Susceptibility to Exercise-Induced Muscle Damage: a Cluster Analysis with a Large Sample

Authors

Affiliations

F. Damas¹, K. Nosaka², C. A. Libardi³, T. C. Chen⁴, C. Ugrinowitsch¹

Affiliation addresses are listed at the end of the article

Key words

- isometric contraction
- muscle soreness
- creatine kinase
- range of motion
- Iengthening contractions
- elbow flexors

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Abstract

We investigated the responses of indirect markers of exercise-induced muscle damage (EIMD) among a large number of young men (N=286) stratified in clusters based on the largest decrease in maximal voluntary contraction torque (MVC) after an unaccustomed maximal eccentric exercise bout of the elbow flexors. Changes in MVC, muscle soreness (SOR), creatine kinase (CK) activity, range of motion (ROM) and upper-arm circumference (CIR) before and for several days after exercise were compared between 3 clusters established based on MVC decrease (low, moderate, and high responders; LR, MR and HR). Participants were allocated to LR (n=61), MR

(n=152) and HR (n=73) clusters, which depicted significantly different cluster centers of 82%, 61% and 42% of baseline MVC, respectively. Once stratified by MVC decrease, all muscle damage markers were significantly different between clusters following the same pattern: small changes for LR, larger changes for MR, and the largest changes for HR. Stratification of individuals based on the magnitude of MVC decrease post-exercise greatly increases the precision in estimating changes in EIMD by proxy markers such as SOR, CK activity, ROM and CIR. This indicates that the most commonly used markers are valid and MVC orchestrates their responses, consolidating the role of MVC as the best EIMD indirect marker.

Introduction

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Bibliography

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Correspondence

Felipe Damas School of Physical Education and Sport University of São Paulo Av. Prof. Mello de Morais 65, São Paulo 05508-030 Brazil Tel.: +55/11/3091 8796 Fax: +55/11/3813 5091 felipedamas@usp.br **v**

Exercise-induced muscle damage (EIMD) is a process that has been studied since the end of 19th and early 20th century with Hough's seminal paper on muscle soreness indicating that the phenomenon occurring in skeletal muscles after strenuous exercise cannot be solely attributed to fatigue [15]. Thenceforth, many studies have been performed to understand EIMD etiology by several investigators through more controlled experiments [6,8,25,27,34]. Most of the studies used more than one marker to represent the EIMD magnitude [6, 10, 25, 28, 33, 37]. The rationale for measuring a set of markers is that EIMD is a phenomenon encompassing various physiological processes and, therefore, each marker would assess at least one of the following processes: a) loss of myofibrillar integrity (shown as Z-band streaming/disruption) [1]; b) extracellularmatrix remodeling or failure in excitation-contraction coupling (reflected in reduced muscle strength) [17,36]; c) connective tissue damage (related to increases in delayed-onset muscle

soreness (SOR) and to decreases in range of motion (ROM)) [18,30]; d) membrane damage (leading to muscle protein leakage into blood-stream such as creatine kinase (CK)) [22]; e) muscle swelling (increasing limb circumference (CIR)) [31]; f) inflammatory events (promoting accumulation of leucocytes in the muscle) [34].

The problem with using a set of markers to describe the same phenomenon is that their responses do not always converge [3, 19, 20]. For example, Chapman et al. [3] demonstrated significant differences between 210 fast vs. 210 slow eccentric contractions for maximal voluntary contraction torque (MVC), CK and ROM after eccentric exercise (ECC), but no difference between conditions for SOR and CIR; Lavender and Nosaka [19] demonstrated that MVC, SOR, CK and ROM responses to ECC are significantly different comparing young and old men, but CIR was similar between ages; Lavender and Nosaka [20] showed similar responses in MVC, CK, ROM and CIR, after ECC in middle-aged vs. young men, but SOR was different between groups. This

disparity among marker responses can be due to large interindividual variability in EIMD outcomes, as several individual factors interfere with individual EIMD susceptibility, such as (but not limited to) previous maximal or submaximal exercises [5], training status [12,23], use of muscles in daily activities [6], flexibility [21], some genetic factors [7,11], and effort exerted during the exercise [5,35]. High inter-individual variability in EIMD marker responses has been found even when participants perform the same exercise [4,9,13,16,24,29,33,35]. In fact, an elegant review [34] suggested that the large inter-individual variability could be responsible for most of the equivocal findings and uncertainties regarding EIMD etiology. Importantly, the large inter-individual variability due to differences in EIMD susceptibility may be a greater problem for convergence in EIMD marker responses when a small sample is used, producing departures from normality and increasing the probability of type II errors. It is therefore possible that using a large number of individuals and classifying them in different levels of susceptibility to EIMD may reduce inter-subject variability and promote a better understanding of EIMD marker responses.

A possible method to overcome the large inter-individual variability is clustering individuals according to post-exercise EIMD marker responses, as this technique significantly minimizes variance within the formed clusters. Through cluster procedure, outcomes are classified by an iterative refinement technique, e.g., k-means algorithm, until it reaches convergence resulting in homogeneous groups. For this classification, it is necessary to use a variable that best represents EIMD magnitude. In this regard, previous studies have suggested that measurements of muscle force-generating capacity, such as MVC, is the best single marker of EIMD [34,37]. In fact, the magnitude of MVC loss seems to be even better than muscle histological analyses obtained from biopsies, as the small specimen obtained from the biopsy does not necessarily reflect the muscle damage along the muscle volume [2, 34, 37]. Therefore, it is reasonable to use changes in MVC as a surrogate marker of EIMD to classify in clusters a large sample of individuals. Such a procedure can ameliorate issues involving high inter-individual variability and small sample sizes that most likely are the reasons for disagreement among EIMD indirect marker responses.

Thus, we used a cluster analysis to stratify a large cohort of individuals in levels of EIMD susceptibility (responsiveness) based on the magnitude of post-exercise MVC loss to reduce interindividual variability and to produce response curves of the EIMD markers to better represent the different physiological processes involved in muscle damage occurrence and recovery. For this purpose, we compared the changes in a set of EIMD indirect markers (MVC, SOR, CK activity, ROM and CIR) between the pre-formed clusters. We hypothesized that clustering individuals by the magnitude of decrease in MVC 1-5 days post-exercise would orchestrate (i.e., 'organize') the responses of other markers and produce typical response curves of all of the other markers, strengthening MVC role as the main EIMD indirect marker. Additionally, as a secondary purpose, we tested if some of the assessed variables were associated with the magnitude of the largest MVC loss 1-5 days post-exercise, indicating possible putative reasons for differences in individual EIMD susceptibility and, therefore, data variability. Tested variables included: baseline MVC strength (indicating the individual capacity to produce force), baseline ROM (indicating individual flexibility), baseline CK activity in the blood (indicating genetic predisposal

to membrane permeability), or MVC change immediately postexercise (indicating individual effort exerted during exercise).

Methods

Experimental design

This was a "retrospective" study in which data from a large cohort of young men (N=286) who performed a bout of unaccustomed elbow-flexor maximal eccentric exercise (ECC) were collapsed and used for the present analyses. They were evaluated for MVC, SOR, CK, ROM and CIR at baseline, immediately after (only for MVC and ROM), and once a day over 120 h after ECC (a subset of 55 individuals were evaluated until 96 h post-ECC). Individuals were stratified into 3 clusters (see Statistics for details) based on the largest reduction in MVC between 1–5 days after ECC, to minimize the confounding effects of fatigue on force production observed immediately after ECC [26].

Participants

Data from 286 young men aged between 18 and 27 years were used in the present study. Exclusion criteria were upper-limb musculoskeletal injuries, use of anti-inflammatory drugs or nutritional supplements, and upper-limb resistance training in the previous 6 months before the commencement of the present study. Participants were asked to refrain from drinking alcohol, and from exercising the elbow flexor muscles from 72 h before and throughout the experimental period. All individuals included herein were participants of our previous studies or of unpublished observations. All of the studies were approved by a local ethics committee, and each participant provided written informed consent before participation in the original studies. Our study met the ethical standards of the International Journal of Sports Medicine [14].

Maximum elbow flexors eccentric (ECC) exercise

We selected maximal ECC exercise (i.e., exercise composed by maximal lengthening contractions) in the present study as it has been shown to induce muscle damage [6,8,25]. All participants performed 30 maximum unaccustomed elbow flexor eccentric actions using an isokinetic dynamometer (Biodex System Pro models 3 or 4, NY, USA) mostly using 90° range of motion and an angular velocity of 30°s⁻¹. Each eccentric action was performed after 1-s maximal isometric contraction and 10-12s rest was given between contractions during which the arm was passively returned to the start position by the isokinetic dynamometer. Participants were positioned on the dynamometer's chair and stabilized by seat belts to avoid extraneous movements. The participants were verbally encouraged to perform maximum effort throughout the range of motion in all repetitions. Importantly, none of the participants had previously performed the same or a similar exercise for the elbow flexor muscles.

Maximal voluntary contraction torque (MVC)

Participants were seated on the isokinetic dynamometer in the same position as for the ECC. The peak torque obtained on either 2 or 3 attempts (2–3 s of contraction) performed at 90° of elbow flexion with 60s rest between attempts or the highest peak concentric torque of a set of four isokinetic concentric repetitions were used for further analysis. The magnitude and time course of changes in peak torque after ECC were reported to be

similar between isometric and isokinetic concentric strength [6]. Furthermore, absolute differences in torque production between isometric and isokinetic concentric contractions did not affect the analysis, because peak torque values were normalized by the pre-ECC values. Strong verbal encouragement was given to the participants during the measurements.

Muscle soreness (SOR)

The level of muscle soreness was evaluated using a 100-mm visual analogue scale (0 mm: no pain at all, 100 mm: unbearable pain). The participants were asked to rate their soreness making a mark on the scale referring to their level of perceived SOR of the elbow flexors upon maximal voluntary extension of the elbow joint. The value in millimeters measured on the scale was used for analysis.

Creatine kinase (CK) activity

Venous blood samples (~5 mL) were drawn from the antecubital vein for the analysis of serum or plasma CK activity. The blood samples were kept at room temperature for 10–15 min to clot for serum, or collected to tubes with heparin for plasma. Samples were centrifuged for 10min, then serum or plasma was transferred to sample tubes, and immediately stored in a -80 °C freezer for later analysis. The samples were analyzed spectrophotometrically using commercially available test kits.

Range of motion (ROM)

Range of motion of the elbow joint was assessed with a goniometer as the difference between fully extended and flexed joint angles. Each participant actively extended the joint (increased joint angle), then attempted to touch the shoulder of the same side with the hand (flexed joint angle) while standing. A goniometer was placed on the following landmarks: lateral epicondyle of the humerus, acromion of the scapula, mid-point between the styloid processes of the ulna and the styloid process of the radius. These anatomical landmarks were marked and remarked with semi-permanent ink throughout the experimental period. 3 measurements were taken for each angle (i.e., extension and flexion angles), and the mean value was used to calculate the ROM.

Upper-arm circumference (CIR)

Circumference was measured at the mid portion of the upper arm (midpoint between the acromion and the olecranon), as the perpendicular perimeter of the longitudinal axis of the humerus while each participant stood with arms relaxed along the body. The mean value of 3 assessments was used for statistical purposes.

Statistical analyses

A k-cluster analysis was used to stratify 286 participants into 3 groups (clusters) based on the largest reduction in MVC (% pre) 1-5 days after ECC. The analysis classified each individual based on initial random means that were the initial cluster centers. The k-means algorithm performed iterations to classify the data using cluster centers as reference values until the formed clusters explained the largest portion of the variance of the data set. A different number of clusters was tested and 3 clusters explained most of the data set variance (i.e., ~90%). Thus, individuals in clusters 1, 2, and 3 were classified as low responders (LR), moderate responders (MR), and high responders (HR), respectively, according to the magnitude of the largest MVC loss. The 3 clusters aligned qualitatively and quantitatively well with the 3 levels of the MVC loss after ECC described elsewhere [34], as they indicate distinct EIMD responsiveness. Once divided in the clusters, their respective descriptive data, baseline and peak values of the EIMD markers were compared between clusters using general linear models with a fixed factor (i.e., cluster). Changes in the EIMD markers between the clusters were compared using a mixed model analysis followed by Tukey-Kramer adjustment for pair-wise comparisons, when appropriate. Pearson's product moment correlation was used for assessing association between variables. Alpha was set at p<0.05. Means are expressed ± SE unless otherwise stated.

Results

• Fig. 1,2 depict all 286 participants' data and means (±SD) in all indirect markers before (• Fig. 1) and after the clustering procedure (• Fig. 2).

Participants were classified into LR (n=61; cluster center: 81.5% of baseline MVC), MR (n=152; cluster center: 60.5% of baseline



Fig. 1 Panels a-e show the individual data distribution and means (±SD) in the EIMD markers before the clustering procedure. MVC, maximum voluntary contraction torque; SOR, muscle soreness; CK, creatine kinase, ROM, range of motion; CIR, upper-arm circumference.



Fig. 2 Panel **a** shows (mean \pm SE) the classification of low responders' cluster (LR), moderate responders' cluster (MR) and high responders' cluster (HR) based on a *k*-cluster analysis using the largest decreases in maximum voluntary contraction torque (MVC) observed 1–5 days after maximum eccentric exercise of the elbow flexors from the baseline (pre = 100%). Panels **b–e** show respectively: peak changes in muscle soreness (SOR, pre = 0 mm), peak creatine kinase (CK) activity, peak changes in range of motion (ROM, pre = 100%); and peak changes in upper-arm circumference (CIR, pre = 0 mm) after maximum eccentric exercise of the elbow flexors in the pre-formed clusters. ^a: significantly (*P*<0.02) different from LR; ^b: significantly (*P*<0.01) different from MR.

Table 1	Participants' characteristics and baseline values	(mean ± SD) of indirect markers	of muscle damage in low, r	noderate and high responders' clusters.
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	Age	Body mass	Height	MVC	SOR	СК	ROM	CIR		
	(years)	(kg)	(cm)	(Nm)	(mm)	(IU/L)	(°)	(mm)		
Low responders (n=61)	21.4±2.3	68.8±12.1	174.2±6.2	36.9±11.5	0±0	137.5±47.4	128.9±9.7	271.9±29.2		
Moderate responders (n = 152)	21.1±1.8	67.7±9.1	172.8±5.5	38.2±11.3	0 ± 0	139.5±43.1	130.2±8.9	269.3±23.1		
High responders ($n = 73$)	21.0±1.9	68.7±7.5	172.0±5.6	38.4±12.4	0 ± 0	136.7±41.9	131.5±9.2	267.2±19.6		
MVC: maximum voluntary contraction torque: SOP: muscle coreners: CK: creatine kinase activity: POM: range of motion: CIP: upper arm circumference										

MVC: maximum voluntary contraction torque; SOR: muscle soreness; CK: creatine kinase activity; ROM: range of motion; CIR: upper-arm circumference

MVC) and HR (*n*=73; cluster center: 42.2% of baseline MVC) clusters (**•** Fig. 2a). Clusters' descriptive characteristics and baseline values were similar among the clusters for all of the variables analyzed (**•** Table 1). As shown in **•** Fig. 2, the average values of the peak change in each marker were different across the clusters showing significantly greater changes from LR, MR to HR for all indirect markers assessed.

• Fig. 3 depicts the changes in MVC, SOR, CK, ROM and CIR post-ECC for each cluster over time and the mean (\pm SE) of all participants with no cluster classification. For the LR, all variables, except CK, showed significant changes from baseline to 96h (p<0.05), but only CIR increased significantly from baseline to 120 h (p<0.001). For the MR and HR, all of the variables changed significantly from baseline to 120 h post-ECC (p<0.001), except for CK. No changes in CK were found at any time point for the LR, but significant increases in CK were evident from 48 h to 120 h post-ECC (p<0.05) for both the MR and HR.

The comparison between clusters demonstrated significant differences between clusters for all of the dependent variables, following a similar pattern: MR showed greater changes compared with LR; and HR showed greater changes than both LR and MR. Specifically, regarding MVC, the MR demonstrated greater decreases than LR (p<0.001), and the HR showed greater decreases than both the LR and MR (p<0.001 for both) (\bullet Fig. 3a). The LR and MR increased SOR similarly, but the HR showed the greater SOR than LR and MR (p<0.05) (\bullet Fig. 3b). Regarding CK, ROM and CIR, the MR showed greater changes than LR (p<0.05), and the HR showed greater changes than LR (p<0.01) (\bullet Fig. 3c-e). Pearson's product moment correlation indicated that the largest MVC decrease 1–5 days after ECC was significantly (p<0.001)

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correlated with peak increases in SOR (r = -0.346), peak increases in CK (r = -0.471), largest decreases in ROM (r = 0.449) and peak increases in CIR (r = -0.494) (**• Fig. 4**).

The largest MVC decrease 1–5 days after ECC was not significantly correlated with the baseline MVC (r= –0.090), baseline ROM (r= –0.115) and baseline CK (r= –0.011). Nevertheless, the largest MVC decrease was significantly (p<0.001) and strongly correlated (r=0.793) with the magnitude of MVC decrease immediately post-ECC (**•** Fig. 5).

Discussion

We used a cluster analysis to stratify a large cohort of young men based on the largest decrease in MVC at 1-5 days after unaccustomed elbow flexors ECC and compared the changes in EIMD indirect markers between clusters. The main finding of the present study was that after the cluster procedure the time responses of all EIMD indirect markers (MVC, SOR, CK activity, ROM and CIR) were in line with the differences in the clusters (i.e., small changes for the LR, larger changes for MR, and the largest changes for HR). This validates the use of muscle soreness, CK activity, ROM and upper arm circumference as surrogate markers of EIMD and consolidates MVC as the main EIMD indirect marker as it orchestrates the responses of the other markers. In addition, we suggest that the incongruence in EIMD marker responses observed in the literature is most probably attributable to large data variability due to lack of stratification of individuals based on the susceptibility to EIMD and the use of the small sample sizes.



Fig. 3 Normalized changes (mean \pm SE) in maximum voluntary contraction torque (MVC) from pre-exercise value of 100% **a**, absolute changes in muscle soreness (SOR) based on visual analogue scale of 100 mm **b**, changes in creatine kinase (CK) activity **c**, normalized changes in range of motion (ROM) from pre-exercise value of 100% **d** and absolute changes in upper-arm circumference (CIR) from pre-exercise value of 0 mm **e** after maximum eccentric exercise of the elbow flexors in low responders' cluster (LR), moderate responders' cluster (MR) and high responders' cluster (HR); and mean values (\pm SE) with no cluster classification. *: significantly (p<0.05) different from baseline (pre) within each cluster; ^a: significantly (P<0.05) different from LR; ^b: significantly (P<0.05) different from MR.



Fig. 4 Correlation between the magnitude of the largest decrease in maximal voluntary contraction torque (MVC) observed at 1–5 days after eccentric exercise from the baseline (pre = 100%) and peak muscle soreness **a**, peak creatine kinase (CK) activity **b**, largest changes in range of motion (ROM, pre = 100%) **c**, and peak changes in upper-arm circumference (CIR, pre = 0 mm) **d**.

As expected, the raw data of this large sample regarding the changes in EIMD markers after ECC present high inter-subject variability (• Fig. 1-3). Additionally, the use of a mean value of the EIMD markers with no cluster classification to indicate EIMD magnitude only masks the large data variability (our data show values close to values of the MR cluster) (**Fig. 3**, dotted lines). In order to minimize inter-individual variability, Paulsen et al. [34] recommended that individuals be classified into groups based on the magnitude of the decrease in muscle force-generation capacity after heavy resistance exercise or maximal ECC. They suggested that a reduction in force-generating capacity < 20% would be linked to low or no morphological/histological indices of damage, but a reduction in force-generating capacity > 50% was generally associated with inflammation and/ or myofibrillar disruptions or even tissue necrosis [34]. Consequently, the authors proposed that a decline < 20%, between 20-50%, and >50% in MVC in the first 24h following ECC corresponded to 'mild', 'moderate', and 'severe' EIMD, respectively [34]. The results of the present study show all 3 categories of responders, as at the first 24h post-ECC, there was a ~16% decrease in MVC for the LR, corresponding to mild damage, a ~38% decrease in MVC for the MR, corresponding to moderate damage, and a ~56% decrease in MVC for the HR, corresponding to severe damage. This demonstrates that individual variability in ECC-induced muscle strength loss is large and should be considered when estimating EIMD magnitude.

This large variability may be one of the main reasons for the lack of agreement between proxy markers of EIMD found previously [3, 19, 20, 37]. Indeed, we clearly demonstrate that the stratification of individuals based on the magnitude of the largest MVC loss at 1–5 days post-exercise decreased the variability inside each cluster and orchestrated the responses of the peak changes (**• Fig. 2**) and time-course responses (**• Fig. 3**) in all EIMD markers of this large cohort of individuals, with LR demonstrating a



Fig. 5 Correlation between the magnitude of the largest decrease in maximal voluntary contraction torque (MVC) observed at 1–5 days after eccentric exercise from the baseline (pre-exercise value of 100%) and the baseline MVC **a**, baseline range of motion (ROM) **b**, baseline creatine kinase (CK) activity **c**, and magnitude of decrease in MVC immediately after eccentric exercise from the pre-exercise value of 100% **d**.

small magnitude of EIMD, MR a greater magnitude of EIMD and HR the greatest magnitude of EIMD. Specifically, the LR showed small decreases in MVC and ROM, and small increases in SOR, CK activity and CIR; while the MR and the HR presented progressively greater changes in all EIMD markers (**•** Fig. 2, 3). Although the magnitude of changes in EIMD markers differs among clusters, the time course of changes in each independent marker was similar among clusters. The lowest MVC values were seen between 0-24 h; the largest changes in SOR and ROM were seen around 48 h (the exception was that the LR cluster depicted the lowest ROM value immediately post-ECC) and CK activity and CIR peaked later, around 96-120 h. However, only the LR showed recovered MVC, SOR and ROM at 120 h after ECC, while MR and HR still depicted decreased MVC and ROM, and significantly increased SOR even at this later time point. It is worth pointing out that SOR increased similarly between LR and MR considering the whole time course (> Fig. 3b) and CK did not increase significantly for the LR cluster (**Fig. 3c**). This indicates that the differences in SOR from low-to-moderate EIMD-eliciting protocols can be small and low responders do not present high membrane permeability. Indeed, the responses of SOR and CK activity has been shown to be troublesome to represent EIMD magnitude [29, 30]. Therefore, caution should be taken when assessing muscle damage magnitude based on SOR and CK activity for low/moderate damage-inflicting protocols. Notwithstanding, when we analyzed peak changes, even LR and MR depicted different levels in SOR (**•** Fig. 2b), corroborating previous data [24]. Additionally, the LR cluster depicted CK activity mean values over 2100 IU/L (mean (SE), 2183.7 (349.7) IU/L) at 96 h post-ECC, which is way above the reference values for healthy young men (upper limit ~170-190IU/L) and thus indicative of EIMD [5, 10, 28]. Overall, our findings suggest that even though MVC, SOR, CK activity, ROM and CIR represent different physiological processes, our cluster procedure with a large sample allowed similar group (clusters) responses among markers, as variability was reduced inside the formed clusters, validating their use as surrogate markers of EIMD. This is relevant as these markers have been widely used to represent EIMD in several studies [6,8,10,25,28,33,37]. Importantly, the classification based on MVC loss orchestrated all marker responses, strengthening the main role that MVC loss has in representing EIMD magnitude. We acknowledge that our conclusion regards the group (clusters) responses, but if we take into account a single individual this might not be the case. As can be noticed in • Fig. 2, participants overlap each other among clusters for all markers (except, obviously, for MVC (O Fig. 2a), which was the marker we used for the clustering procedure). In addition, although correlations between the largest MVC loss 1-5 days post-exercise and peak changes in SOR, CK, ROM and CIR were all significant (probably the large sample size might have contributed to reach significance), the correlation coefficients were not large (greatest absolute r was 0.494), which shows no strong associations between variables (> Fig. 4). This indicates that when individual responses are considered there is no clear linearity among responses of EIMD markers. Therefore, caution should be taken when small samples sizes are used with no possibility of a clear cluster classification of EIMD marker responses. Nevertheless, the present study clearly demonstrates that the stratification of a large sample of individuals by MVC loss coordinates the responses of all other markers, greatly increasing the precision in group-estimating EIMD.

It is important to emphasize that this wide range of EIMD responses occurred despite the lack of differences in baseline values between clusters (> Table 1). Thus, it is reasonable to suggest that baseline values do not seem to influence the post-ECC changes in the variables. Likewise, other analyses lead to similar conclusions, as baseline MVC (individual capacity to produce force), baseline ROM (flexibility) and baseline CK (genetic predisposition to sarcolemma permeability) values were not associated with the magnitude of the largest MVC loss after ECC (Fig. 5). The findings related to strength capacity and genetic predisposition to damage are in agreement with previous observations, as it was demonstrated that baseline MVC did not influence EIMD variables outcomes [35] and identical twins (genetically identical) do not present similar EIMD responses [13]. In contrast with our conclusion regarding flexibility, it was shown that passive muscle stiffness was a modulating factor of EIMD magnitude [21]. This disparity could be due to the muscle group ana-

References lyzed (different muscle groups show different EIMD responses [6]), but the large sample analyzed herein strengthens our findings. It may be that other factors such as previous maximal or submaximal exercise [5], training status [23], use of muscles in daily activities [6], or even the ability to produce strength at long

muscle lengths [32] are more important factors to explain interindividual variability in the susceptibility to EIMD. Nevertheless, we demonstrate that the drop in MVC immediately post-ECC was highly correlated (r~0.8) with the largest MVC loss 1–5 days after ECC, indicating that effort is an important factor to consider regarding an individual's susceptibility to EIMD.

One limitation of our study is that the results and conclusion are applicable to protocols that have measured MVC or have another type of strength assessment. For example, downhill running protocols can elicit a high magnitude of muscle damage and often these studies do not include an MVC measure. Additionally, small sample sizes might be insufficient to adequately classify responders into clusters; therefore, we acknowledge that larger sample sizes are required to derive benefit from our findings.

In conclusion, a large sample size demonstrated a wide range of responses in the EIMD markers after ECC. The stratification (clustering) of individuals based on the largest reduction in strength greatly increases the precision in estimating EIMD by other proxy markers, such as SOR, CK activity, ROM and CIR, validating their extensive use over the years in the literature to indicate the degree of eccentric exercise-induced muscle damage. Importantly, our findings consolidate MVC as the main EIMD indirect marker, as it seems to orchestrate the responses of the other surrogate EIMD markers. Further studies should classify their samples based on their largest MVC decrease in order to reduce variability and thus improve the precision of experimental manipulations that can modulate EIMD.

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Affiliations

¹ School of Physical Education and Sport, University of São Paulo, São Paulo, Brazil

² Biomedical and Health Sciences, School of Exercise, Edith Cowan University, Joondalup, Australia

³ Department of Physical Education, Federal University of São Carlos – UFS-Car, São Carlos, Brazil

⁴Department of Physical Education, National Taiwan Normail University, Taipei City, Taiwan

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