pulmonary fibrosis)
- Diagnosed renal disease
- Congenital or acquired neurological conditions (including dementia), language disorders, repeated or chronic pain conditions (excluding menstrual pain and minor sporadic headaches)
- Diabetes Mellitus
- Symptoms of febrile illness 2 weeks before experiment
- Excessive alcohol consumption (>28 units/week) or use of illicit drugs
- History of smoking within 2 months
- Surgery under general anaesthesia within 3 months
- History of stroke

- Coronary revascularisation
- Haemodialysis or peritoneal dialysis
- Participating in another study for an investigational medicinal product

Sit-to-stand test

SBP did not change over time (main effect of time; $P=0.2254$) but DBP increased over time from sitting to standing (main effect of time; $P=0.0484$), where DBP increased from rest ($78 \pm \text{mmHg}$) to 1 min ($84 \pm 10 \text{ mmHg}$, $P=0.0054$), 2 min ($85 \pm 12 \text{ mmHg}$, $P=0.0026$) and 3 mins ($84 \pm 13 \text{ mmHg}$, $P=0.0242$) of standing. There was no time*group interaction effect for SBP or DBP indicating that both groups responded to sit-to-stand in a similar way. HR increased from sitting to standing (main effect of time, $P<0.0001$). Unexpectedly, the increase in HR from sit-to-stand was greater in the control group versus the long COVID group (main Time*Group effect; $P=0.0258$, supplementary figure 2), where the control group had an increase of $13 \pm 9 \text{ beats/min}$ at 2 mins of standing versus $4 \pm 6 \text{ beats/min}$ in the long COVID group ($P=0.0429$). These data indicate that the prevalence of orthostatic intolerance is similar to controls amongst this cohort of long COVID participants.

49 **Supplementary Tables**

50 **Supplementary table 1:** Medications prescribed to participants in the control and long
51 COVID group. NB: none of these medications were taken on the study visits. Importantly,
52 ivabradine was stopped 48 hours prior to study visits.

	Controls (n=14)	Long COVID (n=14)
Ivabradine (n)	0	4
Statins (n)	1	1
Selective serotonin reuptake inhibitors (n)	2	5
Tamsulosin (prostate hyperplasia, n)	1	1
Proton pump inhibitors (n)	1	3
Pain medication (prescribed for myalgia post COVID; pregabalin, n)	0	1
Bivaracetam (epilepsy, n)	1	0

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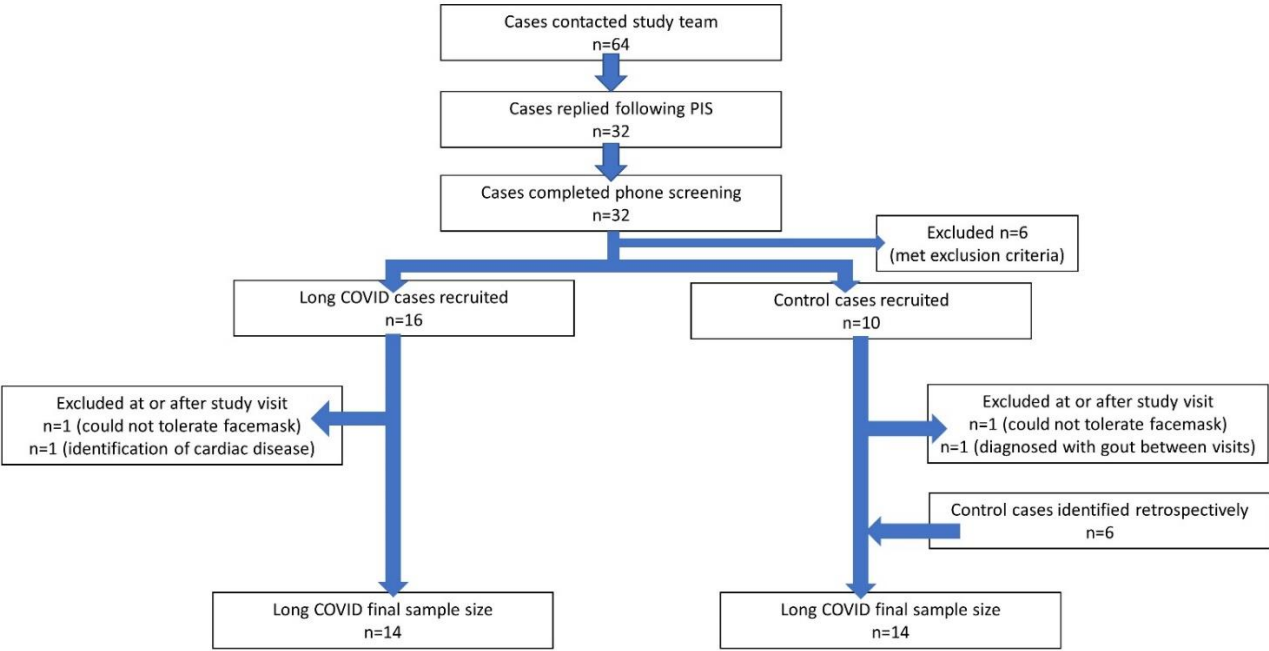
64 **Supplementary table 2:** Blood pressure and heart rate changes during the sit-to-stand
65 test. Data are change from rest.

	Controls	Long COVID	P-value (mixed-effects ANOVA)
SBP (mmHg)			
Δ 1 min	1.2 ± 10.7	6.1 ± 7.9	Time: P=0.5204
Δ 2 min	1.1 ± 8.7	1.9 ± 8.4	Group: P=0.2836
Δ 3 min	-1.6 ± 7.3	3.1 ± 9.8	Time*Group: P=0.7346
DBP (mmHg)			
Δ 1 min	6.9 ± 7.7	5.4 ± 7.2	Time: P=0.1995
Δ 2 min	5.6 ± 9.1	6.5 ± 5.8	Group: P=0.6524
Δ 3 min	2.4 ± 8.8*	7.3 ± 7.3	Time*Group: P=0.0012
Heart rate (beats/min)			
Δ 1 min	10 ± 13	3 ± 6	Time: P=0.1631
Δ 2 min	13 ± 9	4 ± 6	Group: P=0.0698
Δ 3 min	10 ± 9	6 ± 7	Time*Group: P=0.1248

66 Mean ± standard deviation. Mixed model ANOVA showed no group or time effects for
67 systolic blood pressure (SBP) or heart rate. There was an interaction effect for DBP where
68 the change in DBP was smaller after 3 mins vs. the change at 1 min (*P=0.0035).

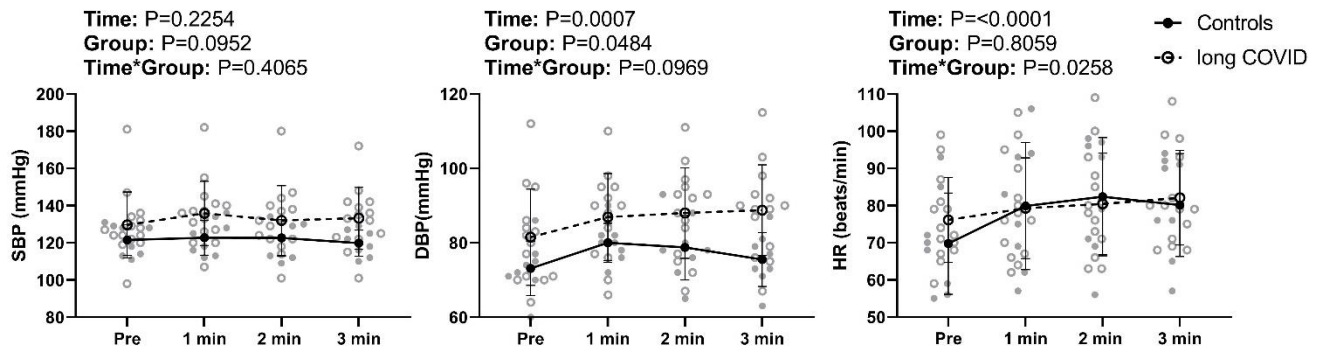
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70 **Supplementary figures**



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72 **Supplementary Figure 1:** Flow chart showing recruitment and excluded cases. Following
73 the participant information sheet (PIS) mailout only 50% of individuals interested replied
74 and completed phone screening.

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Supplementary Figure 2: Sit-to-stand blood pressures and heart rate response in the

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control and long COVID groups. There were no differences in the BP response to standing

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between the groups, however, the control group had a greater increase in HR during

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standing versus the long COVID group. One control and one long COVID participants had

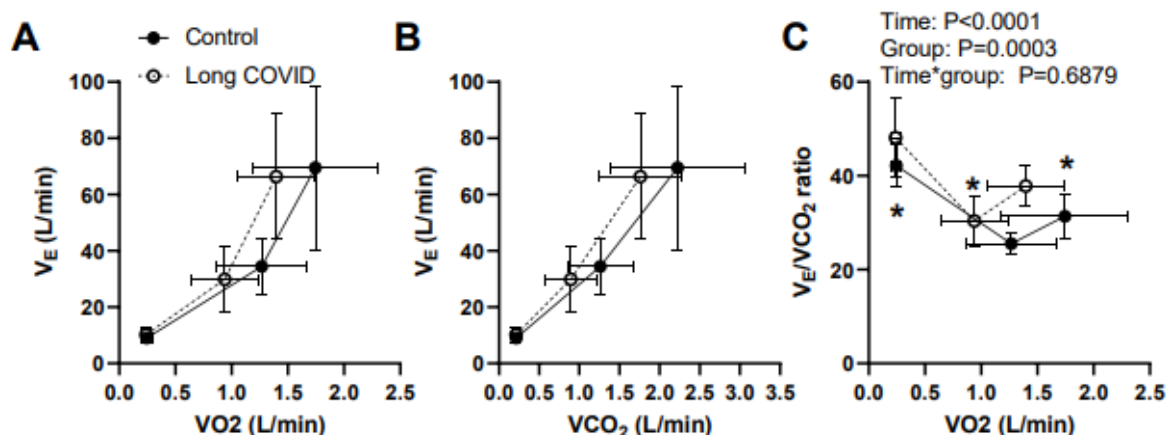
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HR increases above 100 beats/min. SBP; systolic blood pressure, DBP; diastolic BP and

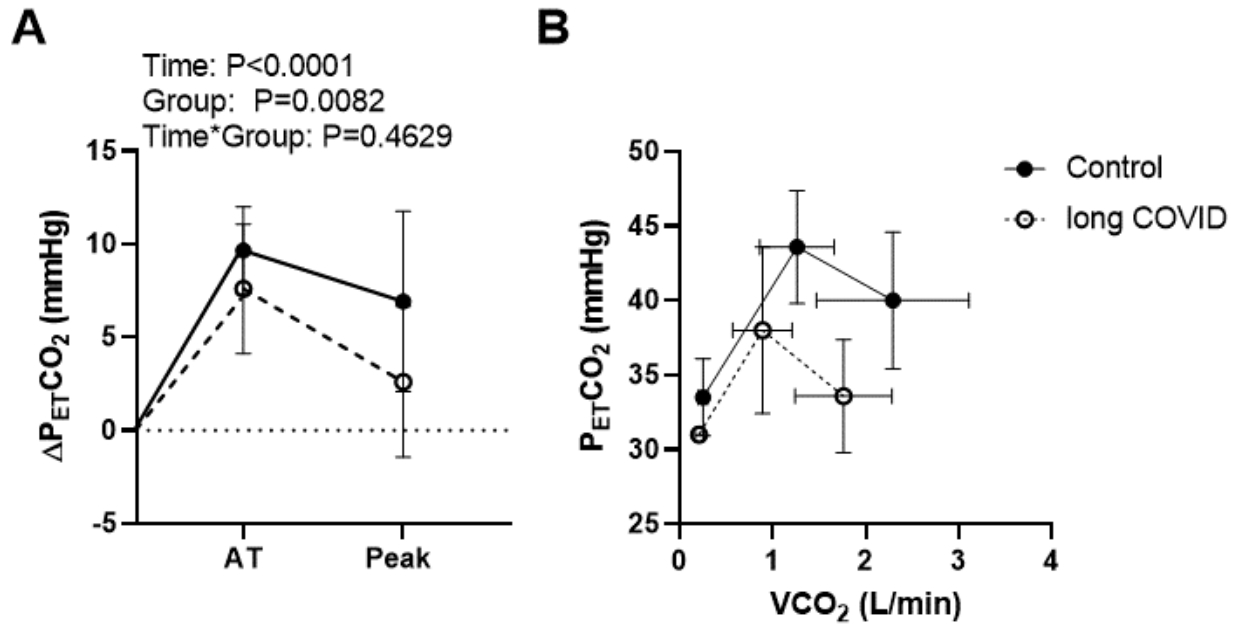
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HR; heart rate. Data are mean $\pm$ standard deviation.

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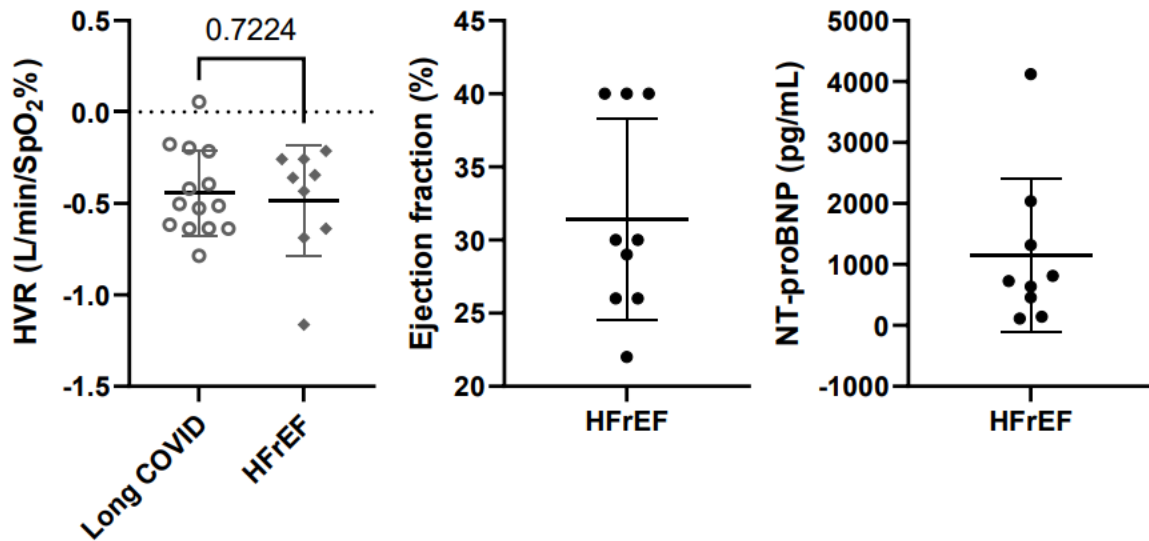
Supplementary Figure 3: Ventilation and heart rate during CPET plotted at 3 timepoints: rest, anaerobic threshold, and peak exercise. Panels A and B show the minute ventilation (V_E) plotted against the volume of oxygen consumed (VO_2) and the volume of CO_2 expired (VCO_2), respectively. Panel C shows the V_E/VCO_2 ratio versus the VO_2 . The mixed model ANOVA shows that V_E/VCO_2 ratio was higher at rest, anaerobic threshold, and peak exercise in the long COVID group. * indicates $P < 0.05$. Rest; $P = 0.0031$, anaerobic threshold; $P = 0.0477$ and peak exercise; $P = 0.0051$ (Bonferroni's multiple comparison test). Mean $\pm$ standard deviation.



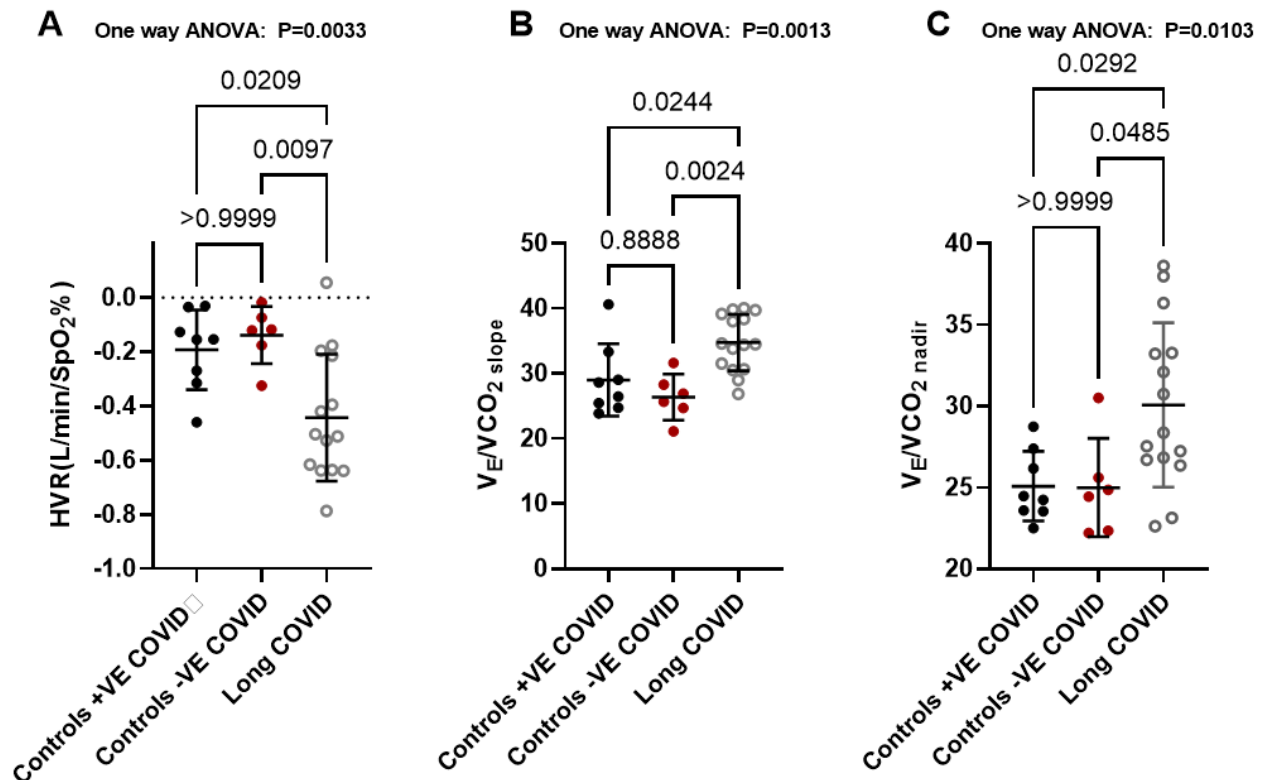
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95 **Supplementary figure 4: A)** Absolute change in partial pressure of end tidal CO_2 ($P_{ET}CO_2$)
 96 from rest to anaerobic threshold (AT) and peak exercise in the control and the long COVID
 97 groups. The long COVID group had a similar increase in $P_{ET}CO_2$ at AT and peak exercise
 98 versus the control group. **B)** The $P_{ET}CO_2$ versus the VCO_2 plotted at three timepoints from
 99 left to right; at rest, at AT and peak exercise in the controls and long COVID group. Data
 100 are mean $\pm$ standard deviation.

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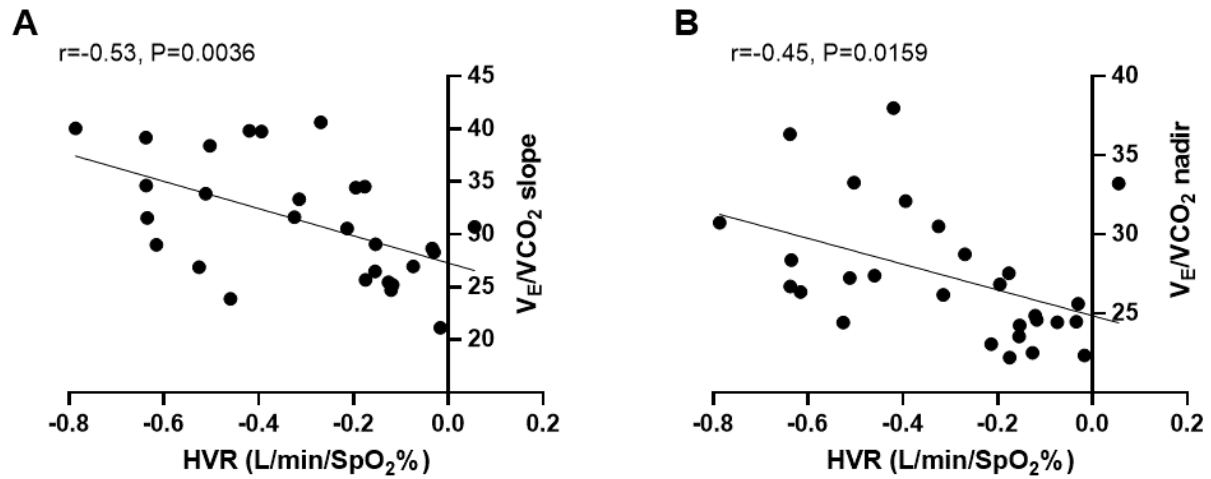
Supplementary Figure 5: The hypoxic ventilatory response in the long COVID participants (n=14) versus a group of participants with heart failure reduced ejection fraction (n=9) measured using the same methods, equipment, location, and study team. The heart failure with reduced ejection fraction (HFrEF) participants are taken from our study comparing carotid chemoreflex function in HFrEF versus heart failure with preserved ejection fraction. The NHS research ethics committee approval number is 18/SW/0241. The HVR was -0.44 ± 0.23 L/min/SpO₂% versus -0.48 ± 0.30 L/min/SpO₂%. Age; 69 ± 11 years, body mass index; 28.7 ± 5.8 kg/m². Panels B and C show the ejection fraction and NT-proBNP in the HFrEF participants. All participants were prescribed treatment for their heart failure (beta-blockers; n=8, angiotensin converting enzyme inhibitors or angiotensin receptor blocker; n=3, sacubitril with valsartan; n=4, aldosterone antagonist; n=6, ivabradine; n=1) which could impact the hypoxic ventilatory response.



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117 **Supplementary Figure 6:** Comparison of the A) hypoxic ventilatory response (HVR), B)
 118 V_E/VCO_2 slope and C) the V_E/VCO_2 nadir amongst controls who had COVID-19 (+VE
 119 COVID), controls who did not have COVID-19 (-VE COVID) and participants who had long
 120 COVID. A difference in all variables persisted between the +VE COVID control and long
 121 COVID participants even when the -VE COVID controls were removed and put in a
 122 separate group. Data passed normality testing (Shapiro-Wilk and visual Q-Q plot
 123 inspection). Bonferroni correction was used for pairwise comparisons.

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127 **Supplementary figure 7:** A) Correlation of the hypoxic ventilatory response (HVR) to the
 128 V_E/VCO₂ slope in all participants. B) Correlation of the HVR to the V_E/VCO₂ nadir in all
 129 participants.