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# Methodology and reliability of respiratory muscle assessment

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**Abstract**

The optimal method for respiratory muscle endurance (RME) assessment remains unclear. This study assessed the test-retest reliability of two RME-test methodologies. Fifteen healthy adults attended the laboratory on four occasions, separated by 5 ± 2 days, and completed each test in a random, "one on two" order. They performed spirometry testing, maximal respiratory pressure assessment and two different RME tests: an inspiratory resistive breathing (IRB) and an isocapnic hyperpnea endurance (IHE) test. Typical error, expressed as coefficient of variation, for IRB maximal inspiratory pressure (MIP) and IHE maximal ventilation were 12.21 (8.85-19.67) % and 10.73 (7.78-17.29) %, respectively. Intraclass correlation coefficients for the same parameters were 0.83 (0.46-0.94) and 0.80 (0.41-0.93), respectively. No correlations were found between RME parameters derived from the IHE and IRB tests (all $p>0.05$). Significant positive correlations were found between both IRB and IHE outcomes and spirometry parameters, MIP and maximal expiratory pressure ($p<0.05$).

Given these results, IRB and IHE appear to be suitable for RME testing in healthy people, although they may reflect different physiological mechanisms (respiratory mechanics and respiratory muscle capacity for IHE test *vs*. inspiratory muscle capacity for IRB test). Future studies are therefore warranted that compare IRB and IHE tests in clinical settings.

**Keywords**: Respiratory muscles; Inspiratory muscles; Expiratory muscles; Respiratory muscle endurance

## 1. Introduction

Similar to other skeletal muscles, respiratory muscle function can be characterised by strength and endurance. Specifically, respiratory muscle strength (RMS) is the ability to produce a maximal force level and is usually estimated by maximal inspiratory and expiratory pressures (MIP and MEP, respectively). Respiratory muscle endurance (RME) is the ability to sustain a high level of work (i.e., ventilation) under isocapnic conditions (Leith, 1976) or to breathe for a prolonged period against a high resistive load (Jones et al., 1985). In clinical practice, respiratory muscle function is commonly assessed from MIP and MEP manoeuvres which are well-tolerated, reliable and can be compared to age- and sex-matched reference values (Black and Hyatt, 1969; Wilson et al., 1984). However, whilst a correlation between RMS and RME is suggested (American Thoracic Society/European Respiratory Society, 2002 statement), the exact relationship remains to be elucidated.

Currently, MIP and MEP measurements are commonly used as an assessment of global respiratory muscle function (American Thoracic Society/European Respiratory Society, 2002 statement; Lotters and Burdof, 2002). However, concerns have been raised regarding the clinical relevance of MIP and MEP. Specifically, maximal pressures are rarely required during daily living and the sensitivity of these assessments, and therefore their ability to detect early impairments in respiratory muscle function, is questionable. For instance, in the early stages of obstructive pulmonary diseases, a training-like effect on RMS is reported, with MIP and MEP remaining normal or even becoming superior to that observed in healthy people (Heinzmann-Filho et al., 2012; Similowski et al., 1991). This highlights the issues with interpreting changes, or the lack thereof, in maximal pressures with regards to respiratory health.

Assessing RME is particularly relevant given its role in enabling ventilation and gas exchange during physical activities. Respiratory muscle fatigue has been shown following intensive

whole-body exercise in healthy adults (Johnson et al., 1997; Verges et al., 2006) and can affect whole-body exercise performance through a respiratory metaboreflex (Dempsey et al., 2006). Thus, evaluating RME may offer an alternative, useful tool with which to identify relevant early clinical changes in respiratory muscle function, though there is no consensus regarding the optimal methodology to assess RME (American Thoracic Society/European Respiratory Society, 2002 statement; Troosters et al., 2009).

Consequently, the development of an incremental protocol, akin to the widely accepted Bruce protocol for exercise testing, has been investigated (American Thoracic Society/European Respiratory Society, 2002 statement). Specifically, while RME tests using an external load predominantly focus on the inspiratory muscles (inspiratory resistive breathing tests (IRB); (American Thoracic Society/European Respiratory Society, 2002 statement; Formiga et al., 2018; Langer et al., 2013), ventilatory endurance tests or isocapnic hyperpnea tests (IHE) involve both inspiratory and expiratory muscle loading by replicating hyperpnea as observed during intense whole-body exercise (American Thoracic Society/European Respiratory Society, 2002 statement; Bai et al., 1984; Mancini et al., 1994; Vincent et al., 2016). RME tests, both IRB and IHE, typically set their workload increments according to maximal voluntary ventilation (MVV) for IHE, or MIP for IRB. Indexes of RME are usually recorded from the last stage completed by participants and are expressed either in absolute values or relative to MVV or MIP (Clanton and Diaz, 1995; Nickerson and Keens, 1982; Verges et al., 2009; Vincent et al., 2016). Given these methodological differences, it should be emphasized that IHE and IRB may assess different physiological mechanisms. Specifically, IHE provides outcomes related to respiratory muscles, lung and chest wall mechanics and can be under-estimated, for instance, in obstructive respiratory disease patients (i.e., Chronic Obstructive Pulmonary Disease; COPD) due to an increased airway resistance and chest wall remodelling (thoracic distension; (Rochester and Arora, 1983). In contrast, IRB, which is used more

frequently, has the advantage of being less influenced by airway resistance but relies on high-intensity, low-speed contractions, which are significantly different from respiratory muscle contractions during spontaneous breathing.

Despite several decades of investigation, the most appropriate method of assessing RME for routine clinical practice remains to be determined, as do the respective reliabilities of the IRB and IHE tests. Therefore, the primary aim of this study was to compare the outcomes from two distinct RME tests (IHE and IRB) and the test-retest reliability associated with such assessments in healthy young adults. The secondary aim was to ascertain the relationships between both RME testing outcomes and clinical parameters such as RMS, lung function and physical activity levels.

## 2. Material and method

### 2.1 Participants

Following written informed consent, fifteen healthy adults (6 females; 25.7 ± 3.0 years) participated in the study. Ethical approval was granted by the local committee (CPP Grenoble Sud Est V) and the study was conducted in accordance with the Declaration of Helsinki.

### 2.2 Study design

Participant's descriptive information is presented in Table 1. Briefly, they visited the laboratory on four separate occasions, separated by 5 ± 2 days, to complete each test in a random, "one on two" order. Specifically, in order to minimize a potential learning effect, a participant assigned to the IRB test during the first visit performed the IHE test for the second visit, then the IRB for the third and the IHE for the last visit.

Prior to performing either RME test, lung function, respiratory muscle strength and physical activity levels were measured. Spirometry measurements were conducted according to standard procedures (Miller et al., 2005). Forced expiratory volume in 1 second ($FEV_{1;}$ $FEV1_{\%pred}$), forced vital capacity (FVC; $FVC_{\%pred}$) and MVV sustained for 12 seconds were measured (Medisoft, Dinant, Belgium) and reported in absolute and % of predicted values. Physical activity was assessed according to the three dimensions of the Baecke questionnaire (e.g., work, leisure and sport levels of physical activity (Baecke et al., 1982)). RMS was assessed by MIP and MEP (MicroRPM, Carefusion, San Diego, California, USA) according to the recommendations for respiratory muscle testing (American Thoracic Society/European Respiratory Society, 2002 statement), with the best of three reproducible values being recorded and expressed in absolute and % predicted values (Wilson et al., 1984). Perceived symptoms of breathlessness and respiratory muscle exertion were determined at task failure in all RME tests using the Borg scale (Borg, 1982).

### 2.3 Respiratory muscle endurance

#### 2.3.1 Isocapnic hyperpnea endurance test (IHE)

The IHE test was performed with the Spirotiger® (Idiag, Fehraltorf, Switzerland), a commercially available device used for respiratory muscle training that allows partial $CO_2$ rebreathing to ensure normocapnia during high ventilatory load (Verges et al., 2009; Villiot-Danger et al., 2011). As previously described (Verges et al., 2009) and according to the latest recommendations (Laveneziana et al., 2019), IHE consists of an isocapnic hyperpnea test with increasing levels of minute ventilation until task failure. Participants were asked to breathe through a mouthpiece connected by a bi-directional valve to a rebreathing bag and ambient air. The volume of the bag was set at 50% of forced vital capacity (FVC) and breathing frequency was adjusted to obtain a minute ventilation ($V_E$) corresponding to 30% MVV for the first stage. Subsequently, breathing frequency was increased every 3 minutes to increase

$V_E$ by 10% MVV. Task failure was determined by the inability of the participant to sustain the $V_E$ level despite strong verbal encouragement. The Spirotiger® provided continuous audio feedback for breathing frequency and visual feedback to control tidal volume throughout the test. RME was determined as the last 3-min stage completed and expressed in maximal absolute $V_E$ and relative to MVV values ($V_{Emax}$ in $L \cdot min^{-1}$ and $V_{Emax}$ in %MVV, respectively). The time to task failure was also recorded.

### 2.3.2. Inspiratory resistive breathing test (IRB)

The IRB test was performed using the Pro2® (Design Net, Smithfield, USA), which is commonly used for inspiratory muscle training and respiratory muscles assessment in both clinical and sports settings (Formiga et al., 2018; Formiga et al., 2019; Hursh et al., 2019). In this study, we used an incremental inspiratory test based on inspiratory repetitions with a resistance modulated by the inspiratory target pressure. The IRB involved a 3-s inspiration / 5-s expiration with the level of inspiratory resistance increasing every 20 respiratory cycles. Participants were asked to inspire through a mouthpiece attached to the Pro2®, with exhalation performed outside the device. Prior to RME testing, MIP was determined using the Pro2® ($MIP_{pro2}$); the first stage started at 30% $MIP_{pro2}$ after which the load was increased by 10% $MIP_{pro2}$ every 20 inspirations. Task failure was defined as the inability to reach or sustain the MIP target for at least 1.5 seconds for three consecutive inspirations despite strong verbal encouragement. The Pro2® provided continuous visual feedback regarding the inspiratory pressure produced by the participants and the target pressure. RME was determined as the last stage of 20 inspirations completed and expressed in maximal absolute inspiratory pressure and relative to the $MIP_{pro2}$ values ($MIP_{max}$ in $cmH_2O$ and $MIP_{max}$ in $\%MIP_{pro2}$, respectively). The time to task failure and the total number of inspirations were also recorded.

## 2.4. Statistical analysis

All variables are reported as mean (standard deviation (SD)). Normality was determined using Kolmogorov-Smirnov test. Paired t-tests or Wilcoxon tests and Pearson or Spearman correlations (according to normality) were used to detect systematic bias and the association between first and second sessions of each testing method (i.e., IHE1, IHE2 and IRB1, IRB2; (Atkinson and Nevill, 1998). Absolute reliability was evaluated according to typical error expressed as a coefficient of variation ($CV_{TE}$; (Hopkins, 2000). Relative reliability was assessed by calculating intraclass correlation (ICC) using a two-way mixed-effects model for single measurement and absolute agreement following the online spreadsheet of Hopkins (2000, 2002). Bland-Altman plots were also used with 95% limits of agreement (LOA) to ascertain differences across the range of values produced between sessions for each RME assessment method (Bland and Altman, 1986). Pearson or Spearman correlations were calculated to investigate the association between the outcomes of IHE and IRB tests, RMS, lung function variables and physical activity index. A multiple linear regression model (i.e., stepwise regression) was conducted to identify relationship between parameters that account in the variation of RME variables ($V_{Emax}$, $MIP_{max}$). Statistical procedures were performed on Statistica version 10 (Statsoft, Tulsa, OK) and SPSS version 25 (IBM corp, Armonk, NY), with the alpha level set at 0.05 for all tests.

## 3. Results

### 3.1 Comparisons of IHE and IRB outcomes

One participant was unable to complete all four experimental sessions due to lack of time. Participants presented supra-normal RMS according to reference values (Wilson et al., 1984), a normal body mass index (22 ± 1.7 $kg \cdot m^{-2}$) and a normal lung function (Table 1). Moreover, all participants tolerated IHE and IRB tests well without any adverse effect.

Individual performances during the last completed stage of the IRB test and the IHE test are shown in Figure 1. No difference was found between end-IHE and end-IRB maximal respiratory levels expressed in relative values ($V_{Emax}$ 60 ± 11 %MVV and $MIP_{max}$ 67 ± 9 $\%MIP_{pro2}$; p = 0.14) or between IRB and IHE end-exercise score of respiratory muscle exertion (p = 0.098). However, end-exercise breathlessness was higher (p < 0.001) and the time to task failure shorter (p = 0.046) during IHE compared to IRB. Sex differences were found for absolute values of $FEV_1$, FVC and MVV, as well as absolute respiratory muscle strength (MIP and MEP) and endurance ($MIP_{max}$ and $V_{Emax}$). However, when expressed in relative values, all of these parameters except MVV were no longer different between sexes (Table 2).

### 3.2 Between days reliability

#### 3.2.1 IHE test-retest

Results of the IHE test-retest reliability are summarised in Table 3. During IHE1 and IHE2, participants reached similar absolute $V_{Emax}$, corresponding to 60 ± 11 and 58 ± 8 %MVV (p = 0.53). No systematic bias was detected between IHE1 and IHE2. Significant correlations were found between IHE1 and IHE2 for $V_{Emax}$ ($r$ = 0.64; p = 0.01), but not for time to task failure ($r$ = 0.52; p = 0.05). With regard to absolute reliability, absolute $V_{Emax}$ demonstrated stronger $CV_{TE}$ agreement than time to task failure between IHE1 and IHE2. Highest relative reliability

(i.e., ICC) was found for absolute $V_{Emax}$. The corresponding Bland-Altman plots illustrating the agreement for absolute IHE $V_{Emax}$ and IHE time to task failure are shown in Figures 2A and 2B, respectively.

### 3.2.2 IRB test-retest

Results of the IRB test-retest reliability are summarised in Table 4. During IRB1 and IRB2, participants reached similar absolute $MIP_{max}$ corresponding to 98.9 ± 19.7 and 94.2 ± 18.7 % $MIP_{Pro2}$, respectively. Systematic bias was detected for breathlessness sensation and time to task failure ($p < 0.05$). A significant correlation between IRB1 and IRB2 was found for absolute $MIP_{max}$ ($r = 0.72$; $p < 0.01$), the total number of inspirations ($r = 0.54$; $p = 0.04$) and the time to task failure ($r = 0.53$; $p = 0.04$). Absolute reliability expressed with $CV_{TE}$ revealed that absolute $MIP_{max}$ had a stronger agreement than the total number of inspirations and IRB time to task failure between IRB1 and IRB2. Highest relative reliability (i.e., ICC) was found for absolute $MIP_{max}$. The corresponding Bland-Altman plots illustrating the agreement for IRB absolute $MIP_{max}$ and IRB time to task failure are shown in Figures 2C and 2D, respectively.

## 3.3 Correlations between RME tests, lung function and physical activity

No correlations were found between RME parameters derived from the IHE and IRB tests (all $p > 0.05$). Regarding IHE correlations, significant relationships were found between end-IHE absolute $V_{Emax}$, MEP and lung function parameters (Figures 3A, 3B and 3C). Moreover, the multilinear regression model indicates that FVC and MVV accounted significantly in the variation of $V_{Emax}$ ($R^2 = 0.43$ and 0.19, $p < 0.05$, respectively). Concerning IRB, significant correlations between end-IRB absolute $MIP_{max}$, MEP and absolute lung function parameters were also found (Figure 4A, 4B and 4C). A significant correlation was also found between end-IRB absolute $MIP_{max}$ and the level of physical activity dimension “Sport index” ($r = 0.54$;

p = 0.04)**.** Furthermore, FVC accounted significantly in the variation of $MIP_{max}$ ($R^2$ = 0.67, p < 0.01).

## 4. Discussion

The current study aimed to determine the reliability of two distinct incremental RME testing methods (IHE and IRB) and to evaluate the relationships between RME outcomes and other physiological parameters (RMS, lung function and physical activity index) in healthy young adults. The main findings were that IHE and IRB reached a comparable level of reliability in terms of $CV_{TE}$ and ICC for absolute $V_{Emax}$ and $MIP_{max}$, the principal measures of interest.

Previous studies using RME testing methods primarily focused on determining maximal outcomes in healthy participants. Specifically, by using an IRB test, Martyn et al. (1987) reported end-test maximal relative MIP values that were between 55 to 75% of MIP at baseline, comparable to the 67 ± 9 $\%MIP_{max}$ in the present study. By using an IHE test, Vincent et al. (2016) reported a higher end-test maximal relative ventilation (74 ± 17 %MVV) in comparison to the present study (60 ± 11 %MVV). Such a discrepancy could be explained, at least in part, by the use of an estimated MVV in Vincent et al. (2016) to predict a target $V_E$ for each test. This may have underestimated $V_E$ and, therefore, overestimated the maximal outcome of the IHE test. This therefore emphasizes the need for rigorous baseline measurements of MIP and MVV parameters for subsequent use in IRB and IHE testing protocols. Indeed, $MIP_{pro2}$ demonstrated excellent absolute and relative reliability, which is in accordance with previous findings that reported maximum static respiratory pressures assessed with a portable device to be both precise and reproducible (Hamnegard et al., 1994). This also highlights the potential utility of the Pro2® device to provide an index of inspiratory strength, in addition of RME.

Our two principal measures of interest, absolute $MIP_{max}$ and $V_{Emax}$, demonstrated the highest test-retest relative reliability between IHE1-IHE2 and IRB1-IRB2, with no systematic bias. Moreover, correlations revealed good relationships between IHE1-IHE2 and IRB1-IRB2. However, only a modest absolute reliability was found for $V_{Emax}$ and $MIP_{max}$, with a $CV_{TE}$ higher than 7% $CV_{TE}$ (Atkinson and Nevill, 1998). Whilst the mean difference between IRB1 and IRB2 $MIP_{max}$ was only -3.5 $cmH_2O$, in a subset of three participants, a greater drop ranging from 21 to 31 $cmH_2O$ was found. Regarding the IHE test, there was one participant with a drop of 44 $L \cdot min^{-1}$ between IHE1 and IHE2, compared to the average difference of 3.2 $L \cdot min^{-1}$ between tests. The explanation for this large intra-individual variability in these participants is unclear but may be related, at least in part, to motivational issues to perform the same test twice.

Previous studies have assessed the reliability of RME testing methods similar to the current IRB test in healthy participants and patients with respiratory diseases (Enright et al., 2006; Formiga et al., 2018; Hart et al., 2002; Larson et al., 1999; Romer and McConnell, 2004). However, there are key methodological differences between the RME assessments and reliability index used in these previous studies that prevent a direct comparison to these results. Firstly, previous reliability studies have not considered isocapnic hyperpnea RME assessment. Larson et al. (1999), Hart et al. (2002) and Romer and McConnell (2004) studied RME reliability of threshold loading apparatus in COPD and healthy participants, reporting considerable variability in the reliability. Specifically, whilst Larson et al. (1999) reported a greater reliability than in the present study, Hart et al. (2002) observed a significantly lower reliability. Differences in sample size and the number of repeats in the test-retest design limit inter-study comparisons, as well as the inter-test period which was as much as 78 ± 82 days in Hart et al. (2002). Furthermore, methodological discrepancies with regards to controlling breathing patterns and the amalgamation of multiple independent studies with separate

research questions to investigate the reliability are important elements to be considered in the interpretation of earlier studies. In COPD, Formiga et al. (2018) reported higher reliability than observed in the current study, with ICC of 0.99 for their primary outcome measure (SMIP: sustainable maximal inspiratory pressure). A key difference in the testing protocol, however, was that the test-retest sessions were on the same day in Formiga et al. (2018), which may impact reliability simply by decreasing heterogeneity and, consequently, affect the ICC results (Atkinson and Nevill, 1998). Nevertheless, one should acknowledge that reliability was moderate in the present study for both IHE and IRB tests. Differences in RME outcomes (assessments and reliability index) reported in the present study highlight that, despite multiple ways available to test the reliability in sport and exercise field, the optimal test applicable across different populations (i.e., healthy, COPD, etc.) is not clear. In this way, our study presents the first results of reliability regarding respiratory muscle assessment with IHE methods. Future research should extend this work to respiratory disease patients. Familiarization sessions may also be required in order to achieve greater reliability when using these kinds of RME testing methods, which are complex to learn within a single session. Nonetheless, significant correlations between maximal outcomes from both IRB and IHE and lung function parameters were found, indicating that those with larger lung volumes and able to produce higher maximal expiratory flow rates are also able to reach higher $V_{Emax}$ and $MIP_{max}$ during RME tests. Conversely, it can be expected that patients with impaired lung function will show reduced RME, although further studies evaluating RME in patients are required. Moreover, while correlations between RME outcomes and lung function may have been limited in the present study due to only healthy young participants with relatively homogenous spirometric values were evaluated, correlations between RME outcomes and lung function might be stronger in patients with more heterogeneous spirometric values (e.g., COPD of various severities).

RME outcomes ($V_{Emax}$ and $MIP_{max}$) also correlated with RMS (MEP only for IHE) showing some relationship between respiratory muscle endurance and strength, although these aspects of RM function are not equivalent. $MIP_{max}$ during IRB also correlated with the sport physical activity dimension, possibly because higher physical activity levels (especially sport) may increase muscle strength and endurance, including RMS. Moreover, we found based on stepwise regression that FVC was the best predictor of RME performance, at least when considering absolute $V_{Emax}$ and $MIP_{max}$ during the IHE and IRB tests, respectively.

Sensations of respiratory muscle exertion reported at exercise cessation were not different between IHE and IRB, while breathlessness perception was significantly higher in IHE. The continuous production of high respiratory flow rates as required during IHE may induce greater breathlessness than repetitive inspiratory efforts at high inspiratory pressures and low flow rates. Conversely, although the difference in RM exertion between IRB and IHE did not reach significance ($p = 0.09$), the IRB test tended to induce higher sensations of RM exertion, probably due to the high resistance participants had to overcome. Altogether, despite IRB and IHE being maximal tests requiring intense effort from participants, they appear to be well-tolerated in healthy people. The slight differences regarding participant's sensations between both testing procedures underline that IRB and IHE probably evaluate distinct respiratory mechanisms. This hypothesis is supported by the lack of correlation between the outcomes of the IRB and IHE tests. This suggests that, despite both tests being designed to evaluate RME, the characteristics of the IHE and IRB tests differ significantly (e.g., respiratory muscle group recruited, type of contraction), leading to different outcomes.

The IHE test is known to require high levels of ventilation that imitate ventilatory efforts during whole body exercise (Rochester and Arora, 1983). Interestingly, the present study found that the IRB test reflected more associations with other respiratory function and physical activity parameters than IHE, despite daily living not requiring people to produce

elevated inspiratory pressure. In this regard, the present results highlight that an IRB method only based on a specific muscular group (i.e., inspiratory muscle) may be sufficient to represent the whole respiratory muscle function in healthy participants. Moreover, it is assumed that IRB reduces the contribution of respiratory mechanics in RME assessment compared to IHE because no ventilatory output is required in such testing. Therefore, IRB may provide better outcomes in pulmonary obstructed patients than the IHE method, in comparison to healthy peers due to its methodology based on repetitive inspiratory contraction which is less sensitive to ventilatory output than IHE (American Thoracic Society/European Respiratory Society, 2002 statement). This emphasises the need to extend the present results to other populations, such as obstructive respiratory diseases, in order to further promote the evaluation of RME in various settings (sport, research, clinical settings). It should be acknowledged that the present study did not assess participants' affinity with each testing method. Nonetheless, participants reported anecdotally that Pro2® device was preferable in terms of providing clearer feedback compared with the Spirotiger®, making the exercise protocol easier for participants and the experimenter. Furthermore, it is important to acknowledge that the present study only incorporated two test-retest sessions for each method (IHE and IRB), which may have impacted on reproducibility outcomes. Future studies should therefore consider multiple repeat sessions to verify our findings.

## 5. Conclusion

This is the first study to compare the reliability and clinical associations of two different methods of RME assessment. Despite comparable test-retest reliability and no differences between the maximal relative criteria provided by both RME testing methods, IHE and IRB outcomes were not related to each other and showed distinct correlations with clinical markers. In this study, $V_{Emax}$ during IHE and $MIP_{max}$ during IRB were the best performance

indicators regarding RME capacity of the participants as supported by the best scores of reliability and the stronger relationships with clinical markers. In order to improve reliability, an initial familiarization session would be advised. Further studies are also required to evaluate reliability and compare both IRB and IHE testing methods in clinical setting (e.g., in patients with respiratory diseases).

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## 8. Figure legends

**Figure 1.**

Individual performances expressed as a percentage of maximal voluntary ventilation or maximal inspiratory pressure attained on the incremental test for each method: Isocapnic hyperpnea endurance (IHE) and Inspiratory resistive breathing (IRB).

**Figure 2.**

Bland-Altman plots of difference *vs.* mean of the two isocapnic hyperpnea endurance tests (IHE; panels A and B) and inspiratory resistive breathing tests (IRB; panels C and D) for absolute minute ventilation ($V_{Emax}$ in $L \cdot min^{-1}$ panel A), IHE time to task failure in minutes (panel B), IRB absolute maximal inspiratory pressure ($MIP_{max}$ in $cmH_2O$, panel C) and IRB time to task failure in minutes (panel D).

**Figure 3.**

Relationship between isocapnic hyperpnea endurance test (IHE) and lung function parameters: absolute maximal minute ventilation ($V_{Emax}$) and absolute forced vital capacity (FVC, panel A), maximal expiratory pressure (MEP, panel B) and absolute maximal voluntary ventilation (MVV, panel C).

**Figure 4.**

Relationship between inspiratory resistive breathing test (IRB) and lung function parameters: absolute maximal inspiratory pressure ($MIP_{max}$) and absolute forced vital capacity (FVC, panel A), maximal expiratory pressure (MEP, panel B) and absolute maximal voluntary ventilation (MVV, panel C).

## 9. Tables

**Table 1.** Participant characteristics

| ***Participant Characteristics*** | |
|---|---|
| Age (y) | 25.7 ± 3.0 |
| Height (cm) | 172.8 ± 9.4 |
| Weight (kg) | 65.9 ± 7.0 |
| ***Physical Activity Index*** | |
| Usual PA index | 2.5 ± 0.5 |
| Sport PA index | 3.8 ± 0.7 |
| Leisure PA index | 3.4 ± 0.8 |
| Global PA index | 9.8 ± 1.6 |
| ***Lung function*** | |
| $FEV_1$ (L) | 4.2 ± 0.8 |
| $FEV_1$ (%) | 103.4 ± 9.0 |
| FVC (L) | 4.8 ± 0.9 |
| FVC (%) | 99.8 ± 8.6 |
| MVV (L) | 182.8 ± 32.1 |
| MVV (%) | 119.9 ± 23.8 |
| ***Respiratory muscle strength*** | |
| MIP ($cmH_20$) | 120.7 ± 32.5 |
| MIP (%pred) | 122.8 ± 27.6 |
| MEP ($cmH_2O$) | 164.1 ± 37.2 |
| MEP (%pred) | 125.9 ± 19.2 |

Values are means ± SD; PA, physical activity; $FEV_1$ forced expiratory volume in one second; FVC, forced vital capacity; MVV, maximal voluntary volume; MIP, maximal inspiratory pressure; MEP, maximal expiratory pressure

**Table 2.** Sex differences in lung and respiratory muscle function

| **Lung function** | **Males (N = 9)** | **Females (N= 6)** |
|---|---|---|
| $FEV_1$ (L) | 4.7 ± 0.5 | 3.5 ± 0.3** |
| $FEV_1$ (%) | 101.3 ± 9.1 | 106.5 ± 8.5 |
| FVC (L) | 5.4 ± 0.6 | 3.9 ± 0.4** |
| FVC (%) | 97.3 ± 9,5 | 103.5 ± 6.0 |
| MVV (L) | 200.7 ± 26.5 | 156.0 ± 17.8* |
| MVV (%) | 106.0 ± 16.1 | 140.7 ± 17.5* |
| **Respiratory muscle strength** | | |
| MIP ($cmH_2O$) | 135.7 ± 32.3 | 98.2 ± 16.9* |
| MIP (%pred) | 117.0 ± 28.0 | 131.4 ± 27.0 |
| MEP ($cmH_2O$) | 188.3 ± 23.3 | 127.8 ± 19.1* |
| MEP (%pred) | 119.9 ± 14.7 | 134.9 ± 23.1 |
| **Respiratory muscle endurance** | | |
| $V_{E^{max}}$ ($L \cdot min^{-1}$) | 125.3 ± 26.9 | 73.3 ± 19.9* |
| $V_{E^{max}}$ (%MVV) | 62.2 ± 10.9 | 51.7 ± 14.7 |
| $MIP_{max}$ $cmH_2O$ | 92.9 ± 21.9 | 76.7 ± 14.9* |
| $MIP_{max}$ (%$MIP_{pro2}$) | 63.3 ± 10.0 | 68.3 ± 13.3 |

Values are means ± SD. $FEV_1$, forced expiratory volume in one second; FVC, forced vital capacity; MVV, maximal voluntary ventilation; MIP, maximal inspiratory pressure; MEP, maximal expiratory pressure. $V_{Emax}$, maximal ventilation minute during IHE test; $MIP_{max}$, maximal inspiratory pressure during IRB test. P values are * for $p < 0.05$ and ** for $p < 0.01$.

**Table 3.** Between-days reliability of parameters measured during the IHE test

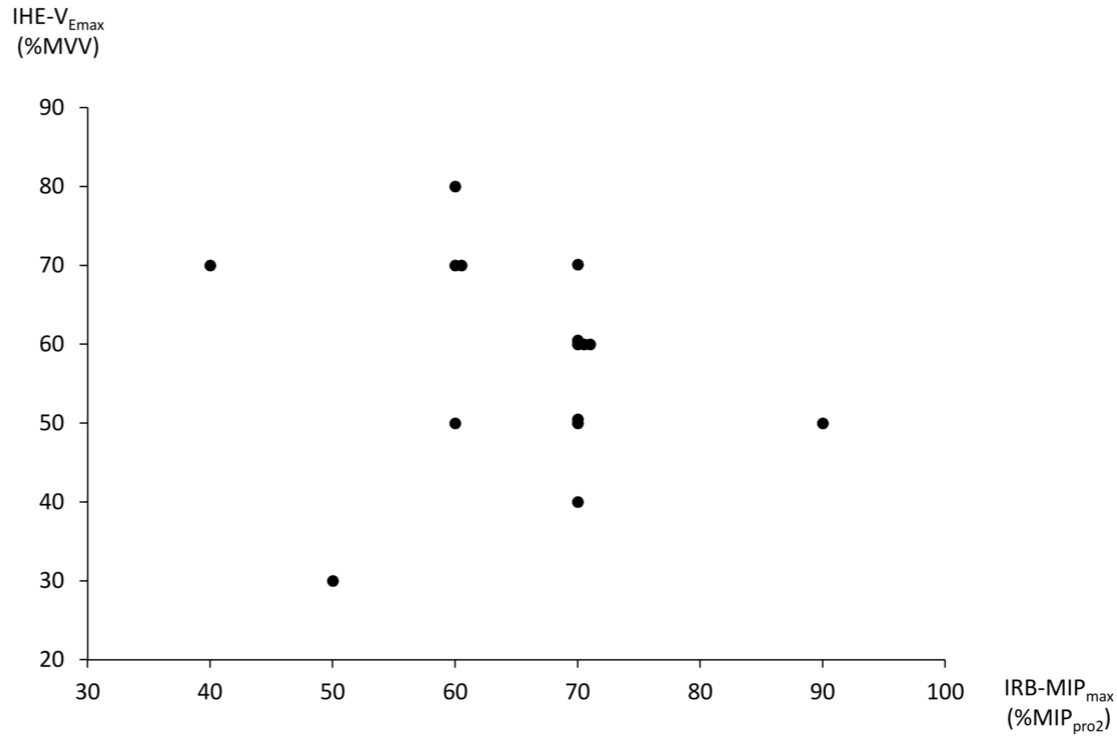
| | IHE 1 | IHE 2 | Mean Change (95% CI) | $CV_{TE}$ (95% CI) | ICC (95% CI) |
|---|---|---|---|---|---|
| $V_{Emax}$ ($L \cdot min^{-1}$) | 110.7 ± 32.3 | 107.5 ± 27.4 | -3.21 (-12.77-6.35) | 10.73 (7.78-17.29) | 0.80 (0.41-0.93) |
| Time to task failure (min) | 11.8 ± 3.7 | 12.4 ± 2.8 | 0.61 (-1.28-2.49) | 19.09 (13.80-30.74) | 0.67 (0.03-0.89) |
| Breathlessness sensation | 14.2 ± 3.8 | 13.7 ± 3.0 | -0.43 (-2.43-1.57) | 17.43 (12.64-28.24) | 0.67 (0.03-0.89) |
| RM exertion | 11 ± 4.3 | 11.1±3.3 | 0.14 (-1.93-2.22) | 22.88 (16.58-36.85) | 0.72 (0.14-0.91) |

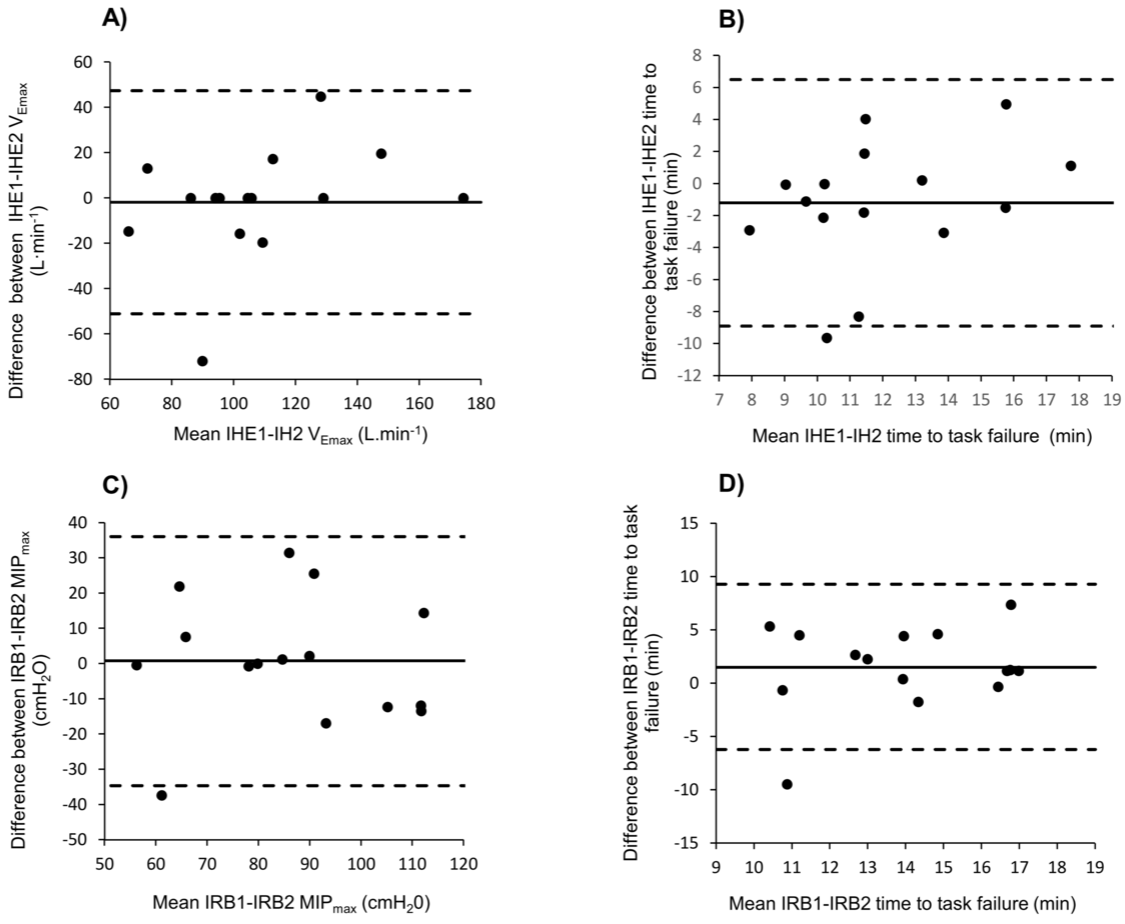
Values are means ± SD; IHE1 and IHE2: first and second isocapnic hyperpnea endurance tests; change in the mean are expressed in percentage of mean values; $CV_{TE}$: typical error expressed as a coefficient of variation; ICC: intraclass correlation coefficient; 95% CI: 95% confidence interval; $V_E$max: maximal minute ventilation; RM: respiratory muscle.

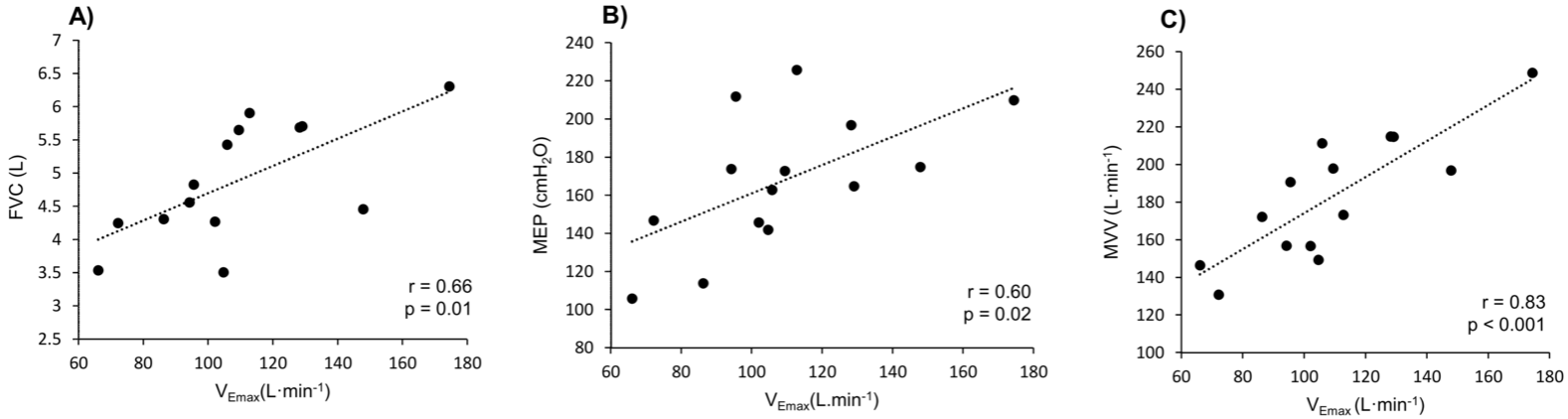
**Table 4.** Between-days reliability of parameters measured during the IRB test

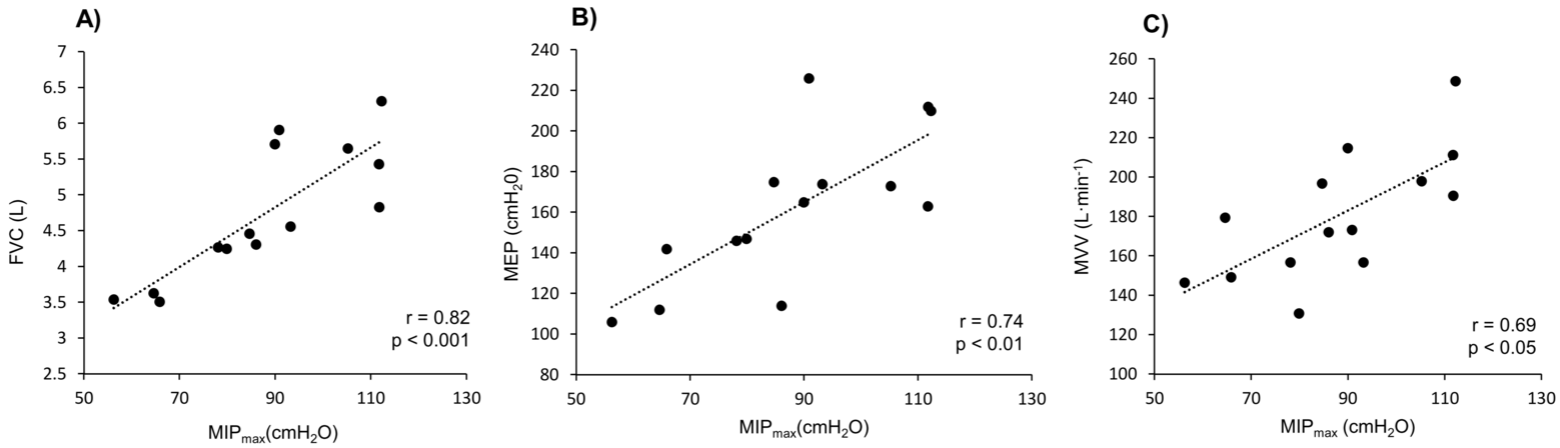
| | IRB 1 | IRB 2 | Mean Change (95% CI) | $CV_{TE}$ (95% CI) | ICC (95% CI) |
|---|---|---|---|---|---|
| $MIP_{pro2}$ | 135.1 ± 29.2 | 135 ± 22.9 | 0.07 (-7.51-7.37) | 6.75 (4.90-10.87) | 0.94 (0.80-0.98) |
| Number of inspirations | 98.9 ± 19.7 | 94.2 ± 18.2 | -4.64 (-15.12-5.83) | 13.3 (9.64-21.42) | 0.70 (0.08-0.90) |
| $MIP_{max}$ ($cmH_2O$) | 89.6 ± 17.2 | 86.1 ± 21.9 | -3.49 (-12.24-5.27) | 12.21 (8.85-19.67) | 0.83 (0.46-0.94) |
| Time to task failure (min) | 15.3 ± 2.6 | 13.0 ± 2.8 | -2.32 (-3.83-0.81) * | 13.03 (9.44-21) | 0.69 (0.03-0.90) |
| Breathlesness sensation | 8.4 ± 2.7 | 10.9 ± 3.8 | 2.50 (0.73-4.27) * | 22.5 (16.35-36.25) | 0.73 (0.15-0.91) |
| RM exertion | 12.7 ± 3.4 | 14.4 ± 3.0 | 1.71 (-0.24-3.67) | 17.57 (12.72-28.31) | 0.62 (-0.05-0.88) |

Values are means ± SD; IRB1 and IRB2: first and second inspiratory resistive breathing endurance tests; change in the mean are expressed in percentage of mean values; $CV_{TE}$: typical error expressed as a coefficient of variation; ICC: intraclass correlation coefficient; 95 % CI: 95% confidence interval; $MIP_{pro2}$: maximal inspiratory pressure measured at baseline with Pro2® device; $MIP_{max}$: maximal inspiratory pressure; RM: respiratory muscle. * significant difference between IRB1 and IRB2 ($p<0.05$)


IHE-$V_{Emax}$
(%MVV)
90
80
70
60
50
40
30
20
30
40
50
60
70
80
90
100
IRB-$MIP_{max}$
(%$MIP_{pro2}$)


A)
Difference between IHE1-IHE2 $V_{Emax}$ (L·min$^{-1}$)
Mean IHE1-IH2 $V_{Emax}$ (L.min$^{-1}$)
B)
Difference between IHE1-IHE2 time to task failure (min)
Mean IHE1-IH2 time to task failure (min)
C)
Difference between IRB1-IRB2 $MIP_{max}$ ($cmH_2O$)
Mean IRB1-IRB2 $MIP_{max}$ ($cmH_20$)
D)
Difference between IRB1-IRB2 time to task failure (min)
Mean IRB1-IRB2 time to task failure (min)


A)
FVC (L)
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
60
80
100
120
140
160
180
$V_{Emax}$(L·min$^{-1}$)
r = 0.66
p = 0.01
B)
MEP (cmH$_2$O)
240
220
200
180
160
140
120
100
80
60
80
100
120
140
160
180
$V_{Emax}$(L.min$^{-1}$)
r = 0.60
p = 0.02
C)
MVV (L·min$^{-1}$)
260
240
220
200
180
160
140
120
100
60
80
100
120
140
160
180
$V_{Emax}$ (L·min$^{-1}$)
r = 0.83
p < 0.001


A)
FVC (L)
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
50
70
90
110
130
MIP$_{max}$(cmH$_2$O)
r = 0.82
p < 0.001
B)
MEP (cmH$_2$0)
240
220
200
180
160
140
120
100
80
50
70
90
110
130
MIP$_{max}$(cmH$_2$O)
r = 0.74
p < 0.01
C)
MVV (L·min$^{-1}$)
260
240
220
200
180
160
140
120
100
50
70
90
110
130
MIP$_{max}$ (cmH$_2$O)
r = 0.69
p < 0.05