

Working title:

The influence of prior activity (warm-up) and inspiratory muscle training upon between and within day reliability of maximal inspiratory pressure measurement

Authors:

Lomax, M. & McConnell, A. K.

Contact details:

Dr Mitch Lomax

Department of Sport & Exercise Science

University of Portsmouth

Spinnaker Building

Cambridge Road

Portsmouth

PO1 2ER

Tel: 02392 845 297

Email: mitch.lomax@port.ac.uk

Professor Alison McConnell

Centre for Sports Medicine & Human Performance

Brunel University

Uxbridge

Middlesex

UB8 3PH

Tel: 01895 266 480

Email: alison.mcconnell@brunel.ac.uk

Corresponding author:

Dr Mitch Lomax, Department of Sport and Exercise Science, University of Portsmouth, Spinnaker Building, Cambridge Road, Portsmouth, PO1 2ER.

Abstract

Background: A specific inspiratory muscle ‘warm-up’ (IWU) prior to assessment of maximal inspiratory mouth pressure (P_Imax) may reduce the number of measurements required to obtain reproducible, representative estimates of P_Imax. The influence of inspiratory muscle training (IMT) upon this phenomenon is unknown. **Objective:** Compare the impact of an IWU on the between and within day reliability of P_Imax before and after IMT. **Method:** Eight participants were assessed on four separate occasions; two trials preceded IMT and two followed it. At each assessment, the highest of three initial efforts was recorded as the pre-IWU value (PI). The highest of 9 subsequent efforts that followed two sets of 30 breaths at 40% PI was recorded as P_Imax. Following 4 weeks of IMT the trials were repeated. **Results:** IWU increased PI by 11-17% ($p \leq 0.01$) irrespective of IMT status. After IWU, five to six efforts were required to determine P_Imax, irrespective of IMT status. P_Imax was similar between the two trials pre-IMT and the two trials post-IMT ($p \geq 0.05$), and was 21% higher post-IMT ($p \leq 0.01$). The coefficient of variation was excellent pre- and post-IWU, both before (1.9% and 0.6%, respectively) and after IMT (1.1 % and 0.3%, respectively). Limits of agreement and sample sizes for effect sizes $\leq 10\%$ were substantially smaller post-IWU in all trials. **Conclusions:** 1) IWU enhances the between day reliability of P_Imax measurement, and this is unaffected by IMT, 2) judgements regarding acceptability in relation to P_Imax reliability should be made in relation to analytical goals and we present data to facilitate this.

Key words: maximal inspiratory pressure, warm-up, inspiratory muscle training.

Introduction

Assessment of inspiratory muscle pressure can broadly be divided into effort-dependent and effort-independent tests [see ref 1 for a review of this area]. Although there are advantages and disadvantages to both, volitional methods, specifically mouth pressure measures of maximal inspiratory pressure (P_Imax) i.e. Mueller manoeuvres, are simple and quick to perform in both laboratory and field based settings. They are also non-invasive and demonstrate good fidelity with intra-thoracic pressure [1]. It is therefore not surprising that P_Imax is favoured in situations where a more holistic evaluation of inspiratory muscle function is required [2].

However, within trial maxima and/or between day P_Imax are affected by the number of efforts performed [3, 4], and this has led to P_Imax being disregarded as a meaningful assessment of inspiratory muscle function [5]. We believe that this is unfortunate, because P_Imax is one of only two truly holistic measures of inspiratory muscle function available, and it is also the only measure of function that is reflective of both the central and peripheral factors that influence inspiratory muscle function. Our group has previously suggested that the use of a specific inspiratory muscle ‘warm-up’ (IWU) reduces the number of measurements required in order to obtain reproducible, representative estimates of P_Imax [6].

The mechanistic basis for the increase in P_Imax observed post-IWU is yet to be resolved, but a number of suggestions have been made. Specifically an IWU: 1) may accelerate the task learning effect associated with repeated Mueller manoeuvres [4, 6], 2) may increase peripheral excitability [7], 3) may increase the synergy between active inspiratory muscles [8], or 4) may exert its influence by a combination of these factors. Whatever the cause(s) at least some of the variability of routine P_Imax measurements between trials reflects the failure to minimise the influence of these

factors. If this is the case it is reasonable to postulate that the between day reliability of P_Imax, and the number of efforts required to elicit P_Imax (rather than a submaximal value (P_I)), will be improved by the use of an IWU. In addition, if the effect of the IWU were primarily task learning and enhanced synergy between active inspiratory muscles, then one would predict that the effect of an IWU would diminish after a period of inspiratory muscle training (IMT) [9].

Thus, the present study sought to compare the influence of an IWU on the between and within day reliability of P_Imax, as well as the effect of IMT upon between and within day reliability of P_Imax.

Methods

Participants

Following ethics committee approval from the Institutional Review Board at Brunel University and informed written consent, eight healthy, active participants (7 females and 1 male) volunteered for the study. Mean \pm standard deviation (SD) for age was 29.1 ± 6.3 years, height 167.4 ± 6.1 cm, and body mass 73.3 ± 13.1 kg.

Procedure

Following two habituation trials each participant took part in a series of 4 identical trials on separate days. During habituation subjects were required to perform a series of P_Imax manoeuvres. Each subject's technique was deemed proficient when the highest of three manoeuvres [1] was within 5 cmH₂O of one another [10]. Trials 1 and 2 were undertaken before a four week period of IMT, and trials 3 and 4 post-IMT (one subject did not complete trial 2); all testing took place within a total of 6-7

weeks. For all participants, P_{Imax} was measured using a calibrated hand held mouth pressure meter (Micro Medical, Rochester, UK) from residual volume using a flanged PVC mouthpiece (P.K. Morgan Ltd, Gillingham, UK), and with the nose occluded.

The highest of three Mueller manoeuvres pre-IWU was recorded as PI. Immediately following this, an IWU was administered according to the methods of Volianitis et al. [6]. Briefly, a spring-loaded threshold inspiratory muscle trainer (POWERbreathe®, Gaiam Ltd, UK) was used to administer the IWU consisting of two sets of 30 breaths at 40% PI. The first bout of 30 breaths was followed by a 60-second rest during which a single PI was undertaken; after which, the second bout of 30 breaths was completed. The interim PI permitted the resistance on the inspiratory muscle trainer to be adjusted to 40% of the new PI, as appropriate [6, 10].

Following the IWU each participant undertook an additional series of 9 Mueller manoeuvres (based on Volianitis et al. [6]). As with pre-IWU, all efforts were undertaken whilst seated and 60-seconds rest separated each effort. Overall (pre- and post-IWU) a total of 12 Mueller manoeuvres were performed.

Following the completion of trials 1 and 2, participants undertook 4-weeks of pressure threshold IMT (POWERbreathe®, Gaiam Ltd, UK). Participants completed 2 sets of 30 repetitions daily at a load equivalent to 50% of the highest post-IWU P_{Imax} [11-13]. To set-up the inspiratory muscle trainer with the starting load, a 0.8 mm diameter needle was inserted into a flanged PVC mouthpiece connected to an inspiratory muscle trainer, which in-turn was connected to a mouth pressure meter (Morgan, Precision Medical, UK). This set-up allowed the inspiratory muscle trainer to be set at 50% of P_{Imax} by adjusting the tension of the spring until the valve opened at the correct pre-determined pressure. Each week the tension on the inspiratory muscle trainer was increased to ensure the load continued to reflect ~50% of each

participant's P_Imax. Between these weekly assessments, participants were instructed to increase the tension in the spring when the subjective load during the last five breaths of the thirty no longer felt progressively harder. Each participant completed an IMT diary so that adherence and training load progression could be monitored.

Statistical analysis

A two-way repeated measure ANOVA (condition x trial) with Bonferroni adjustment was used to assess the impact of IWU (condition) and IMT training status (trial) on P_I and P_Imax. Where significance was found, planned comparisons were conducted using one-way repeated measure ANOVAs with Bonferroni adjustments. A paired t-test was used to assess the impact of IWU on each individual trial and a one-way repeated measure ANOVA compared the number of efforts required to achieve the highest P_Imax post-IWU between trials. These analyses were conducted using version 15 of SPSS (SPSS Inc, Chicago Ill, USA). Significance was set at $P \leq 0.05$.

As heteroscedasticity was evident in P_Imax (i.e. the magnitude of random error was related to the mean value of P_Imax), ratio limits of agreement were used to assess the bias or systematic error (general learning effect) and random error (biological, mechanical variation and chance factors) in P_I and P_Imax between trials [14, 15]. 95% confidence intervals (CI) for bias and random error were calculated to determine worst case scenarios for P_I and P_Imax, as well as to estimate the sample sizes required for 1%, 5%, 10%, 20% and 30% effect sizes with a power of 0.9, using an Excel spreadsheet based on the calculations of Zar [16]. Worst case scenario data were calculated based on the lowest ((bias ÷ agreement ratio)) x P_I or P_Imax) and highest ((bias x agreement ratio)) x P_I or P_Imax) values [17] pre- and post-IWU. Using logarithmically transformed data, the coefficient of variation (CV) for P_I and P_Imax, pre- and post-IMT, was calculated as the standard deviation of the differences

between pre- and post-IWU divided by the mean of PI/PI_{max}, multiplied by 100 [14]. In addition, to assess the mean difference and relative consistency (i.e. ordering) of PI_{max} values over repeated measures, intraclass correlation coefficients (ICC) (2-way random model) were determined using the methods of Vincent [18]. PI and PI_{max} values were separated into 4 blocks of 3 efforts: block 1 = efforts 1-3; block 2 = efforts 4-6; block 3 = efforts 7-9; block 4 = efforts 10-12 (therefore efforts 1-3 occurred pre-IWU and efforts 4-12 occurred post-IWU). ICCs were calculated: 1) pre-IWU between trials 1 and 2, and between trials 3 and 4; 2) post-IWU between trials 1 and 2, and between trials 3 and 4; and 3) blocks 1-4 per trial. This permitted between and within trial comparisons. Significance was set at $P \leq 0.05$.

Results

The recorded PI/PI_{max} was significantly different between trials ($P = 0.000$) and between conditions ($P = 0.000$). An interaction effect between trial was also observed ($P = 0.002$) with PI_{max} being 21% higher post-IMT (reported inspiratory muscle training compliance was excellent (95%)). Further analysis revealed that trials 1 and 2 (both pre-IMT) were similar pre- ($P = 1.000$) and post-IWU ($P = 1.000$), with trials 3 and 4 (both post-IMT) also being similar pre- ($P = 1.000$) and post-IWU ($P = 0.126$) (see table 1).

The IWU significantly increased PI ($P \leq 0.01$) in all trials i.e. pre- and post-IMT (trial 1: $t = -7.648$, $P = 0.000$; trial 2: $t = -6.871$, $P = 0.000$; trial 3: $t = -10.089$, $P = 0.000$; trial 4: $t = -13.137$, $P = 0.000$) (see table 1) and the magnitude of improvement was similar between trials (trial 1: $11.6 \pm 9.3\%$; trial 2: $17.3 \pm 8.0\%$; trial 3: $16.4 \pm 5.9\%$; trial 4: $14.4 \pm 4.9\%$; $P = 0.207$). In addition, the number of attempts required to attain PI_{max} (i.e. post-IWU PI values) was similar between trials (trial 1: 6 ± 1.7

attempts; trial 2: 5 ± 2.7 attempts; trial 3: 6 ± 3.1 attempts; trial 4: 6 ± 1.9 attempts; $P = 0.909$).

The IWU also reduced (but did not abolish) heteroscedasticity, and yielded better limits of agreement, irrespective of IMT status (see table 2). Consequently, when an IWU preceded P_{Imax} determination, the sample size required for an effect size of between 1-10% was reduced (see table 3). Table 4 depicts worst-case scenario data calculations, based upon the bias and random error estimates. It is clear from these data there is an improvement in between day reliability when measurements are preceded by the IWU.

Intraclass correlation coefficients were excellent for each block per trial i.e. within trial, varying between 0.981 and 0.996. Although between trial ICCs and CVs were very good, there was a non-significant trend for these to be better post-IWU (see table 1).

Discussion

The aim of this study was to determine whether an IWU and/or IMT, enhanced the reliability of P_{Imax} assessment. The main findings were; 1) the data confirmed that of previous studies showing that the inclusion of an IWU increased PI by 11-17%, but generated the new finding that this increase was independent of IMT status; 2) although P_{Imax} was increased up to 21% post-IMT, IMT status had no impact upon either the number of attempts required to attain P_{Imax} (between 5 and 6), or the sample size estimates for significant effects; 3) CVs and ICCs were slightly enhanced post-IWU, although they were deemed excellent by statistical standards, irrespective of IWU and IMT status; 4) the limits of agreement were substantially narrower post-

IWU; and 5) the sample sizes necessary to detect effect sizes of 1-10% were substantially reduced post-IWU, irrespective of IMT status.

The 11-17% increase in PI post-IWU is similar to that reported by others [6-8, 19, 20]. Although we cannot delineate the precise mechanisms behind this increase, we have previously suggested that motor unit activation increases post-IWU [7, 8] and inspiratory muscle coordination is enhanced such that synergy between the diaphragm and accessory inspiratory muscles is increased [8]. An alternative explanation would be the negation of a learning effect following the IWU [4, 6]. The fact that the IWU generated similar changes pre- and post-IMT suggests that the most likely explanation for the observed enhancement of PI is neurophysiological [7], rather than being the result of task or motor learning. Had the latter made a contribution to the improvements in PI, we would expect the efficacy of the IWU to diminish after a period of IMT, in line with the expression of these factors that occurs following strength training [9].

Our data suggest that it takes 5-6 attempts to obtain P_{Imax} post-IWU, irrespective of IMT status. Although this is greater than that reported by Volianitis et al. [6] and Hawkes et al. [8], who both reported reliable measurements in the first attempt post-IWU, attainment of P_{Imax} should not be inferred from attainment of reliability. For example, Aldrich & Spiro [21] reported that PI (50% of P_{Imax}) and P_{Imax} values could be reliably reproduced using a criterion of the best of three efforts within 5% or 5 cmH₂O, which is the traditional criterion adopted in the respiratory muscle literature for such measurements [7, 8, 10, 11, 22, 23]. We observed excellent within trial ICCs in the first three manoeuvres preceding IWU i.e. block 1 (0.988-0.996) and the first three manoeuvres after IWU i.e. block 2 (0.981-0.995); however, the highest PIs were not observed until block 3 (attempts 4-6 post-IWU) (ICCs of 0.988-0.996).

Such observations raise interesting questions regarding how reliability is defined in respiratory muscle strength assessment and the criteria that should be adopted. For example, in the study of Aldrich & Spiro [21] the within trial CV was substantially lower during maximal efforts than sub-maximal efforts (6% vs. 13%, respectively) despite reliability being deemed present using the traditional criteria of $\leq 5\%$ or 5 cmH₂O. Although we observed slightly better between trial CVs and ICCs post-IWU vs. pre-IWU (see table 1), the CVs and ICCs were excellent [see refs 18 & 24], with and without IWU. This occurred despite a mean increase of 12% (mean of trials 1 and 2) to 15% (mean of trials 3 and 4) following IWU.

Ultimately, the question regarding acceptable or unacceptable levels of reliability needs to be placed in the context of the analytical goals. For example, inspiratory muscle fatigue has been shown to result in a decline in P_{Imax} of 5-30% following a range of exercise modes, durations and intensities [12, 22, 25-31]. If the baseline measure of PI is 10% lower than P_{Imax}, and the study is designed to examine a phenomenon likely to result in a reduction in P_{Imax} of 10%, then the underestimation of baseline P_{Imax} has significant implications for data interpretation.

By using analytical goals as one's guide, it is possible to determine whether the limits of agreement are sufficiently narrow for practical purposes, and hence whether a given level of variability, combined with the proposed sample size, and a given effect size, are acceptable or not [14]. By calculating random error (e.g. chance and measurement factors) and bias (e.g. learning effects) we were able to construct worst-case scenario values for PI and P_{Imax} and, in turn, to determine the sample size required for a given effect size [14,17].

It should be noted that we did not use the standard error of measurement (SEM), which is a common method of assessing absolute reliability. This is because, unlike the CV, the SEM assumes the absence of heteroscedasticity [see refs 14 & 24 for a

review of these issues]. The presence of heteroscedasticity in our data is a caution against selecting ICCs to determine relative reliability. This is because high levels of between participant variability can mask large between trial variability [15]. Although we observed excellent between and within trial ICCs, it should be noted that the range of PImax values observed between participants was quite high (see table 4), and this likely enhanced the ICCs.

In the present study random error was reduced (and to a greater extent than bias) by the IWU, irrespective of IMT status (see table 2). As random error reflects the magnitude of difference in PI/PImax per comparison, fewer participants were therefore required to observe a change in PI post-IWU [14]. Unsurprisingly, the 95% CIs were substantially narrower post-IWU (see table 2). Specifically, when the worst case scenarios were expressed as a percentage of the highest or lowest PImax value, the possible range post-IWU was approximately one quarter of that observed pre-IWU at both the lower and higher ends irrespective of IMT status (see table 4). Consequently, the estimated sample size was substantially reduced from an effect size of $\leq 10\%$ (see table 3).

Only one other study [17] has used limits of agreement to evaluate the between day reliability of PI. However, this study did not include an IWU as part of the assessment procedure. Although they reported less bias (0.977) than that observed in the present study (1.007-1.016), the random error was larger than that observed in the present study (1.051 vs. 1.028 in the present study) demonstrating less agreement. As a result, the sample size required for effect sizes of 1% and 5% was smaller in the present study, but only after the IWU (68 vs. 22 and 3 vs. 1, respectively).

Our data indicate that with effect sizes in excess of 10% an IWU has no impact on sample size. As inspiratory muscle strength may increase by around 19-44% following pressure threshold IMT [12, 13, 22, 23, 32-35], it may be argued that the

inclusion of an IWU (in fully habituated participants) is not necessary because the effect size is very large. However, IMT studies to date have focused on examining the influence of a single regimen of IMT, typically with a training load of 50-60% P_Imax. Future studies might examine subtle differences elicited by different training regimens. Under these conditions, more precise estimates of PI may be required in order to detect correspondingly smaller magnitude effects between training conditions. Our data suggest that the use of IWU would enhance the prospects of detecting such differences.

In conclusion, we have shown that in healthy participants who are fully habituated to performing Mueller manoeuvres, it is possible to obtain P_Imax following 5 to 6 efforts post-IWU, irrespective of IMT status. Although we were unable to delineate the mechanistic basis for the increase in PI post-IWU (or post-IMT), our results suggest that it is not due to task or motor learning. The data also demonstrate that using an IWU creates narrower limits of agreement for PI, which may be of great importance in studies seeking to identify small effect sizes. These data support the benefits of administering an IWU prior to the determination of baseline P_Imax, and confirm that judgements regarding the reliability of the resulting measurements be viewed in relation to analytical goals.

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Table 1. Pre- & post-IMT trial-re-trial data for PI & P_Imax pre- & post-IWU: mean, standard deviation (cmH₂O) & between trial CV (%) & ICC

PI/PImax	Trial 1	Trial 2	Mean difference	CV	ICC
Pre-IMT					
Pre-IWU	122 ± 30.3	117 ± 32.5	5.13 ± 9.8	1.9	0.971
Post-IWU	133 ± 29.2 ^{**}	135 ± 28.6 ^{**}	-2.00 ± 3.0	0.6	0.996
Post-IMT					
Pre-IWU	141 ± 29.1 [†]	141 ± 31.9 ^{††††}	-0.13 ± 7.2	1.1	0.901
Post-IWU	161 ± 29.6 ^{**††††}	163 ± 29.94 ^{**††††}	-2.0 ± 2.5	0.3	0.990

^{**}($P \leq 0.01$) different to pre-IWU, ^{††}($P \leq 0.01$) different to trial 1, ^{†††}($P \leq 0.01$) ($P \leq 0.05$) different to trial 2

Table 2. Ratio limits of agreement for pre- & post-IMT PI & PImax: log transformed

	Bias Ratio	SE ^a	95% CI ^b	Random error Ratio	SE ^a	95% CI ^b	Lower L oA ^c	Upper LoA ^c
Pre-IWU								
Trials 1 & 2	1.034	0.016	0.000-0.068	1.099	0.017	0.905-0.977	1.100	1.173
Trials 3 & 4	1.002	0.019	-0.037-0.042	1.116	0.020	0.857-0.940	1.076	1.160
Post-IWU								
Trials 1 & 2	1.007	0.005	-0.003-0.017	1.028	0.005	0.968-0.990	1.025	1.046
Trials 3 & 4	1.016	0.005	0.006-0.026	1.028	0.005	0.978-0.999	1.034	1.055

Note: ^astandard error; ^bconfidence interval, ^climits of agreement,

Table 3. Estimated sample size for effects of 1-10%, pre- & post-IWU on PI & PImax

	1%	5%	10%
Pre-IWU			
Trials 1 & 2	267	11	3
Trials 3 & 4	365	15	4
Post-IWU			
Trials 1 & 2	22	1	1
Trials 3 & 4	22	1	1

Table 4. Worst-case scenario data for PI & PImax (cmH₂O) based on the lowest & the highest PI/PImax values per comparison

Comparison	Lowest	Highest	Possible range		Possible range (% difference)	
			lowest	highest	lowest	highest
Pre-IWU						
Trials 1 & 2	95	189	89-108	178-215	21.3	20.8
Trials 3 & 4	112	199	101-125	179-223	23.8	24.6
Post-IWU						
Trials 1 & 2	109	196	107-113	192-203	5.6	5.7
Trials 3 & 4	135	220	133-141	217-230	6.0	6.0