Title: The effects of inspiratory muscle training in older adults

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Running title: Inspiratory muscle training in older adults

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ABSTRACT

Purpose: Declining inspiratory muscle function and structure and systemic low-level inflammation and oxidative stress may contribute to morbidity and mortality during normal ageing. Therefore, we examined the effects of inspiratory muscle training (IMT) in older adults on inspiratory muscle function and structure and systemic inflammation and oxidative stress, and re-examined the reported positive effects of IMT on respiratory muscle strength, inspiratory muscle endurance, spirometry, exercise performance, physical activity levels (PAL) and quality of life (QoL). **Methods:** Thirty-four healthy older adults (68 ± 3 years) with normal spirometry, respiratory muscle strength and physical fitness were divided equally into a pressure-threshold IMT or sham-hypoxic placebo group. Before and after an 8 week intervention, measurements were taken for dynamic inspiratory muscle function and inspiratory muscle endurance using a weighted plunger pressure-threshold loading device, diaphragm thickness using B-mode ultrasonography, plasma cytokine concentrations using immunoassays, DNA damage levels in peripheral blood mononuclear cells (PBMC) using Comet Assays, spirometry, maximal mouth pressures, exercise performance using a six minute walk test, PAL using a questionnaire and accelerometry, and QoL using a questionnaire. **Results:** Compared to placebo, IMT increased maximal inspiratory pressure (+34 ± 43%, $P = 0.008$), diaphragm thickness at residual volume (+38 ± 39%, $P = 0.03$), and peak inspiratory flow (+35 ± 42%, $P = 0.049$), but did not change other spirometry measures, plasma cytokine concentrations, DNA damage levels in PBMC, dynamic inspiratory muscle function, inspiratory muscle endurance, exercise performance, PAL nor QoL. **Conclusion:** These novel data indicate that in healthy older adults IMT elicits some positive changes in inspiratory muscle function and structure, but does not attenuate systemic inflammation and oxidative stress, nor improve exercise performance, PAL or QoL. **Keywords:** inspiratory muscle structure, inspiratory muscle function, cytokines, oxidative stress.

INTRODUCTION

Paragraph Number 1 The work of breathing is increased in older adults at rest and during exercise. With healthy ageing there is a progressive decrease in total respiratory system compliance resulting in flow limitation, air trapping and an increase in residual volume which flattens the curvature of the diaphragm, shifting its length-tension relationship to a shorter length and placing it at a mechanical disadvantage (21). This contributes to a reduction in inspiratory muscle force and endurance, whilst increasing the oxygen cost of breathing (21). Functionally, this can contribute to increased dyspnea during everyday tasks, limit exercise performance, and lead to reduced physical activity levels (PAL) and quality of life (QoL) (17, 22).

Paragraph Number 2 Respiratory muscle training in healthy older adults improves respiratory muscle strength and endurance, spirometry measures, exercise tolerance, PAL and QoL (3, 4, 19, 43). However, this research has either lacked statistical power (3) or has risked participant bias by failing to employ a placebo (PLA) group (4, 19, 43). Inspiratory muscle training (IMT) in younger adults also increases respiratory muscle size, as evidenced by increases in diaphragm thickness (T_{di}) measured with B-mode ultrasonography (13), but whether this occurs in older adults is unknown.

Paragraph Number 3 We have recently shown in young healthy adults that increased respiratory muscle work at rest increases plasma interleukin-1β (IL-1β) and interleukin-6 (IL-6) concentrations, and that IMT reduces the plasma IL-6 response to increased respiratory muscle work (30, 31). Our data suggest that when the work of breathing is increased, the respiratory muscles directly contribute to systemic inflammation and that IMT can reduce the evoked plasma IL-6 response. IL-1β can impair striated muscle function (27), whilst both IL-

1β and IL-6 may have a role in muscle repair and regeneration following injury (40). IL-1β can also stimulate myogenesis (12), whereas IL-6 acts to stimulate lipolysis (44), hepatic glucose output (14), glucose uptake in the contracting myocytes (10), and satellite cell proliferation (25, 40). Ageing is also associated with systemic low-level inflammation and oxidative stress, and both may contribute to morbidity and mortality (9, 26). Systemic inflammation is linked to arthritis, cancer, and cardiovascular and neurodegenerative disease (9), whilst oxidative stress can cause damage to lipids, proteins and nucleic acids (26). Whole-body resistance or endurance training in healthy older adults can attenuate resting systemic cytokine concentrations (33) and oxidative stress (39). However, whether IMT elicits similar effects in older adults remains unknown.

Paragraph Number 4 Therefore, the aim of this study was to examine the effects of IMT in healthy older adults on inspiratory muscle function and structure and systemic inflammation and oxidative stress, and to re-examine the reported positive effects of IMT on respiratory muscle strength, inspiratory muscle endurance, spirometry, exercise performance, PAL and QoL.

METHODS

Participants

Paragraph Number 5 Thirty-six participants aged 65-75 years were recruited according to the exclusion criteria used to define "healthy" older participants for exercise studies (16). Participants arrived at the laboratory after an overnight fast (morning visits) or 4 h postprandially (afternoon visits) having abstained from alcohol and caffeine for 24 h before testing.

Experimental design

Paragraph Number 6 The study adopted a randomized, PLA-controlled design. All procedures were conducted in accordance with the Declaration of Helsinki, and the study was approved by the Nottingham Trent University Human Ethics Committee. Before experimental trials, all participants undertook a screening and familiarization session. In the screening session, height, body mass, blood pressure, spirometry and maximal mouth pressures were measured according to published guidelines (2, 29) and a full 12-lead electrocardiogram was performed. At least 1 week later, participants undertook a familiarization session of all testing procedures. For the experimental trials, participants attended the laboratory on two separate occasions, separated by 7 days, at the same time of day before and after an 8 week intervention. During the first visit, spirometry and maximal mouth pressures were measured and participants performed a six minute walk test (6MWT) and completed a questionnaire to determine QoL. Participants were also given an accelerometer to measure PAL, and were re-familiarized with the testing procedures for the second visit. During the second visit, participants returned the accelerometer, a blood sample was taken and body fat, T_{di}, dynamic inspiratory muscle function and inspiratory muscle endurance were assessed. Participants also completed a questionnaire to measure PAL.

Participants were then randomly, and equally, divided into an IMT or PLA group. Randomization was concealed from the participants. One week before the end of the intervention, participants were re-familiarized with all the testing procedures. Following the intervention, participants repeated the experimental trials in the same order.

Maximal dynamic inspiratory muscle function

Paragraph Number 7 Maximal dynamic inspiratory muscle function was assessed as described previously (37, 38) using a weighted plunger threshold loading device (24). Maximal inspiratory pressure at zero flow (P_{0max}) was initially measured. The maximal value recorded for both inspiratory pressure (P_0) and flow ($\dot{V}_I$) at each $\%P_0$ was used for analysis. Inspiratory muscle power ($\dot{W}_I$) was calculated as the product of P_0 and $\dot{V}_I$. The maximum rate of inspiratory pressure development (MRPD) was assessed during an inspiratory effort at P_{0max}.

Inspiratory muscle endurance

Paragraph Number 8 Inspiratory muscle endurance was assessed as described previously using a weighted plunger threshold loading device (24). Loading started at 10 (males) or 5 (females) cmH_2O and was increased by 5 cmH_2O every minute until task failure. An audio metronome paced breathing frequency (15 breaths·min^{-1}) and duty cycle (0.5).

Diaphragm thickness

Paragraph Number 9 T_{di} was assessed using B-mode ultrasonography (Phillips ATL HDI 5000; ATL Ultrasound, Washington, USA) according to published guidelines (2). Measurements were obtained in triplicate at residual volume ($T_{di.RV}$) and total lung capacity ($T_{di.TLC}$) and during a Müeller maneuver from residual volume ($T_{di.CONT}$). The diaphragm

thickening ratio ($T_{di.TR}$) was calculated as: $T_{di.RV}$ / $T_{di.CONT}$. Lung volumes were estimated from flow signals measured using a Fleisch no. 3 pneumotachograph. Ultrasound images were synchronized with flow signals using a custom-built trigger.

Six minute walk test

Paragraph Number 10 Exercise performance was assessed using a 6MWT according to published guidelines (1, 8). After the test, measurements were immediately taken for cardiac frequency (f_C) and estimated arterial oxygen saturation (SpO_2) using fingertip pulse oximetry (Model 8600; Nonin, Minnesota, USA), and rating of perceived exertion (RPE) for dyspnea and leg discomfort using Borg's modified CR10 scale (5).

Accelerometry

Paragraph Number 11 Accelerometry was measured (Model GT1M; Actigraph Manufacturing Technology, Florida, USA) for 7 days during waking hours according to published guidelines (32). The accelerometer was removed for sleep and bathing only. Based on previous work (11), data were reduced to provide bands of PAL.

Percentage body fat and questionnaires

Paragraph Number 12 Percentage body fat was measured using bioelectrical impedance (Bodystat 1500; Bodystat, Isle of Man, UK) according to published guidelines (28). PAL and QoL were evaluated using the Physical Activity Scale for the Elderly (PASE) (42) and the Older People's Quality of Life Questionnaire (OPQOL-35) (7), respectively. Both PASE and OPQOL-35 are scored so that higher scores equate to a higher PAL or QoL, and total scores from all the components of the questionnaires can range from 0->400 and 35-175, respectively.

Collection of blood for assays and isolation of peripheral blood mononuclear cells

Paragraph Number 13 Whole blood samples (~10 mL) were taken at rest from an antecubital vein. Blood was immediately transferred into pre-cooled tubes (SARSTEDT, Leicester, UK) containing either EDTA for plasma cytokines or lithium heparin for peripheral blood mononuclear cells (PBMC), which were isolated using density gradient centrifugation as described previously (31).

Plasma cytokine assay

Paragraph Number 14 Plasma cytokine concentrations were measured in duplicate using an ultrasensitive electrochemiluminescence multiplex immunoassay (Meso Scale Discovery, Maryland, USA). To exclude inter-assay variation, baseline and post-intervention cytokines from both groups were measured during the same assay. The intra/inter-assay coefficient of variation (CV) were 10% and 14%, respectively. If the lowest limit of detection was not met from the cytokine analyses, participant data (from baseline and post-intervention) were excluded from the analysis.

Measurement of systemic oxidative stress

Paragraph Number 15 Systemic oxidative stress was determined using the Comet Assay which measures oxidative DNA damage in PBMC. DNA damage (DNA single strand breaks and alkali labile lesions) in PBMC was determined in duplicate using the alkaline Comet Assay and the modified alkaline Comet Assay as described previously (31). The inter-assay CV was <10%.

Inspiratory muscle training and placebo interventions

Paragraph Number 16 The intervention lasted 8 weeks. The IMT group performed 30 consecutive dynamic inspiratory efforts twice daily using an inspiratory pressure-threshold device (POWERbreathe® Classic series 1st generation; Gaiam Ltd, Southam, UK). The initial training load was 50% maximal inspiratory pressure (MIP). Thereafter, participants periodically increased the load so that 30 maneuvers could only just be completed. Each inspiratory effort was initiated from residual volume and participants strove to maximize tidal volume. This regimen is known to be effective in eliciting an adaptive response (23, 30, 37). The PLA group used a sham hypoxic trainer which was identical to that used by the IMT group, except that the resistance spring was removed and the lower chamber was loosely packed with aquarium gravel, which was promoted to the participants as being oxygen absorbent, thus reducing the oxygen content of inspired air and mimicking altitude exposure (23). Participants were instructed to breathe normally for 30 consecutive breaths twice daily through the device and to not increase their normal breathing effort. The resistance of the device was <5 cmH_2O, a pressure known to elicit negligible changes in inspiratory muscle function (38). Spirometry and maximal mouth pressures were assessed at 2 and 4 weeks during the intervention. During these visits, correct training technique and load (to ensure it was maintained at 50% MIP) was evaluated in both IMT and PLA groups and the "oxygen absorbent" gravel in the PLA device was also replaced. During the post-intervention period the IMT and PLA groups performed their intervention 2 $d \cdot wk^{-1}$, which is sufficient to maintain improvements in inspiratory muscle function following IMT (37). These maintenance sessions were performed 48 h before and 48 h after experimental trials. All participants completed a training diary throughout the study to record adherence to the prescribed intervention and whole-body training sessions.

Statistical analyses

Paragraph Number 17 Statistical analyses were performed using SPSS for Windows (IBM, Illinois, USA). We based our sample size on the resting plasma IL-6 concentrations observed in older adults (2.5 ± 0.5 $pg \cdot mL^{-1}$) (9) and the reductions observed following IMT in our previous studies (30, 31). From this we estimated that a sample size of 16 participants in each IMT and PLA group would have a power of 80% to detect a 0.5 $pg \cdot mL^{-1}$ reduction in resting plasma IL-6 concentrations for an α of 0.05. A repeated measures ANOVA was used to analyze the effects of 'intervention' (pre- vs. post-'treatment') and 'treatment' (IMT vs. PLA). Main effects of intervention and intervention x treatment interactions were further explored by analyzing IMT and PLA groups separately using paired *t*-tests between baseline and post-intervention. Reliability was assessed using CV. Statistical significance was set at $P < 0.05$. Results are presented as mean ± SD.

RESULTS

Baseline measurements and intervention

Paragraph Number 18 Results are presented for 34 participants who completed the study. Participants had normal spirometry and maximal mouth pressures (Table 2) and physical fitness (Table 4). Participant characteristics were unchanged post-intervention (Table 1). Compliance with the intervention was excellent with 97 ± 5 (IMT) and 96 ± 7% (PLA) of sessions completed. Inspection of training diaries revealed that habitual whole-body exercise remained constant in both groups.

[Table 1]

Spirometry and maximal mouth pressures (Table 2)

Paragraph Number 19 There were main effects of intervention for forced vital capacity (FVC) ($P < 0.001$), forced expiratory volume in 1 s (FEV_1)/FVC ($P = 0.01$), peak inspiratory flow (PIF) ($P < 0.001$) and MIP ($P = 0.001$). Subsequent paired *t*-tests revealed that FVC decreased ($P = 0.02$) and FEV_1/FVC increased ($P = 0.002$) after PLA only. These changes in FVC and FEV_1/FVC were not different from those after IMT (intervention x treatment interactions of $P = 0.358$ and $P = 0.469$, respectively). The 26% increase in PIF after IMT ($P = 0.001$) exceeded (intervention x treatment interaction, $P = 0.049$) the 12% increase observed after PLA ($P = 0.027$). MIP increased from 82 ± 27 cmH_2O at baseline to 97 ± 23, 100 ± 23 and 103 ± 23 cmH_2O ($P = 0.001$) after 2, 4 and 8 weeks of IMT only and these changes exceeded those after PLA (intervention x treatment interaction, $P = 0.008$).

[Table 2]

Dynamic inspiratory muscle function, inspiratory muscle endurance and diaphragm thickness (Table 3)

Paragraph Number 20 There were main effects of intervention for P_{0max} ($P < 0.001$), MRPD ($P = 0.001$) and optimal inspiratory pressure (P_{0opt}) ($P = 0.011$) and a strong trend for maximum inspiratory muscle power ($\dot{W}_{Imax}$) ($P = 0.052$). Subsequent paired *t*-tests revealed that P_{0max} increased after both IMT ($P = 0.001$) and PLA ($P = 0.023$), whereas increases in P_{0opt} ($P = 0.015$) and MRPD ($P = 0.004$) were observed after IMT only. The changes in P_{0opt} and MRPD were not different from those after IMT (intervention x treatment interactions, $P = 0.057$ and $P = 0.352$, respectively), although there was a trend in P_{0opt}. There was a main effect of intervention for inspiratory muscle endurance ($P < 0.001$) and subsequent paired *t*-tests revealed an increase after IMT only ($P = 0.001$). This change was not different from that after PLA (intervention x treatment interaction, $P = 0.125$). There was a main effect of intervention for $T_{di.RV}$ ($P < 0.001$) and $T_{di.TR}$ ($P = 0.002$) and an intervention x treatment interaction for $T_{di.RV}$ ($P = 0.03$). Subsequent paired *t*-tests revealed that $T_{di.RV}$ increased ($P = 0.001$) whilst $T_{di.TR}$ decreased ($P = 0.016$) after IMT only. The change in $T_{di.TR}$ was not different from that after PLA (intervention x treatment interaction, $P = 0.368$).

[Table 3]

Exercise performance, physical activity levels and quality of life

Paragraph Number 21 There were no main or interaction effects for any 6MWT measurements, apart from an intervention x treatment interaction for f_C ($P = 0.011$) (Table 4). Subsequent paired *t*-tests revealed that f_C increased after PLA only ($P = 0.014$). There were no main or interaction effects for QoL or PAL measured with accelerometry and the PASE. At baseline the total QoL score was 134 ± 7 and 137 ± 9, and the total PAL score measured with PASE was 174 ± 56 and 181 ± 69 in IMT and PLA groups, respectively. Moderate to

vigorous physical activity measured by accelerometry was 46 ± 23 and 37 ± 17 counts·min^{-1} at baseline in IMT and PLA groups, respectively.

[Table 4]

Plasma cytokines and DNA damage levels in peripheral blood mononuclear cells

Paragraph Number 22 Plasma cytokines and DNA damage levels in PBMC were unchanged in both groups post-intervention (Table 5).

[Table 5]

DISCUSSION

Main findings

Paragraph Number 23 This is first study to examine the effects of IMT in older adults on inspiratory muscle function and structure and systemic inflammation and oxidative stress. We also re-examined the reported positive effects of IMT on respiratory muscle strength, inspiratory muscle endurance, spirometry, exercise performance, PAL and QoL. The main findings were that in a population of healthy older adults with normal spirometry, respiratory muscle strength and physical fitness and compared to PLA, IMT increased MIP, $T_{di.RV}$ and PIF, but did not change other spirometry measures, plasma cytokine concentrations, DNA damage levels in PBMC, dynamic inspiratory muscle function, inspiratory muscle endurance, 6MWT distance, PAL nor QoL.

Plasma cytokines and DNA damage in peripheral blood mononuclear cells

Paragraph Number 24 IMT did not change resting systemic plasma cytokine concentrations or DNA damage levels in PBMC. Baseline plasma cytokine concentrations in the present study were similar to those previously reported in older adults (9) and the %DNA damage in PBMC was greater than we previously reported in younger adults (31), thus demonstrating systemic inflammation and oxidative stress in our participants. Whether there was a reduction in local cytokines and/or DNA damage levels in PBMC within the inspiratory muscles cannot be excluded. A diaphragmatic or intercostal biopsy would allow this area to be explored. However, this is a very invasive measurement that requires open surgery or thoracscopy under general anaesthesia and, therefore, for these healthy individuals this would be unethical. It is possible that the improvements in inspiratory muscle function and structure following IMT may not have reached a threshold necessary to elicit a systemic reduction in cytokines and/or DNA damage levels in PBMC. Previous studies have shown that whole-body

resistance or endurance training can decrease resting systemic cytokine concentrations (33) and oxidative stress (39). However, the respiratory muscles only weigh ~960 g (15) and represent ~3% of total body mass (36). Thus, IMT only targets a small muscle group and although they do contribute to systemic cytokine concentrations during increased respiratory muscle work in younger adults (30, 31), IMT appears to not attenuate these under resting conditions.

Diaphragm thickness

Paragraph Number 25 The increase in $T_{di.RV}$ after IMT is similar to the 8% increase in $T_{di.TLC}$ previously reported after 4 weeks of IMT in younger adults (13). Our findings also support the 21% increase in type II fiber size in the external intercostals of chronic obstructive pulmonary disease patients after 5 weeks of IMT (35). Together these findings suggest that IMT elicits a hypertrophic response in the inspiratory muscles. MIP has been widely used in IMT studies as an estimate of inspiratory muscle strength. Since MIP is somewhat technique-dependent, it is argued that IMT-induced increases in MIP primarily reflect a learning effect (34). Our data argue against this, and instead suggest that increased T_{di} may contribute to an increase in MIP.

Spirometry

Paragraph Number 26 Except for an increase in PIF, spirometry measures were unchanged after IMT. This contrasts previous studies reporting increases in vital capacity after voluntary isocapnic hyperpnea training (4), and FVC and peak expiratory flow after concurrent IMT/expiratory muscle training (43). Belman and Gaesser (4) and Watsford and Murphy (43) failed to provide a mechanism for the changes in spirometry, but we suggest that their findings reflect a learning effect rather than an adaptation. This notion is supported by the

observed increase in PIF and P_{0max} after PLA, despite a rigorous familiarization of the maneuvers. Along with the increase in f_C following the 6MWT after PLA, this highlights the requirement to employ a legitimate placebo group that will impact participant expectation and motivation.

Maximal inspiratory pressure

Paragraph Number 27 The 26% increase in MIP after IMT is consistent with the 21-39% increases reported in comparably aged adults (65-71 years) after IMT (3, 19, 43). A rapid 18% increase in MIP, suggestive of neural adaptation, was observed after just 2 weeks of IMT in the present study. This has been reported previously in younger participants with 14% and 28% increases in MIP observed after 1 and 2 weeks of IMT, respectively (3, 18).

Exercise performance

Paragraph Number 28 The 6MWT distance was unchanged after IMT and PLA. This contrasts with other IMT studies reporting improvements in 6MWT distance (19) and endurance time during treadmill walking at the first ventilatory threshold (3). These discrepancies may be explained by different baseline endurance training status. Huang et al. (19) reported a baseline 6MWT distance that was 90 ± 16% of predicted (41), suggesting that the cardiorespiratory response may have been limited. Conversely, 6MWT distance in the present study was 102-103% of predicted (41). If a functional consequence of IMT is to improve 6MWT distance in older adults, then it may be those with a low baseline status that show the greatest improvement (6). Indeed, a recent meta-analysis suggests that participants with a low baseline physical fitness level experience, compared to trained participants,

greater improvements in exercise performance/capacity following respiratory muscle training (20).

Physical activity levels

Paragraph Number 29 PAL was unchanged after IMT. This is the first study to report PAL measured with the PASE, but PAL measured with accelerometry has demonstrated increases in moderate to vigorous physical activity in six older adults after IMT (3). Aznar-Lain et al. (3) attributed this to an increase in exercise intensity as other accelerometry measures were unchanged. Since baseline PAL measured with accelerometry and the PASE in the present study were higher than those previously reported in older adults (11, 42), this may have limited the potential for PAL to increase following IMT.

Quality of life

Paragraph Number 30 Baseline QoL in the present study was similar to that previously reported in older adults (7). The unchanged QoL after IMT contrasts with the findings of Huang et al. (19) who observed an increase in the physical subcategory of the SF-36 QoL questionnaire. Differences in the type of questionnaire used (SF-36 vs. OPQOL-35), or the economical or health (mental and physical) status of participants, could account for these discrepancies.

Conclusion

Paragraph Number 31 In conclusion, IMT in healthy older adults with normal spirometry, respiratory muscle strength and physical fitness and compared to PLA, increased MIP, T_{di}

and PIF, but did not change other spirometry measures, plasma cytokine concentrations, DNA damage levels in PBMC, dynamic inspiratory muscle function, inspiratory muscle endurance, 6MWT distance, PAL nor QoL. These novel data indicate that the inspiratory muscles of healthy older adults are not a major limiting factor to PAL or exercise performance, nor do they impact on resting systemic inflammation and oxidative stress. Conversely, IMT may exert a greater influence in older adults with inspiratory weakness and/or low levels of physical fitness, or patients with chronic obstructive pulmonary disease or asthma who experience an elevated work of breathing and systemic inflammation and oxidative stress. Such studies remain to be conducted and thus offer an attractive avenue for future investigation.

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