Sources of Variability in Musculo-Articular Stiffness Measurement

Massimiliano Ditroilo\(^1,2\), Mark Watsford\(^3\), Aron Murphy\(^3\), Giuseppe De Vito\(^1,4\)

\(^1\) School of Public Health, Physiotherapy and Population Science, University College Dublin, Dublin, Ireland, \(^2\) Department of Sport, Health and Exercise Science, Faculty of Science, University of Hull, Hull, United Kingdom, \(^3\) Sport and Exercise Group, UTS: Health, University of Technology, Sydney, Australia, \(^4\) Institute for Sport and Health, University College Dublin, Dublin, Ireland

**Abstract**

The assessment of musculo-articular stiffness (MAS) with the free-oscillation technique is a popular method with a variety of applications. This study examined the sources of variability (load applied and frequency of oscillation) when MAS is assessed. Over two testing occasions, 14 healthy men (27.7±5.2 yr, 1.82±0.04 m, 79.5±8.4 kg) were measured for isometric maximum voluntary contraction and MAS of the knee flexors using submaximal loads relative to the individual’s maximum voluntary contraction (MAS\(_{\text{MVC}}\)) and a single absolute load (MAS\(_{\text{ABS}}\)). As assessment load increased, MAS\(_{\text{MVC}}\) (coefficient of variation (CV) = 8.1–12.1%; standard error of measurement (SEM) = 51.6–98.8 Nm\(^{-1}\)) and frequency (CV = 4.8–7.0%; SEM = 0.060–0.075 s\(^{-1}\)) variability increased consequently. Further, similar levels of variability arising from load (CV = 6.7%) and frequency (CV = 4.8–7.0%) contributed to the overall MAS\(_{\text{MVC}}\) variability. The single absolute load condition yielded better reliability scores for MAS\(_{\text{ABS}}\) (CV = 6.5%; SEM = 40.2 Nm\(^{-1}\)) and frequency (CV = 3.3%; SEM = 0.039 s\(^{-1}\)). Low and constant loads for MAS assessment, which are particularly relevant in the clinical setting, exhibited superior reliability compared to higher loads expressed as a percentage of maximum voluntary contraction, which are more suitable for sporting situations. Appropriate sample size and minimum detectable change can therefore be determined when prospective studies are carried out.

**Introduction**

Musculo-articular stiffness (MAS) measured with the free-oscillation technique is a comprehensive measurement of joint stiffness which includes the stiffness of the muscle-tendon unit, skin, ligaments and articular capsule, along with a number of other mechanical and neuromuscular factors [1]. The assessment of MAS has implications for muscular performance, injury occurrence and gender differences [1]. The construct validity of the free-oscillation technique has been ascertained with a positive linear relationship between MAS and rate of torque development [2] and a negative relationship between MAS and either electromechanical delay [3] or performance augmentation (as a result of the pre-stretch action) [4]. Reliability of the method has also been established in a number of papers, with an overall level of absolute reliability higher than the relative reliability [1].

When assessing MAS with the free-oscillation technique, the joint is modelled as a single-degree of freedom spring-mass system with a damping element, with an assumption of linearity of the model [5,6]. Despite some evidence of nonlinearity of the damped acceleration signal [7], the vast majority of studies using the free-oscillation technique adopted a linear model, which is easier to use and has been granted construct validity [1]. The stiffness value is obtained as follows [4,6]:

\[
k = m \left(4\pi^2 f^2 + \gamma^2\right) \quad \text{(eq.1)}
\]

where \(k\) is the MAS (N·m\(^{-1}\)), \(m\) is the load supported (kg), \(f\) is the damped natural frequency (s\(^{-1}\)) and \(\gamma\) is the coefficient of damping (s\(^{-1}\)). It was previously demonstrated that \(\gamma\) is negligible as it contributes less than 1% to the total stiffness value [8]. Therefore, eq. 1 can be approximated as:

\[
k = m \cdot 4\pi^2 f^2 \quad \text{(eq.2)}
\]

Accordingly, \(k\) varies linearly with mass and exponentially with frequency variation.

While frequency is a measured variable which depends on the elastic characteristics of the structure assessed [9], mass is the load added, along with the weight of the body segment under analysis. Typically, either fixed loads or multiple loads expressed as a percentage of maximal voluntary contraction (MVC) are utilised with the aim of reproducing the loads supported during functional activities [3]. Specifically, if an investigation applies a repeated-measures design, absolute assessment loads may be appropriate [10]. Conversely, when comparing MAS between individuals, relative loads must be used to prevent bias due to differences in mass and strength, as occurs when comparing males and females.
[8], subjects of different body mass [11] or athletes of different levels [2].

By virtue of equation 2, it is evident that a component of any change in stiffness originates from a change in frequency, while an additional element comes from the variability in MVC assessment and its consequent use in determining the submaximal load. The latter is exacerbated when MAS is measured prior to and following an intervention which has probably altered the level of MVC [12,13].

Whilst two studies have identified the issue of a dual source of error [3,14], the proportional contribution of each component to the overall MAS variability at varying loads has yet to be established. Accordingly, the aim of this study was to determine and quantify the sources of variance when MAS is assessed with multiple loads relative to MVC, or a constant absolute load.

Methods

Fourteen men (27.7±5.2 yr, 1.82±0.04 m, 79.5±8.4 kg), physically active but not involved in competitive sport, volunteered to participate in this study. They gave written informed consent and avoided any strenuous physical activity 24 hours prior to each testing session. Ethical clearance was granted from the Ethical Committee of University College Dublin (Ireland). Two series of tests, separated by at least 2 weeks, were carried out and administered randomly, both requiring 2 sessions within 7 days. Participants were tested for MVC of the knee flexors (KF) and MAS using submaximal loads relative to the individual’s MVC (MAS%MVC) over two sessions. In a further two sessions the participants were tested for MAS using a single, absolute load (MASABS). MAS of the KF has been commonly assessed due to its use for later analysis. The absence of bursts of electrical activity of the muscles (measured via surface electromyography of biceps femoris), along with the presence of damped oscillations were used to assist in judging an acceptable trial.

The acceleration signals were filtered using a fourth order Butterworth filter with a cut-off frequency of 4 Hz, and the frequency of the first cycle of oscillations was determined along with the average frequency of the four trials, which was considered for later analysis. For the two sessions of MAS%MVC assessment, loads of 15, 30, 45, 60% of the specific MVC measured on each day were used in a non-randomized order. At the two constant load sessions to assess MASABS, a load of 6.5 kg was applied as described above. This load corresponded to approximately 35% of MVC.

Results are expressed as mean ± SD. Coefficient of variation (CV) of load, frequency and MAS variables were calculated as:

\[ \frac{s}{\text{mean}} \times 100 \] (eq.3)

where \( s \) is the standard deviation and mean is the mean of the two results obtained on testing session 1 and 2. Standard error of measurement (SEM) was also calculated as

\[ s \sqrt{1 - ICC} \] (eq.4)

where \( s \) is the square root of the total sum of squares divided by ‘number of observations - 1’ (as in an ANOVA analysis) and ICC is the intraclass correlation coefficient. Confidence limits of CV and SEM were also determined [17,18]. Repeated measure ANOVA was used to examine the difference between CVs obtained at various loads (15, 30, 45, 60% of MVC and constant load) for two dependent variables (frequency and MAS). When a significant effect was found, the post-hoc Tukey’s method was used to identify where significant differences lay.

Systematic bias between testing sessions was analyzed using a paired t-test and an alpha level of \( p<0.05 \) was considered statistically significant for all tests.

The calculations related to the propagation of errors (uncertainties that attend all measurements) [19] were used to assess the theoretical specific contribution of variation related to mass (CVm) and frequency (CVf) to the overall MAS variation (CVMAS). With reference to equation 2, the error related to \( k \), \( m \) and \( f \) are \( \delta k \), \( \delta m \) and \( \delta f \), respectively, whereas there is no error associated to \( 4\pi^2 \). Since \( \delta m \) and \( \delta f \) are independent from each other, \( \delta k \) can be estimated as follows [19]:

\[ \frac{\delta k}{k} \approx \sqrt{\left(\frac{\delta m}{m}\right)^2 + \left(\frac{2\delta f}{f}\right)^2} \] (eq.5)

As \( \delta k/k \), \( \delta m/m \), and \( \delta f/f \) are percentage errors, CV can be substituted into equation 5 to obtain:

PLOS ONE | www.plosone.org 2 May 2013 | Volume 8 | Issue 5 | e63719
The statistical analysis was performed using Microsoft Office Excel® 2007 and Statistica software, version 9.1 (StatSoft LTD, Bedford, UK).

**Results**

The mean ± SD of MVC for KF was 180.6 ± 42.7 and 192.1 ± 49.9 N on testing session 1 and 2, respectively, with no significant difference (p = 0.15). No systematic bias was detected (p>0.05) in any of the variables considered. The results of load, frequency and MAS from the two testing sessions, along with the reliability coefficients, are summarized in tables 1, 2 and 3, respectively. Notably, while CV was constant for the load variable (6.7%), MAS (table 3) and frequency (table 2) CV increased as load increased, however the ANOVA post-hoc analysis did not show a significant difference (p = 0.15 to 0.44). In contrast, when a constant load was used, CV was considerably lower in MAS (table 3) and frequency (table 2) variables compared to multiple loads and the post-hoc analysis revealed to be significant different from MAS%MVC60% (6.5 vs 12.6% and 3.3 vs 7.0%, respectively; p<0.05). A similar pattern was observed for SEM, which increased as load increased for all variables, though when a constant load was used it was reduced, on average, by 33% (0.039 vs 0.060 s⁻¹) to 48% (0.039 vs 0.075 s⁻¹) (frequency, table 2) and by 22% (40.2 vs 51.6 Nm⁻¹) to 59% (40.2 vs 90.8 Nm⁻¹) (MAS, table 3).

Further, the load CV (table 1) was slightly higher than the frequency CV (table 2) at low loads, whereas the trend was reversed at high loads (i.e. 60% of MVC).

**Discussion**

This study quantified the contribution of variance in each component of MAS assessment. Although no statistical differences were detected between the two testing sessions for the variables examined, the current assessment procedures exhibited a CV of up to 12.6% for MAS. This magnitude of variability is certainly relevant when longitudinal changes in MAS are examined with reference to injury occurrence [15,20], training [12] or fatigue [13]. Specifically, when conducting longitudinal or comparative research designs where stiffness is hypothesized to be influenced by a particular intervention, condition or timeframe, the identification of this variability dictates the required magnitude of change for a meaningful outcome. The level of variability in this study is slightly higher than that reported for different musculature which required different set-ups [4,21]. This is probably due to the specific set-up needed for the assessment of the knee flexors, which is undoubtedly less comfortable for the participants than the assessment of the ankle flexors or the knee extensors.

The repeatability of the MVC measurement affects the assessment load added to the system, which consequently contributes variance to the MAS%MVC. Further, variability in the frequency of oscillation also affects the precision of MAS%MVC results. Based on equation 4, it can be empirically proven that with the level of variability reported in this study (8 to 12.5%), CVMAS is calculated as approximately the sum of CVF and CVm, despite CVF being multiplied by 2. The minor discrepancy between the reported results and the theoretical equation is likely due to the
That despite the apparent trend, CVMAS and CVf did not reach statistical significance and this can be attributed to the high inter-subject variability as expressed by the confidence limits for CV and SEM.

The fact that the tables only report mean values which exhibit medium to large confidence limits. CVMAS and CVf exhibited a clear trend towards an increase as the assessment load increased and this could at least be partially explained as an impaired ability to maintain a stable position and the augmented physiological tremor that could contribute to variance in equation 2. As depicted in figure 4, under such conditions the CVMAS was calculated as the CVf x 2. Interestingly, the use of a constant load yielded a very good level of reliability in frequency and MASABS (tables 2 & 3), noticeably higher than that reported for the multiple load assessment and statistically significant when compared to a load corresponding to 60% of MVC. The lower variability can conceivably be attributed to the more standard conditions of testing administration for MASABS, which probably elicited more stable responses from the subjects. Further, the MAS%MVC testing sessions were far longer and involved a number of measurements that fatigue may have increased the overall variability of MAS%MVC% compared to MVC ABS, as emerged in a recent examination of sub-maximal force up to 60% of MVC [24].

Table 1. Summary of the loads corresponding to different percentages of maximal voluntary contraction, along with a constant load of 6.5 kg, adopted during the two testing sessions.

| Mean (SD) (kg) | Reliability coefficients |
|---------------|--------------------------|
| T1            | T2           | CV (%) | 95% CL | SEM (kg) | 95% CL |
| MAS%MVC15%    | 2.76 (0.65)  | 2.94 (0.76) | 6.7 | 3.5–9.8 | 0.19 | 0.13–0.31 |
| MAS%MVC30%    | 5.52 (1.31)  | 5.88 (1.53) | 6.7 | 3.5–9.8 | 0.37 | 0.26–0.62 |
| MAS%MVC45%    | 8.28 (1.96)  | 8.81 (2.29) | 6.7 | 3.5–9.8 | 0.56 | 0.39–0.94 |
| MAS%MVC60%    | 11.04 (2.61) | 11.75 (3.05) | 6.7 | 3.5–9.8 | 0.74 | 0.52–1.26 |
| MASABS        | 6.5          | 6.5       | \    | \       | \    | \       |

Coefficients of reliability are also displayed.

MAS%MVC = musculo-articular stiffness measured with a load corresponding to a percentage of maximal voluntary contraction; MASABS = musculo-articular stiffness measured with a constant absolute load; T1, T2 = testing session number 1 and 2; CV = coefficient of variation; SEM = standard error of measurement; CL = confidence limits for CV and SEM.

doi:10.1371/journal.pone.0063719.t001

Table 2. Summary of the frequency results obtained with a load corresponding to different percentages of maximal voluntary contraction, along with a constant load of 6.5 kg, over two testing sessions.

| Mean (SD) (s⁻¹) | Reliability coefficients |
|-----------------|--------------------------|
| T1              | T2           | CV (%) | 95% CL | SEM (s⁻¹) | 95% CL |
| MAS%MVC15%      | 1.615 (0.163) | 1.622 (0.168) | 4.8 | 2.8–6.7 | 0.060 | 0.040–0.114 |
| MAS%MVC30%      | 1.570 (0.162) | 1.530 (0.179) | 5.2 | 3.3–7.1 | 0.065 | 0.046–0.111 |
| MAS%MVC45%      | 1.407 (0.156) | 1.414 (0.124) | 6.4 | 3.9–8.8 | 0.071 | 0.051–0.118 |
| MAS%MVC60%      | 1.392 (0.093) | 1.343 (0.116) | 7.0 | 3.9–10.1 | 0.075 | 0.048–0.165 |
| MASABS          | 1.462 (0.104) | 1.442 (0.125) | 3.3* | 2.1–4.5 | 0.039 | 0.029–0.060 |

Coefficients of reliability are also displayed.

MAS%MVC = musculo-articular stiffness measured with a load corresponding to a percentage of maximal voluntary contraction; MASABS = musculo-articular stiffness measured with a constant absolute load; T1, T2 = testing session number 1 and 2; CV = coefficient of variation; SEM = standard error of measurement; CL = confidence limits for CV and SEM.

* = significantly different from MAS%MVC60% (p<0.05).

doi:10.1371/journal.pone.0063719.t002

Table 3. Summary of the musculo-articular stiffness results obtained with a load corresponding to different percentages of maximal voluntary contraction, along with a constant load of 6.5 kg, over two testing sessions.

| Mean (SD) (Nm⁻¹) | Reliability coefficients |
|------------------|--------------------------|
| T1               | T2           | CV (%) | 95% CL | SEM (Nm⁻¹) | 95% CL |
| MAS%MVC15%       | 588.2 (147.6) | 653.7 (179.6) | 9.7 | 4.8–14.8 | 51.6 | 35.5–94.3 |
| MAS%MVC30%       | 889.3 (221.1) | 859.8 (253.4) | 8.1 | 3.4–12.7 | 60.3 | 43.2–99.4 |
| MAS%MVC45%       | 949.1 (182.8) | 959.7 (207.2) | 10.9 | 6.4–15.5 | 85.9 | 60.9–145.9 |
| MAS%MVC60%       | 1068.7 (276.2) | 1016.9 (218.6) | 12.6 | 6.3–18.8 | 98.8 | 63.7–217.6 |
| MASABS           | 790.0 (127.9) | 779.7 (126.3) | 6.5* | 4.1–8.9 | 40.2 | 29.7–62.6 |

Coefficients of reliability are also displayed.

MAS%MVC = musculo-articular stiffness measured with a load corresponding to a percentage of maximal voluntary contraction; MASABS = musculo-articular stiffness measured with a constant absolute load; T1, T2 = testing session number 1 and 2; CV = coefficient of variation; SEM = standard error of measurement; CL = confidence limits for CV and SEM.

* = significantly different from MAS%MVC60% (p<0.05).

doi:10.1371/journal.pone.0063719.t003
In conclusion, the assessment of MAS% MVC is affected by a combination of variability in determination of the load applied and the frequency recorded, with approximately the same magnitude of contribution for each variable. In contrast, the MASABS variability was consistently lower than MAS% MVC variability. Based on the results presented, low and constant loads for MAS assessment yielded good to excellent levels of reliability and are thus ideal to implement in longitudinal research. This is particularly relevant in the clinical setting which typically uses one constant relatively low assessment load [25]. The assessment of MAS with multiple higher loads, which is more suitable for sporting situations [12], incorporates higher error. Such information can provide practitioners with a detailed understanding of the strengths and limitations of the methodology. This may require a compromise between the need to increase test specificity and the need to reduce the measurement error. The results obtained in the current study are particularly relevant for clinicians, physical therapists, conditioning coaches and sports scientists who are involved in athlete screening and physical development. The CV (in percentage) and the SEM (using the same units as the variable of interest) provide a useful measure of the expected trial-to-trial noise in the data, which is relatively unaffected by the inter-subject variability. Further, knowledge regarding the source and magnitude of error in assessment procedures can inform decision making regarding the usefulness of such screening procedures, including determination of appropriate sample size and minimum detectable change.

**Author Contributions**
Conceived and designed the experiments: MD MW AM GDV. Performed the experiments: MD. Analyzed the data: MD MW AM GDV. Contributed reagents/materials/analysis tools: MD. Wrote the paper: MD MW AM GDV.

**References**

1. Ditroilo M, Watsford M, Murphy A, De Vito G (2011) Assessing musculotendinous stiffness using free oscillations: theory, measurement and analysis. Sports Med 41: 1019–1032.
2. Watsford M, Ditroilo M, Fernandez-Pena E, D’Amor G, Lucertini F (2010) Muscle stiffness and rate of torque development during sprint cycling. Med Sci Sports Exerc 42: 1324–1332.
3. Ditroilo M, Watsford M, De Vito G (2011) Validity and inter-day reliability of a free-oscillation test to measure knee extensor and knee flexor musculotendinous stiffness. J Electromyogr Kinesiol 21: 492–498.
4. Walshe AD, Wilson GJ, Murphy AJ (1996) The validity and reliability of a test of lower body musculotendinous stiffness. Eur J Appl Physiol Occup Physiol 73: 332–339.
5. Shorten MR (1987) Muscle Elasticity and Human Performance. Med Sport Sci 25: 1–18.
6. McNair PJ, Wood GA, Marshall RN (1992) Stiffness of the hamstring muscles and its relationship to function in anterior cruciate ligament deficit individuals. Clin Biomech (Bristol, Avon) 7: 131–137.
7. Coveney VA, Hunter GD, Spriggs J (2001) Is the behaviour of the leg during oscillation linear? J Biomech 34: 827–830.
8. Blackburn JT, Riemann BL, Padua DA, Guskiewicz KM (2004) Sex comparison of extensibility, passive, and active stiffness of the knee flexors. Clin Biomech (Bristol, Avon) 19: 36–43.
9. Panjabi MM, White III AA (2003) Vibrations. In: Panjabi MM, White III AA, editors. Biomechanics in the musculoskeletal system. Philadelphia (Pa, USA): Churchill Livingstone. pp. 131–150.
10. Granata KP, Wilson NE, Massimini AK, Gabriel R (2004) Active stiffness of the ankle in response to inertial and elastic loads. J Electromyogr Kinesiol 14: 599–609.
11. Farina A, Gabriel R, Abrantes J, Brac M, Moreira H (2009) Triceps-surae musculotendinous stiffness: relative differences between obese and non-obese postmenopausal women. Clin Biomech (Bristol, Avon) 24: 866–871.
12. Spurrs RW, Murphy AJ, Watsford ML (2003) The effect of photometric training on distance running performance. Eur J Appl Physiol 89: 1–7.
13. Ditroilo M, Watsford M, Fernandez-Pena E, D’Amor G, Lucertini F, et al. (2011) Effects of fatigue on muscle stiffness and intermittent sprinting during cycling. Med Sci Sports Exerc 43: 837–845.
14. McLachlan KA, Murphy AJ, Watsford ML, Rees S (2006) The interday reliability of leg and ankle musculotendinous stiffness measures. J Appl Biomech 22: 296–304.
15. Watsford ML, Murphy AJ, McLachlan KA, Bryant AL, Cameron ML, et al. (2010) A prospective study of the relationship between lower body stiffness and hamstring injury in professional Australian rules footballers. Am J Sports Med 38: 2056–2064.
16. Winter D (2005) Anthropometry. In: Winter D, editor. Biomechanics of motor control and human movement. New York: Wiley-Interscience pp. 59–85.
17. Atkinson G, Nevill AM (1998) Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. Sports Med 26: 217–230.
18. Weir JP (2005) Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. J Strength Cond Res 19: 231–240.
19. Taylor JR (2002) Propagation of Uncertainties. In: Taylor JR, editor. An Introduction to Error Analysis. Mill Valley (CA, USA): University Science Books · Oxford University Press. pp. 40–80.
20. Jennings AG, Seedhom BB (1988) The measurement of muscle stiffness in anterior cruciate injuries—an experiment revisited. Clin Biomech (Bristol, Avon) 13: 130–140.
21. Murphy A, Watsford ML, Coutts AJ, Pine MJ (2003) Reliability of a test of musculotendinous stiffness for the triceps-surae. Physical Therapy in Sport 4: 175–181.
22. Wilson GJ, Wood GA, Elliott BC (1993) The relationship between stiffness of the musculature and static flexibility: an alternative explanation for the occurrence of muscular injury. Int J Sports Med 12: 403–407.
23. Jaskolski A, Andrzejewska R, Marusiak J, Kiel-Sajewicz K, Jaskolska A (2007) Similar response of agonist and antagonist muscles after eccentric exercise revealed by electromyography and mechanomyography. J Electromyogr Kinesiol 17: 568–577.
24. Misenard O, Mottet D, Perrey S (2009) Factors responsible for force steadiness impairment with fatigue. Muscle Nerve 40: 1019–1032.
25. Bell DR, Blackburn JT, Norcross MF, Ondrak KS, Hudson JD, et al. (2012) Estrogen and muscle stiffness have a negative relationship in females. Knee Surg Sports Traumatol Arthrosc 20: 361–367.