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Early View

Research letter

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The skeletal muscle metaboreflex: a novel driver of ventilation, dyspnoea and pulmonary haemodynamics during exercise in pulmonary arterial hypertension

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TAKE HOME MESSAGE

During exercise, the skeletal muscle metaboreflex is enhanced in pulmonary arterial hypertension and drives excess ventilation, increased pulmonary artery pressure, and increases the perception of dyspnoea.

WORD COUNT

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To the Editor:

Impairment of exercise capacity, predominately limited symptomatically by dyspnoea [1], affects most patients with pulmonary arterial hypertension (PAH) despite current therapies [2] with significant implication for patients, adversely impairing health-related quality of life [3] and clinical prognosis [4]. However, the underpinning physiologic mechanisms behind dyspnoea and exercise limitation remain incompletely understood. Skeletal muscle metabolic and microcirculatory deficits are present in PAH [2] and likely lead to earlier and more pronounced accumulation of metabolites during exercise. We hypothesised that this would augment the activation of group III/IV afferents responsive to metabolites present in exercising limb muscles (i.e., muscle metaboreflex), and provide a novel driver for hyperventilation [5], pulmonary arterial pressure [6] and sensations of dyspnoea [7] in PAH.

Ventilation, pulmonary artery pressure and the perception of dyspnoea were determined during isolated muscle metaboreflex activation (MMA) in 14 PAH patients (9 female, mean age 48.4 ± 12.8 years), recruited, following provision of written informed consent, from the Greenlane Pulmonary Vascular Service, Auckland, New Zealand. Their responses were compared to 14 age- and sex-matched healthy controls (9 female, mean age 49.4 ± 14.2 years). Participants were free of other comorbidities that may limit exercise capacity or cause exertional dyspnoea. This study was approved by the Health and Disability Ethics Committee (HDEC 2022 FULL 12454), prospectively registered (Australia New Zealand Clinical Trials Registry, ACTRN12622000493741), and conducted according to the Declaration of Helsinki (2013).

MMA was achieved using a standard technique of post-exercise circulatory occlusion (PECO) following handgrip exercise, which involves inflation of a cuff on the exercising arm to supra-systolic pressures prior to cessation of handgrip, thereby trapping metabolites within the muscle

during recovery [8]. Participants performed two trials of 8 minutes (MMA and control), in a random order. Each trial consisted of a two minute baseline period, followed by two minutes of right-sided static handgrip (handgrip dynamometer; ADInstruments, Bella Vista, NSW, Australia) to 35% of maximal voluntary contraction, followed by four minutes of rest (final two minutes termed recovery period). In one trial (MMA trial) PECO was performed for the first two minutes of recovery (MMA period). In the control trial participants underwent recovery without MMA, with the first two minutes termed the 'free-flow' period.

Minute ventilation ($\dot{V}_E$) and end-tidal gas partial pressures were measured with a pneumotachometer (Hans Rudolph, Shawnee, KS, USA) and gas analyser (ADInstruments) respectively, connected via a mouthpiece (with nose-clip). Subjective dyspnoea ratings for each trial period were assessed using the Borg 0-10 dyspnoea scale [9]. Transthoracic echocardiographic (Vivid S70, GE Healthcare, Chicago, IL, USA) assessment of right ventricular outflow tract (RVOT) acceleration time (AT), and RVOT velocity time integral (VTI) were performed and mean pulmonary artery pressure (mPAP) estimated from AT using Kitatabake's equation [10]. Assuming a constant RVOT cross-sectional area, percent changes from baseline right ventricular stroke volume were estimated from RVOT-VTI [11], and percent changes in cardiac output (CO) were estimated by multiplying RVOT-VTI by heart rate. Mean responses from baseline, for each variable, were calculated for the second minute of each trial period.

Time series data were examined using mixed models for repeated measures analysis. Normality was verified by visualisation. Where appropriate, multiple comparison *post-hoc* analyses were performed with unpaired t-test and Bonferroni correction. Data are expressed as mean ±

standard deviation (SD). Statistical significance was defined as $p<0.05$. Statistical analysis was performed using SPSS version 29 (IBM, Armonk, NY, USA).

Of PAH aetiologies, nine were idiopathic, three connective tissue disease associated, one drug induced and one congenital heart disease associated. WHO functional class of PAH patients were I (n=3), II (n=8) and III (n=3). Mean parameters on most recent right heart catheterisation were mPAP 42.9 ± 13.3 mmHg, pulmonary vascular resistance (PVR) 5.5 ± 3.2 WU and CO 6.4 ± 1.6 $L \cdot min^{-1}$. Three patients were on monotherapy (one on PDE5 inhibitor [PDE5i], two on calcium channel blockers [CCB]), eight on dual-combination therapy (PDE5i and endothelin receptor antagonist, alongside CCB in one), and three on triple-combination therapy with prostacyclin analogues.

MMA stimulated an excess ventilatory response with a four-fold larger increase in ventilation in PAH compared to healthy controls ($\Delta\dot{V}_E$ 2.23 ± 1.90 vs. 0.42 ± 1.36 $L \cdot min^{-1}$ respectively; p=0.012; Figure 1A). Whilst baseline $P_{ET}CO_2$ was lower in PAH (37 ± 4.9 vs. 41 ± 3.3mmHg respectively), there was also a more pronounced decline with MMA in PAH ($\Delta P_{ET}CO_2$ -2.43 ± 1.98 vs. -1.23 ± 1.15 mmHg respectively; Group p=0.030). In contrast, without MMA, no between group difference was observed, with similar $\dot{V}_E$ recovery to baseline in PAH and healthy controls, occurring during the free-flow period ($\Delta\dot{V}_E$ 0.51 ± 1.29 vs. 0.85 ± 1.55 $L \cdot min^{-1}$ respectively; Group p=0.743; Figure 1A), and no difference in free-flow period $P_{ET}CO_2$ responses ($\Delta P_{ET}CO_2$ -1.01 ± 0.96 vs. -0.52 ± 0.67 respectively, Group p=0.469). Accordingly, the MMA ventilatory response (determined for each individual as the ventilatory response from baseline of the MMA period, minus the ventilatory response during the corresponding free-flow period of the control trial) was higher in PAH versus controls (1.72 ± 1.42 vs. -0.434 ± 1.42 $L \cdot min^{-1}$ respectively; unpaired t-test p<0.001; Figure 1D).

MMA sustained the handgrip exercise-induced increase in mPAP in PAH but not healthy controls (ΔmPAP 10.34 ± 9.49 vs. 0.40 ± 5.02% of baseline respectively; Group p=0.017; Figure 1B). This seemingly occurred through increased PVR, rather than a flow-mediated effect, as CO recovered to baseline during MMA. Without MMA during free-flow recovery, mPAP returned to baseline similarly in PAH and healthy controls (Figure 1B). The MMA mPAP response (calculated analogous to the ventilatory response) was numerically greater in PAH than healthy controls (11.51 ± 12.84 vs. 3.03 ± 8.99%, respectively; unpaired t-test p = 0.077; Figure 1D).

The impact of MMA on dyspnoea perception in PAH was marked, with doubling of Borg dyspnoea score responses compared to healthy controls (ΔBorg 1.89 ± 1.21 vs. 0.81 ± 1.13 units, respectively; p<0.001; Figure 1C), with a clinically important mean difference of 1.08 units [12]. In PAH, the mean Borg dyspnoea score reported during handgrip from both trials was positively correlated with the magnitude of MMA ventilatory response (Pearson correlation coefficient r=0.581, p=0.029), though not with the MMA mPAP response (r=0.404, p=0.247).

Our results further elucidate the physiologic mechanisms of dyspnoea in PAH, demonstrating the role of skeletal muscle metaboreflex activation. Classically the predominant factors limiting exercise are understood to be impaired CO response to exercise due to high right ventricular (RV) afterload, and excess ventilation stimulated primarily by high physiologic dead-space ratio (V_D/V_T) and reduced arterial partial pressure of CO_2 (P_aCO_2), causing sensations of dyspnoea [13], often exacerbated by impaired breathing mechanics augmenting the work of breathing [14]. We demonstrate the metaboreflex to be an additional driver of excess

ventilation, and enhanced perception of dyspnoea [13]. Furthermore, by exacerbating already high PVR and RV afterload, thereby limiting CO, skeletal muscle underperfusion may result, potentially further heightening skeletal muscle dysfunction and fatigue, metabolite build-up and thus metaboreflex responses in a positive feedback loop.

A limitation of this study is use of non-invasive echocardiography rather than the gold-standard right heart catheterisation. RVOT AT was used instead of tricuspid regurgitant velocity to estimate mPAP due to superior recoverability and correlation to invasively measured mPAP during exercise, as previously reported [15]. We recognise that $P_{ET}CO_2$ may further dissociate from P_aCO_2 in PAH, and as P_aCO_2 was not measured in this study, we cannot definitely conclude the augmented ventilatory response to metaboreflex activation is excessive relative to CO_2 production (i.e. lowers P_aCO_2). Thirdly, the metabolic environment of the muscle interstitium during exercise was not measured, and thus we are unable to elucidate the mechanisms by which the ventilatory response to metaboreflex activation is augmented in PAH, whether by greater metabolite accumulation, by higher metaboreflex sensitivity (peripherally or centrally) or a combination of these.

In summary, the novel findings of this study are that metaboreflex activation evokes exaggerated ventilatory and pulmonary vascular responses in PAH and causes clinically meaningful increases in ratings of perceived dyspnoea. This study raises the possibility that metaboreflex afferents represent a novel physiologic mechanism to target (e.g. pharmacologically or by exercise training) for improving exertional dyspnoea in PAH.

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CONFLICTS OF INTEREST

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DATA AVAILABILITY STATEMENT

Study data are available from the corresponding author upon reasonable request.

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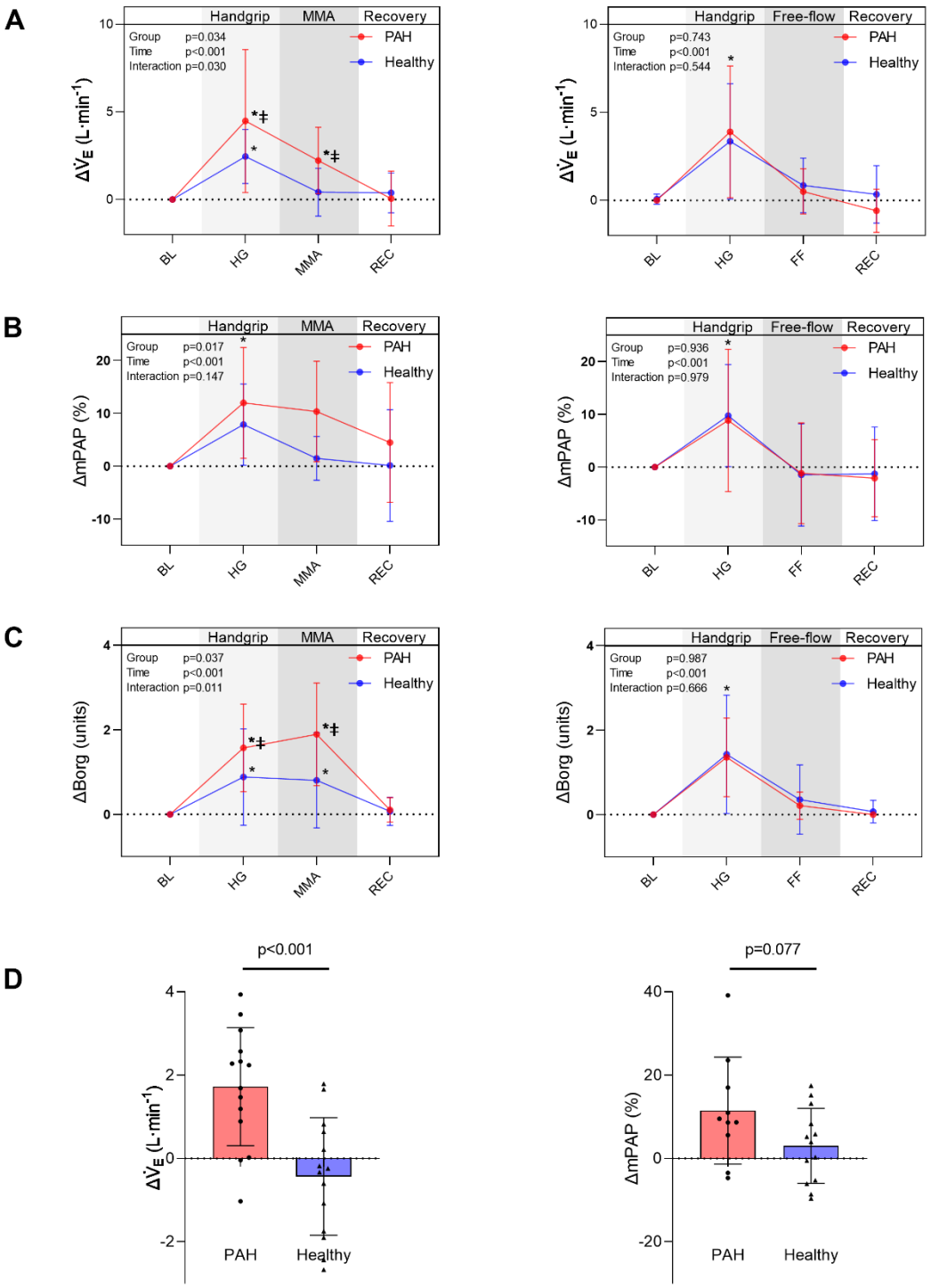

Changes in minute ventilation (A, ΔVE), mean pulmonary artery pressure (B, ΔmPAP) and Borg dyspnoea score (C, ΔBorg) from baseline in MMA trial (left) and control trial (right) in healthy control and PAH participants. Panel D shows metaboreflex responses for ventilation (VE; left) and mean pulmonary artery pressure (mPAP; right) in PAH (red) and healthy control (blue) participants. Circles denote individual PAH patients, triangles denote individual healthy controls. Abbreviations: BL, baseline; HG, handgrip; FF, freeflow; MMA, muscle metaboreflex activation; REC, recovery; N = 14 in all variables for PAH and healthy controls, apart from ΔmPAP in PAH where N = 10. * denotes p<0.05 compared to baseline. ǂ denotes p<0.05 PAH compared to healthy controls. Values are means ± standard deviations.