

# **Spatio-temporal clustering of lumbar intervertebral flexion interactions in 127 asymptomatic individuals**

**Mehdi Nematimoez<sup>1</sup> Alexander Breen<sup>2</sup>, Alan Breen<sup>2</sup>**

<sup>1</sup> Department of Sport Biomechanics, University of Bojnord, Iran

<sup>2</sup> Faculty of Science and Technology, Bournemouth University, Poole BH12 5BB, UK

Corresponding author:

Mehdi Nemtimoez

nemati80@gmail.com;

Department of Sport Biomechanics,

University of Bojnord,

Esfarayen road,

Bojnord, North Khorasan,

9453155111, Iran

Word count: 2604

## Abstract

Little information is available about the movement interactions among lumbar vertebral levels that could be applied when considering interventions. The purpose of this study was therefore to categorize asymptomatic participants based on the clustering of spatial and temporal intervertebral kinematic variables during lumbar flexion. Lumbar segmental interactions (L2-S1) were evaluated in 127 asymptomatic participants during flexion using fluoroscopy. First, four variables were identified consisting of: 1. Range of motion (ROMC), 2. Peaking time of the first derivative for separate segmentation (PTFDs), 3. Peaking magnitude of the first derivative (PMFD), and 4. Peaking time of the first derivative for stepwise (grouped) segmentation (PTFDss). These variables were used to cluster and order the lumbar levels. The number of participants required to constitute a cluster was chosen as 7. Participants formed eight (ROMC), four (PTFDs), eight (PMFD), and four (PTFDss) clusters, which included 85%, 80%, 77%, and 60% of them, respectively, according to the above features. For all clustering variables, angle time series of some lumbar levels showed significant differences between clusters. However, in general, all clusters could be categorized based on the segmental mobility contexts into three main groups as incidental macro clusters: the upper (L2-L4>L4-S1), middle (L2-L3<L3-L5>L5-S1) and lower (L2-L4<L4-S1) domains. There are spatial and temporal segmental interactions and between-subject variability in asymptomatic participants. In addition, the differences in angle time series among the clusters have provided evidence of feedback control strategies, while the stepwise segmentation facilitates consideration of the lumbar spine as a system and provides supplementary information about segmental interactions. Clinically, these facts could be taken into account when considering any intervention, but especially fusion surgery.

**Keyword: Cluster, lumbar spine flexion, spatial, temporal, interaction**

# **Spatio-temporal clustering of lumbar intervertebral flexion interactions in 127 asymptomatic individuals**

## **Abstract**

Little information is available about the movement interactions among lumbar vertebral levels that could be applied when considering interventions. The purpose of this study was therefore to categorize asymptomatic participants based on the clustering of spatial and temporal intervertebral kinematic variables during lumbar flexion. Lumbar segmental interactions (L2-S1) were evaluated in 127 asymptomatic participants during flexion using fluoroscopy. First, four variables were identified consisting of: 1. Range of motion (ROMC), 2. Peaking time of the first derivative for separate segmentation (PTFDs), 3. Peaking magnitude of the first derivative (PMFD), and 4. Peaking time of the first derivative for stepwise (grouped) segmentation (PTFDss). These variables were used to cluster and order the lumbar levels. The number of participants required to constitute a cluster was chosen as 7. Participants formed eight (ROMC), four (PTFDs), eight (PMFD), and four (PTFDss) clusters, which included 85%, 80%, 77%, and 60% of them, respectively, according to the above features. For all clustering variables, angle time series of some lumbar levels showed significant differences between clusters. However, in general, all clusters could be categorized based on the segmental mobility contexts into three main groups as incidental macro clusters: the upper (L2-L4>L4-S1), middle (L2-L3<L3-L5>L5-S1) and lower (L2-L4<L4-S1) domains. There are spatial and temporal segmental interactions and between-subject variability in asymptomatic participants. In addition, the differences in angle time series among the clusters have provided evidence of feedback control strategies, while the stepwise segmentation facilitates consideration of the lumbar spine as a system and provides supplementary information about segmental interactions. Clinically, these facts could be taken into account when considering any intervention, but especially fusion surgery.

**Keyword: Cluster, lumbar spine flexion, spatial, temporal, interaction**



## Introduction

Mechanical load on the lumbar spine could influence the risk of low back pain (LBP). According to the global burden of disease study, between 1990–2013, LBP's prevalence and years lived with disability (YLDs) both increased by 57% (Atun, 2015). It is one of the top ten for prevalence and the first leading cause of YLDs in 188 and 86 countries (45 to 50 developed countries) respectively. In addition, LBP ranks high (second or third) among other disabilities in 67 countries (Atun, 2015). Hence, the cause and treatment of LBP becomes difficult when it becomes chronic (CLBP), and in some case requires stabilization surgery (Fritzell et al., 2001). Given the role of lumbar surgery in restoring normal biomechanical function, understanding the normal biomechanical function of the lumbar spine is necessary. Intervertebral mobility and movement patterns are thought to be factors influencing return to normal function and reduction of disability. However, some studies have reported adjacent segment degeneration and decrease in maximum forward flexion as result of spinal fusion surgery (Nagata et al., 1993; Stief et al., 2015) thus, any decrease in the mobility of a lumbar level could increase mobility and load on its adjacent segments.

To investigate spinal mobility and movement patterns, a range of variables and procedures have been used that consider simultaneously lumbar intervertebral motion and their interactions. This information might be used to determine fusion surgery strategies (Lawrence et al., 2012). Range of motion (ROM) is a common variable to consider mobility and is different between lumbar levels. It may be used to reveal interactions between and across levels. Inflexion point timing may also provide valuable information about temporal interplay and can be extracted from the intervertebral angle's first derivative during motion at individual and grouped levels (i.e., separate and stepwise). Specifically, the inflexion point is one data point that shows changes in the angle's curve concavity and rate of change (Nematimoez and Thomas, 2022).

Some studies have attempted to characterize lumbar motion patterns using non-medical imaging systems (e.g., motion capture and inertial sensors). Utilizing different variables, various lumbopelvic motion patterns have been attributed to some individual and task

characteristics (Zawadka et al., 2018). Pries et al., (2015) studied the lumbar/pelvic (L/P) ratio in 309 young to elder asymptomatic subjects (134 males and 175 females). Although this ratio was highly variable among participants, opposite trends were observed in the L/P ratio for older versus younger males, while during the middle and late phases of flexion, the contribution of the pelvis was reportedly larger in females than in males (Pries et al., 2015). The direction of motion can also change the coordination of these two segments; indeed, a greater in-phase pattern has been reported during trunk flexion than return (Zhou et al., 2016). Pal et al. (2007) have found that the pelvis initiated flexion motion later than the lumbar spine (i.e.,  $25.9 \pm 4.4\%$  vs.  $16.0 \pm 3.5\%$ ). They also reported a subgroup of participants (20%,  $n=4/20$ ) with a similar pattern during both flexion and return phases (Pal et al., 2007). Tafazzol et al., (2014) demonstrated simultaneous rhythms for the pelvis and lumbar spine except that for the early and final stages of flexion the pelvis was dominant, with implications for reducing lumbar loads (Tafazzol et al., 2014). Lumbopelvic motion patterns have also been studied as clinical tools for discriminating asymptomatic and low back patients (Kim et al., 2013; Sanchez-Zuriaga et al., 2015). However, technical differences between the data acquisition systems used (e.g. medical imaging vs motion analysis) may, for the time being, make conclusions about the nature of lumbopelvic spatio-temporal interactions difficult.

The lumbar segments generally tend to work in tandem or in compensation (du Rose and Breen, 2016). Breen et al. (Breen and Breen, 2018) suggested less intervertebral motion sharing inequality and less variability as indices for a pain free lumbar spine and Gatton et al. (Gatton and Pearcy, 1999) demonstrated between-subject variability and complexity of interactions among lumbar vertebrae in a small participant sample ( $n=14$ ). Additionally, discussion has arisen around whether segmental flexion and extension patterns should be simultaneous or sequential (Breen and Breen, 2018; du Rose and Breen, 2016; Gatton and Pearcy, 1999; Lawrence et al., 2012; Nematimoez and Thomas, 2022). In a study that categorized ninety adults into four and three groups according to the degree of total ROM of flexion and extension respectively, the greatest

segmental contributions to lumbar flexion occurred in the upper segments (Miyasaka et al., 2000). Wong et al. (Wong, Leong, Chan, Luk, & Lu, 2004) also observed more flexibility in the cranial region for one hundred subjects, reporting that physiologic ROM decreased in people aged 51 years or older. However, the lumbar kinematics literature is inconsistent on this, leading to difficulties with clinical interpretations, with different authors measuring different kinematic variables and numbers of vertebrae.

The purpose of this study was therefore to categorize intervertebral angular motion in a substantial dataset of asymptomatic subjects based on a group of clustering criteria utilizing spatial and temporal variables recorded during continuous motion for individual and segmentally grouped flexion. It was hypothesized that the lumbar segments' ROM and angle time series would be different among clusters during the flexion phase of movement.

## Methods

### Database

The data used for this analysis were obtained from an open access database (Breen and Breen, 2022). The database contains continuous vertebral midplane angles collected from 127 asymptomatic participants as they performed a controlled lumbar flexion and return bending protocol. The demographic characteristics of the participants are presented in Table 1.

The methodology used for data acquisition and image analysis has been previously described in Breen et al. (2021) (Breen et al., 2021). Briefly, participants underwent a controlled motion task, with their hips constrained, while low dose fluoroscopic recordings were taken of the L2-S1 levels during continuous motion (Fig. 1). A semi-automated image tracking process was used to determine the position of each vertebra (L2, L3, L4, L5, and S1) in each recorded image during the flexion and return trials. The

midplane angles of each vertebra were then extracted and smoothed using Tikhonov regularization (Eilers, 2003) to reduce noise in the output.

Table 1

Fig 1

## Clustering procedure

In this study, four clustering variables were considered based on movement interactions among lumbar levels. This was accomplished using calculations of ROM and the intervertebral angle's first derivative with separate (L5-S1, L4-L5, L3-L4, L2-L3) and stepwise (L5-S1, L4-S1, L3-S1, L2-S1) segmentation procedures (Nematimoez and Thomas, 2022). Following this, the peak of the first derivatives was determined for both segmentation procedures (Fig. 2).

Fig. 2

Next, four variables were obtained: 1. Range of motion ( $\text{Angle}_{\max} - \text{Angle}_{\min}$ ) (ROMC), 2. Peaking time of the first derivative for separate segmentation (PTFDs), 3. Peaking magnitude of the first derivative (PMFD), and 4. Peaking time of the first derivative for stepwise segmentation (PTFDss). A custom algorithm was used for clustering the individuals by considering an interaction among all four segmental values. For each participant, there were four values as input. There was no set limitation for the number of clusters to assess all unique interactions for each variable. The following provides a detailed description of a clustering algorithm.

Assuming a variable (i.e., ROMC, PTFDs, PMFD, PTFDss) passes through the algorithm with 127 data points (number of participants,  $n=127$ ) as input (Fig.3); first, the interaction among segments (i.e., A, B, C, and D) were coded from 1 to 4, reflecting the spatial or temporal values (i.e.,  $1=\min < 2 < 3 < \max=4$ ) by sub-algorithm for segments. (At this stage, there was no set limitation for similar coding of the segments and all equal values could take the same code (e.g., 1111)). This interaction coding continues



until  $n$  reaches 127. Next, the clustering procedure is begun, and after determining the unique code, the frequency of each of them provides a criterion for creation of a cluster ( $n \leq 7$ ). This is based on sample size in some experimental studies (Widmer et al., 2019) and provides insight for recruiting participants from similar populations. All of the above calculations were done in MATLAB R2016b (MathWorks Inc., Natick, US).

Fig. 3

## Statistical analysis

ROM and angle were considered as dependent variables in comparing clusters in each method; thus, separate one-way ANOVA was conducted in SPSS software (version 23, SPSS Inc. Chicago, IL). In addition, statistical parametric mapping (SPM) was used to compare between clusters' time series data (spm1d.stats.ttest), utilizing the open source spm1d package (www.spm1d.org) in MATLAB (MathWorks Inc., Natick, US). For all the aforementioned tests, alpha was set at 0.05.

## Results

Participants were made up of slightly more males than females, covered a wide age range (21-70 years) and had a mean BMI of 24 (sd 2.8). (Table 1). They were distributed in eight (ROMC), four (PTFDs), eight (PMFD), and four (PTFDss) clusters according to spatial and temporal variables (Fig. 4) which included 85%, 80%, 77%, and 60% of subjects, respectively (Table 2). In the following paragraphs, the main effects were reported for each clustering variable.

Fig. 4

Table 2

ANOVA and SPM analyses showed significant differences for the ROMC variable. Clusters 1 and 7 (C1 and C7) included the highest proportion of participants (Table 2 and Fig. 5a). PTFDs demonstrated a top-down delay for 57% of participants ( $n=73$ ); also, this method showed significant differences for the ROM. However, SPM did not exhibit significant differences for the L5-S1 angle time series (Fig. 5b). For PMFD, all

the ROM and angle time series were significant (Fig. 5c). For this variable, C4 covered the highest percent of subjects (19%, n=24). For PTFDss, L3-L4 and L2-S1 were not significant for the ROM differences. In addition, the L5-S1 and L2-S1 angle time series' were not significant (Fig. 5d).

#### Fig. 5

According to the SPM results, differences among clusters mainly appeared after 10% of the flexion cycles for the ROM (Fig. 6a). For PTFDs, the L5-S1 angle time series showed non-significant differences; indeed, this variable revealed top-down initiation sequences of the significant periods (Fig. 6b). For PMFD, four segments exhibited significant time series periods after 22% of flexion cycles, except for L3-L4 where the first 55% was significant (Fig. 6c). Finally, only L4-L5 was highly significant for clustering using the PTFDss variable (Fig. 6d).

#### Fig. 6

PMFD and ROMC included the smallest ( $n=14$ ,  $C8=31.78\pm9.82$ ) and largest ( $n=7$ ,  $C3=45.37\pm0.74$ ) ROMs for L2-S1, respectively. In effect, all clusters could be categorized based on the segmental mobility contexts in three main groups as macro clusters: the upper ( $L2-L4>L4-S1$ ), middle ( $L2-L3<L3-L5>L5-S1$ ) and lower ( $L2-L4<L4-S1$ ) domains (Table 2). ROMC and PMFD contained all of these macro clusters; nevertheless, the PTFDs and PMFDss variables did not include the middle and lower categories, respectively (Table 2).

## Discussion

The aim of this study was to categorize asymptomatic subjects based on clustering criteria utilizing spatial and temporal variables during flexion. All clustering variables distributed the participants into distinguishable groups. In addition, SPM showed significant differences in angle time series for all of these methods. All clusters could be categorized based on the segmental mobility in the upper, middle, and lower levels domains.

First of all, there are some reasons for utilizing the discrete variables for inputs into the clustering procedure. Although continuous variables are more informative, analyzing the segmental interactions and interpreting the results are time-consuming and complicated (Ronan et al., 2016). Thus, discrete variables were selected for the clustering procedure due to ease of implementation and interpretation of the results in clinical situations. However, the continuous test (SPM) was included in the statistical analysis for evaluation of the segmental angle of each clustering variable (i.e., ROMC, PTFDs, PMFD, PTFDss).

Clustering by ROMC variable showed interactive variability and the numbers of unreported unique patterns ( $n < 7$ ) were lower than the other clustering variables. For this variable, C1 and C8 showed the upper segmental mobility contexts while C2 and C4 demonstrated lower segmental mobility. ROM increased through L2-S1 levels for C1. Whereas some studies have supported upper and lower segmental mobility, with discordant results (Miyasaka et al., 2000; Teyhen et al., 2007; Wong et al., 2004), the current data ( $n=127$ ) showed mainly middle segmental mobility (Breen et al., 2021). In the current study, the participants exhibited upper (30%), middle (36%), and lower (14%) segmental mobility contexts. Thus, ROM partly reveals difficulties for distinguishing a discerning factor for healthy and LBP people pertaining to ROM.

The PTFDs variable showed that more than half of the participants have a top-down dominant pattern, evidencing the existence of a lag time among lumbar segments. This pattern has also been suggested by others utilizing different recording and/or calculation methods (Breen and Breen, 2020; du Rose and Breen, 2016; Harada et al., 2000; Kanayama et al., 1996). Interestingly, PTFDs are therefore amenable to being considered as a pattern recognition variable. In the present study, 'dominant' means the notable temporal contribution of a segment while other segments may contribute to movement competency (Fig. 7a). Indeed, the sophisticated neural system controls the smoothness of movement through a segmental movement cascade for the multi-segmental structure (Williams et al., 2013). This variable also proves the discrepancy

between the spatial and temporal analyses; in other words, the top-down dominant pattern did not show top-down or bottom-up increases in ROM.

### Fig. 7

As ROMC, the PMFD variable had more clusters than the others and somehow distributed participants into all three segmental mobility categories. Using this variable, C1 and C7 showed lower segmental mobility that demonstrated sequential and alternative (L2L3>L4L5>L3L4>L5S1) top-down increasing and decreasing ROM. The relationship between PMFD and ROM is hard to justify. Nevertheless, it seems that C1 is more complicated for lower lumbar fusion surgery; this pattern imposes higher risk of injury to lower segments, due to the critical role of ROM and velocity in degeneration processes (Ren et al., 2016).

The numbers of unique patterns ( $n < 7$ ) were highest for PTFDss and L3-L4 and L2-S1 ROM did not show significant differences. It seems this method is suitable for distributing the participants in the homogenous L3-L4 ROM clusters. In addition, PTFDss provides information about the action and interaction of each level by considering cumulative effects; for example, in C3, L2-L3 and L3-L4 showed dynamic integrity (L5S1>L4S1>L3S1=L2S1). C2 demonstrated the top-down dominant pattern (L5S1>L4S1>L3S1>L2S1) in which each level has an effect on the whole lumbar spine. On the other hand, in C4, the L2-L3 and L3-L4 movements did not show any effect on the lumbar spine dynamic (L5S1>L4S1=L3S1=L2S1) (Fig. 7b). These results would be valuable and logical reasons to assess fused segment kinematics and to use stepwise segmentation in clinical environments (Caserta et al., 2002).

The segmental mobility context would be applicable to regulate fusion surgery and to consider as a possible predictive risk factor. Some studies have reported that lumbar fusion increases ROM in and load on the adjacent segments (Nagata et al., 1993; Stief et al., 2015). In this way the fused lumbar motion segment has dominant mobility in the upper or lower segments that may lead to adjacent segment disorder (ASD). Clinically, dynamic stabilization would be a safer procedure because small ROM increases in the

adjacent segments are not a risk factor for ASD (Tachibana et al., 2017). Although the interactions among lumbar segments may be diminished by multiple fused segment surgery, some clusters and segments exhibited the potential for fusion regardless of ROM. For example, PTFDss (C2: n=43, ~34%) showed a top-down dominant pattern, thus dynamic fusion is a sensible strategy while for C4, hybrid (rigid and semi-rigid) L2-L4 fusion could be suggested because of dynamic integrity. Besides the segmental mobility context may predict the risk of injury (e.g., ROMC: C1 and C8), given that causality of ASD, and the literature has suggested regional vulnerability (upper vs. lower) (Battie et al., 2004). However, little information is available about the lumbar mobility of patients before degeneration or fusion surgery. These contentions would prompt further consideration.

The SPM results would be evidence of feedback control strategies revealed by clustering variables. Biomechanically, spine stability faces a challenge from increasing lumbar flexion. In this situation, segmental motion is intimately associated with feedback control making the spine more robust (Reeves et al., 2007). Indeed, follower load has been addressed as such a stabilization mechanism for the lumbar spine that simulates compressive muscle action (Patwardhan et al., 1999). The multifidus muscle has been characterized as a stabilizer due to its physiological cross-section and short fiber length (Ward et al., 2009). A microgravity exposure study demonstrated correlation between multifidus' morphology and L2-L3, L3-L4, and L4-L5 ROMs (Bailey et al., 2017). Consequently, any between-subject differences could change the motor control strategy and lead to kinematic variability. Multifidus' morphology differences may arise from conditioning level and inappropriate exercise even in professional athletes (Hides et al., 2008). These situations were also possible in the participants of the current study.

To our knowledge, this is the first study to consider the spatial and temporal interactions among lumbar segments in depth using four cluster variables in asymptomatic subjects. Furthermore, stepwise segmentation provides a specific analysis procedure to consider the whole lumbar spine. On the other hand, there are further limitations than those that Breen et al. (Breen et al., 2021) have mentioned for the current study, such as the unknown effects of conditioning and history of LBP for each cluster. However, although

staff, students, and visitors of the university volunteered for this study, it is reasonable to postulate that the vast majority of subjects had similar characteristics. Because of the need to meet the desired criterion (i.e., at least seven participants for each cluster), some patterns were not reported. Nevertheless, this criterion was determined based on the minimum acceptable number of participants in previous studies (Widmer et al., 2019). Finally, segmental mobility and motion pattern contexts are suggested as criteria in future studies

In summary, asymptomatic subjects were clustered utilizing spatial and temporal variables and showed variability in interactions among lumbar segments. Furthermore, clustering variables could be categorized based on segmental mobility contexts into the upper, middle, and lower domains as macro clusters. PTFDs, as patterns, showed more than half of participants to have a top-down dominant pattern based on the lag time between lumbar segments. In addition, this method confirmed the discrepancy between spatial and temporal analysis. While the relationship between PMFD and ROM is hard to interpret, the PMFD variable distributed participants in all three segmental mobility categories. PTFDs' results would be valuable and logical variables with which to assess fused segment kinematics and to use stepwise segmentation in clinical environments. Angle time series differences would be evidence of feedback control strategies revealed by clustering variables. Finally, segmental mobility and motion pattern contexts are suggested as criteria in future studies.

### **Conflict of interest statement**

The authors declare no conflicts of interest.

### **References**

- Atun, R., 2015. Transitioning health systems for multimorbidity. *Lancet* 386, 721-722.  
Bailey, J.F., Miller, S.L., Khieu, K., O'Neill, C.W., Healey, R.M., Coughlin, D.G., Sayson, J.V., Chang, D.G., Hargens, A.R., Lotz, J.C., 2017. From the international space station to the clinic: how prolonged unloading may disrupt lumbar spine stability. *The spine journal : official journal of the North American Spine Society*.

Battie, M.C., Videman, T., Parent, E., 2004. Lumbar disc degeneration: epidemiology and genetic influences. *Spine (Phila Pa 1976)* 29, 2679-2690.

Breen, A., Breen, A., 2018. Uneven intervertebral motion sharing is related to disc degeneration and is greater in patients with chronic, non-specific low back pain: an in vivo, cross-sectional cohort comparison of intervertebral dynamics using quantitative fluoroscopy. *European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society* 27, 145-153.

Breen, A., Breen, A., 2020. Dynamic interactions between lumbar intervertebral motion segments during forward bending and return. *J Biomech* 102, 109603.

Breen, A., Breen, A., 2022. Reference Database of Continuous Vertebral Flexion and Return. *Open Science Framework*. <https://osf.io/a27py/>.

Breen, A., De Carvalho, D., Funabashi, M., Kawchuk, G., Page, I., Wong, A.Y.L., Breen, A., 2021. A Reference Database of Standardised Continuous Lumbar Intervertebral Motion Analysis for Conducting Patient-Specific Comparisons. *Front Bioeng Biotechnol* 9, 745837.

Caserta, S., La Maida, G.A., Misaggi, B., Peroni, D., Pietrabissa, R., Raimondi, M.T., Redaelli, A., 2002. Elastic stabilization alone or combined with rigid fusion in spinal surgery: a biomechanical study and clinical experience based on 82 cases. *European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society* 11 Suppl 2, S192-197.

du Rose, A., Breen, A., 2016. Relationships between lumbar inter-vertebral motion and lordosis in healthy adult males: a cross sectional cohort study. *BMC Musculoskelet Disord* 17, 121.

Eilers, P.H., 2003. A perfect smoother. *Anal Chem* 75, 3631-3636.

Fritzell, P., Hagg, O., Wessberg, P., Nordwall, A., Swedish Lumbar Spine Study, G., 2001. 2001 Volvo Award Winner in Clinical Studies: Lumbar fusion versus nonsurgical treatment for chronic low back pain: a multicenter randomized controlled trial from the Swedish Lumbar Spine Study Group. *Spine (Phila Pa 1976)* 26, 2521-2532; discussion 2532-2524.

Gatton, M.L., Percy, M.J., 1999. Kinematics and movement sequencing during flexion of the lumbar spine. *Clinical Biomechanics* 14, 376-383.

Harada, M., Abumi, K., Ito, M., Kaneda, K., 2000. Cineradiographic motion analysis of normal lumbar spine during forward and backward flexion. *Spine (Phila Pa 1976)* 25, 1932-1937.

Hides, J.A., Stanton, W.R., McMahon, S., Sims, K., Richardson, C.A., 2008. Effect of stabilization training on multifidus muscle cross-sectional area among young elite cricketers with low back pain. *The Journal of orthopaedic and sports physical therapy* 38, 101-108.

Kanayama, M., Abumi, K., Kaneda, K., Tadano, S., Ukai, T., 1996. Phase lag of the intersegmental motion in flexion-extension of the lumbar and lumbosacral spine. An in vivo study. *Spine (Phila Pa 1976)* 21, 1416-1422.

Kim, M.H., Yi, C.H., Kwon, O.Y., Cho, S.H., Cynn, H.S., Kim, Y.H., Hwang, S.H., Choi, B.R., Hong, J.A., Jung, D.H., 2013. Comparison of lumbopelvic rhythm and flexion-relaxation response between 2 different low back pain subtypes. *Spine (Phila Pa 1976)* 38, 1260-1267.

Lawrence, B.D., Wang, J., Arnold, P.M., Hermsmeyer, J., Norvell, D.C., Brodke, D.S., 2012. Predicting the risk of adjacent segment pathology after lumbar fusion: a systematic review. *Spine (Phila Pa 1976)* 37, S123-132.

Miyasaka, K., Ohmori, K., Suzuki, K., Inoue, H., 2000. Radiographic analysis of lumbar motion in relation to lumbosacral stability. Investigation of moderate and maximum motion. *Spine (Phila Pa 1976)* 25, 732-737.

Nagata, H., Schendel, M.J., Transfeldt, E.E., Lewis, J.L., 1993. The effects of immobilization of long segments of the spine on the adjacent and distal facet force and lumbosacral motion. *Spine (Phila Pa 1976)* 18, 2471-2479.

Nematimoez, M., Thomas, J.S., 2022. The effect of head movement restriction on the kinematics of the spine during lifting and lowering tasks. *Ergonomics* 65, 842-856.

Pal, P., Milosavljevic, S., Sole, G., Johnson, G., 2007. Hip and lumbar continuous motion characteristics during flexion and return in young healthy males. *European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society* 16, 741-747.

Patwardhan, A.G., Havey, R.M., Meade, K.P., Lee, B., Dunlap, B., 1999. A follower load increases the load-carrying capacity of the lumbar spine in compression. *Spine (Phila Pa 1976)* 24, 1003-1009.

Pries, E., Dreischarf, M., Bashkuev, M., Putzier, M., Schmidt, H., 2015. The effects of age and gender on the lumbopelvic rhythm in the sagittal plane in 309 subjects. *J Biomech* 48, 3080-3087.

Reeves, N.P., Narendra, K.S., Cholewicki, J., 2007. Spine stability: the six blind men and the elephant. *Clinical Biomechanics* 22, 266-274.

Ren, D., Hu, Z., Yuan, W., 2016. The Study of Cobb Angular Velocity in Cervical Spine during Dynamic Extension-Flexion. *Spine (Phila Pa 1976)* 41, E410-415.

Ronan, T., Qi, Z., Naegle, K.M., 2016. Avoiding common pitfalls when clustering biological data. *Sci Signal* 9, re6.

Sanchez-Zuriaga, D., Lopez-Pascual, J., Garrido-Jaen, D., Garcia-Mas, M.A., 2015. A comparison of lumbopelvic motion patterns and erector spinae behavior between asymptomatic subjects and patients with recurrent low back pain during pain-free periods. *Journal of manipulative and physiological therapeutics* 38, 130-137.

Stief, F., Meurer, A., Wienand, J., Rauschmann, M., Rickert, M., 2015. Has a Mono- or Bisegmental Lumbar Spinal Fusion Surgery an Influence on Self-Assessed Quality of Life, Trunk Range of Motion, and Gait Performance? *Spine (Phila Pa 1976)* 40, E618-626.

Tachibana, N., Kawamura, N., Kobayashi, D., Shimizu, T., Sasagawa, T., Masuyama, S., Hirao, Y., Kunogi, J., 2017. Preventive Effect of Dynamic Stabilization Against Adjacent Segment Degeneration After Posterior Lumbar Interbody Fusion. *Spine (Phila Pa 1976)* 42, 25-32.

Tafazzol, A., Arjmand, N., Shirazi-Adl, A., Parnianpour, M., 2014. Lumbopelvic rhythm during forward and backward sagittal trunk rotations: combined in vivo measurement with inertial tracking device and biomechanical modeling. *Clinical Biomechanics* 29, 7-13.

Teyhen, D.S., Flynn, T.W., Childs, J.D., Kuklo, T.R., Rosner, M.K., Polly, D.W., Abraham, L.D., 2007. Fluoroscopic video to identify aberrant lumbar motion. *Spine (Phila Pa 1976)* 32, E220-229.

Ward, S.R., Kim, C.W., Eng, C.M., Gottschalk, L.J.t., Tomiya, A., Garfin, S.R., Lieber, R.L., 2009. Architectural analysis and intraoperative measurements demonstrate the unique design of the multifidus muscle for lumbar spine stability. *J Bone Joint Surg Am* 91, 176-185.

Widmer, J., Fornaciari, P., Senteler, M., Roth, T., Snedeker, J.G., Farshad, M., 2019. Kinematics of the Spine Under Healthy and Degenerative Conditions: A Systematic Review. *Annals of biomedical engineering* 47, 1491-1522.

Williams, J.M., Haq, I., Lee, R.Y., 2013. A novel approach to the clinical evaluation of differential kinematics of the lumbar spine. *Manual therapy* 18, 130-135.

Wong, K.W., Leong, J.C., Chan, M.K., Luk, K.D., Lu, W.W., 2004. The flexion-extension profile of lumbar spine in 100 healthy volunteers. *Spine (Phila Pa 1976)* 29, 1636-1641.

Zawadka, M., Skublewska-Paszkowska, M., Gawda, P., Lukasik, E., Smolka, J., Jablonski, M., 2018. What factors can affect lumbopelvic flexion-extension motion in the sagittal plane?: A literature review. *Hum Mov Sci* 58, 205-218.

Zhou, J., Ning, X., Fathallah, F., 2016. Differences in lumbopelvic rhythm between trunk flexion and extension. *Clinical Biomechanics* 32, 274-279.



## Table captions

Table 1. Demographic information of the subjects with and without considering gender.

Number: the number of the subjects; SD: standard deviation

Table 2. Clustering variables, patterns and ROM's means and standard deviations for each cluster and variable.

ROMC: range of motion clustering; PTFDs: peaking time of first derivative for separate segmentation; PMFD: peaking magnitude of first derivative; PTFDss: peaking time of first derivative for stepwise segmentation; C: cluster's number; m: male; f: female; SD: standard deviation; D: segmental mobility domain; U: upper; M: middle; L: lower; Frq: number of participants to each clustering variable; N: total number of participants that distributed by each variable in clusters; F: f value for one way ANOVA; Sig: Significant; bolded number: main significant difference. L5S1 (red), L4L5 (orange), L3L4 (blue), L2L3 (green).

## Figure captions

**Fig. 1.** Motion protocol used for fluoroscopic image acquisition. (LEFT) Initial neutral standing position, (MIDDLE) 60° Flexion, (RIGHT) Example fluoroscopic image from the neutral position

**Fig. 2.** "Mean of first derivatives of angular motion (velocity) of L2 to S1 during flexion and return for all participants (n=127)." (a) Intervertebral velocity (L5-S1 (black), L4-L5 (red), L3-L4 (blue), L2-L3 (magenta)). (b) S1 stepwise segmentation (S1ss: L5-S1 (black), L4-S1 (red), L3-S1 (blue), L2-S1 (magenta)).

**Fig. 3.** A custom algorithm for clustering the individuals by considering an interaction among all four segmental values: n: number of subjects, Seg: segment, Max: maximum, 2th: second value ordered from maximum to minimum, 3th: third value ordered from maximum to minimum, Min: minimum, F: frequency of unique codes.

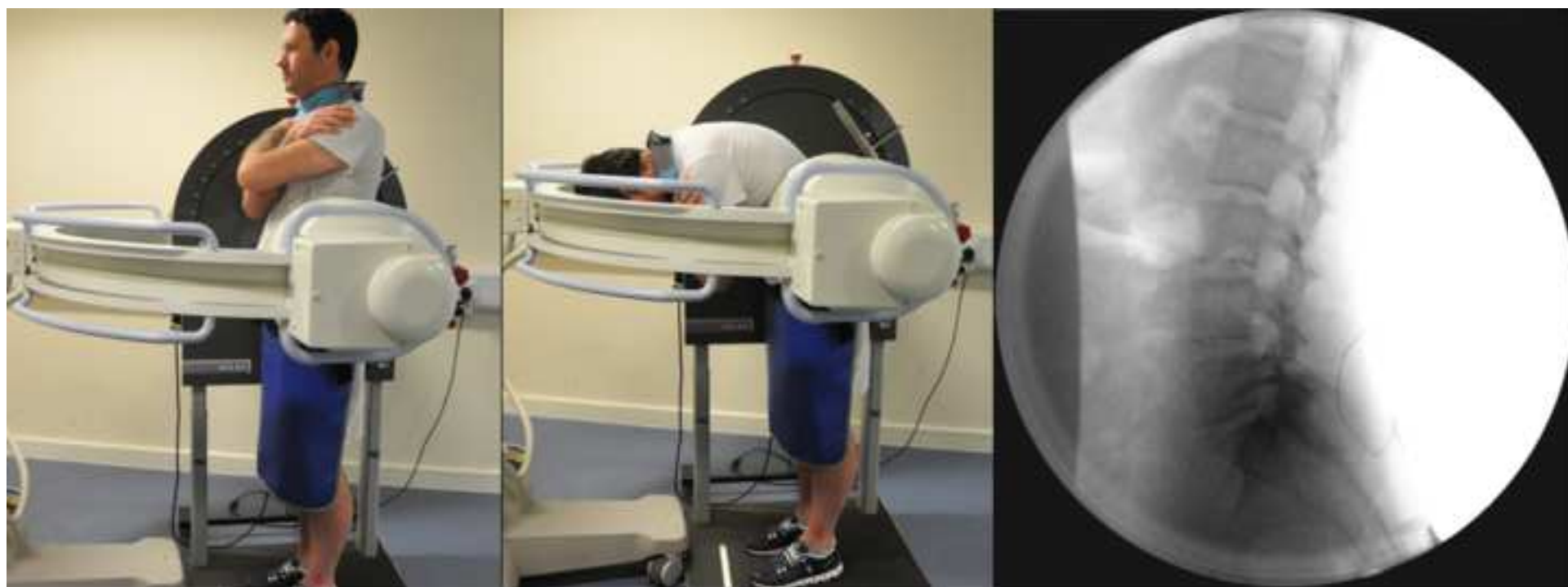
**Fig. 4.** Distribution of participants in clusters (C1, C2, C3..., Cn) by different variables: a) range of motion clustering (ROMC); C1 ('<' red), C2 ('diamond' blue), C3 ('O' black), C4 ('>' red), C5 ('\*' blue), C6 ('square' red), C7 ('^' black), and C8 ('O' blue). b) Peaking time of first derivative with separate segmentation (PTFDs); C1 ('diamond' black), C2 ('O' magenta), C3 ('>' blue), C4 ('\*' red). c) peaking magnitude of first derivative (PMFD); C1 ('<' red), C2 ('diamond' blue), C3 ('O' black), C4 ('>' red), C5 ('\*' blue), C6 ('square' red), C7 ('^' black), C8 ('O' blue). d) Peaking time of first derivative with stepwise segmentation (PTFDss); C1 ('\*' red), C2 ('diamond' blue), C3 ('O' black), C4 ('>' red).

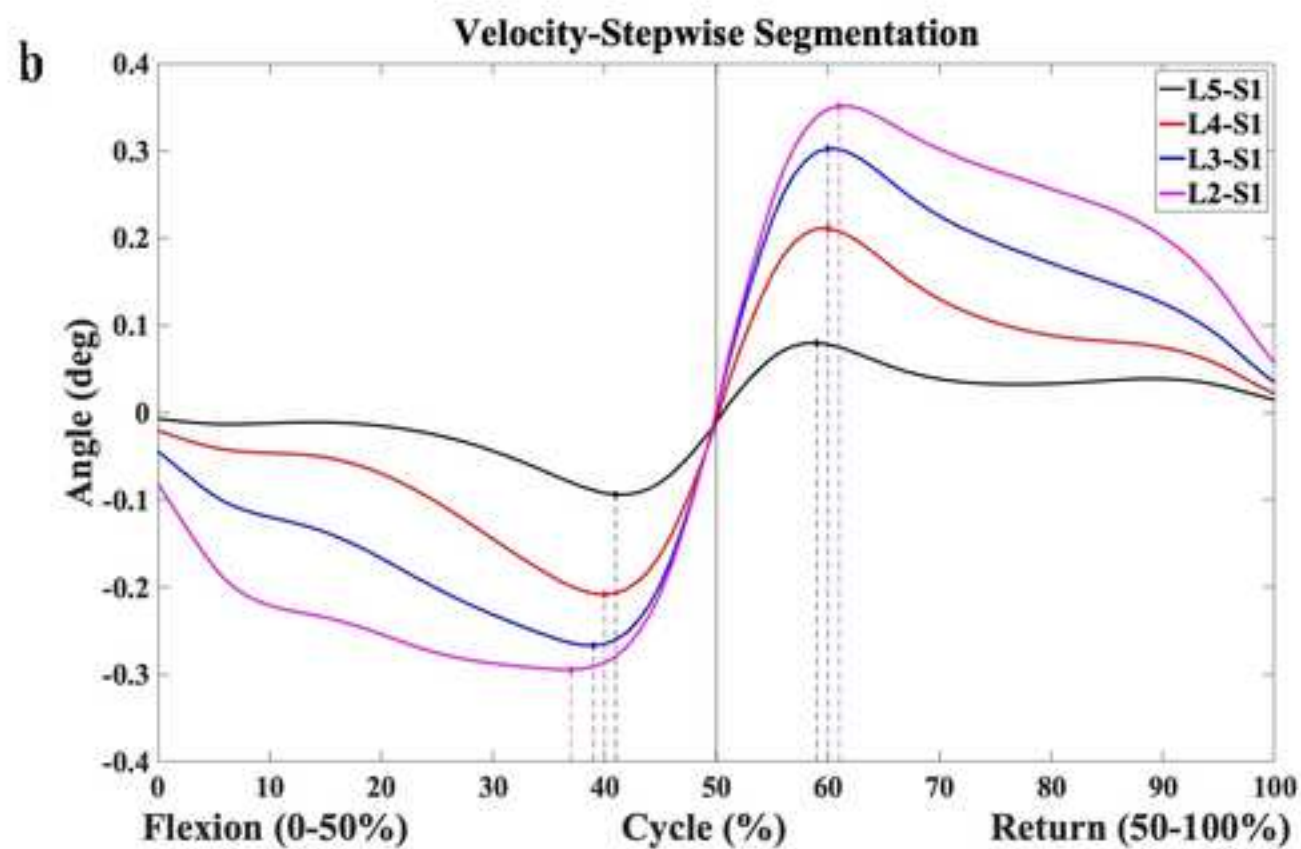
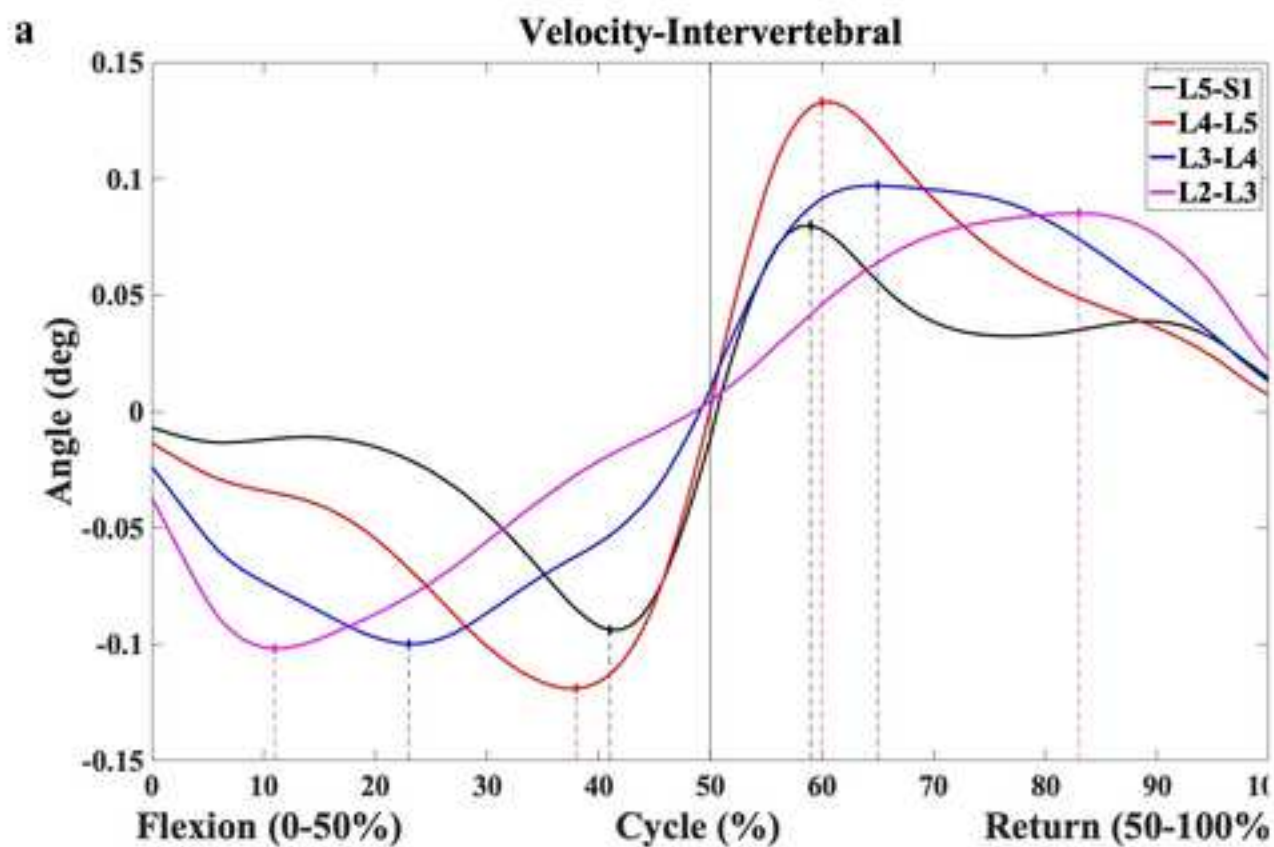
**Fig. 5.** Segmental comparison of intervertebral angle (L5-S1, L4-L5, L3-L4, L2-L3) among clusters of each variable (ANOVA) by statistical parametric mapping (SPM) during flexion movement (%); shadowed areas show significant differences: a) range of motion clustering (ROMC); b) peaking time of first derivative with separate

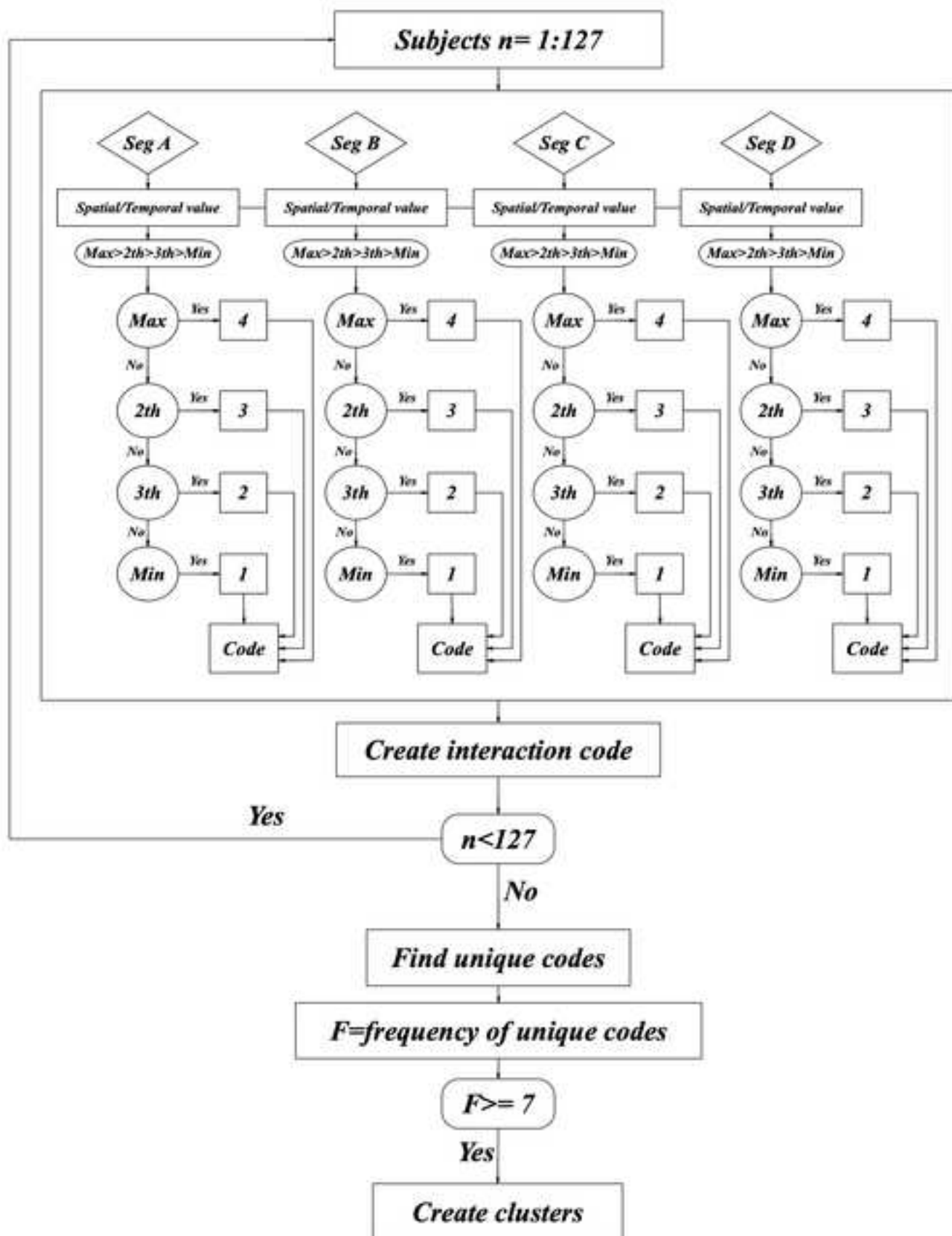
segmentation (PTFDs); c) peaking magnitude of first derivative (PMFD); d) Peaking time of first derivative with stepwise segmentation (PTFDss).

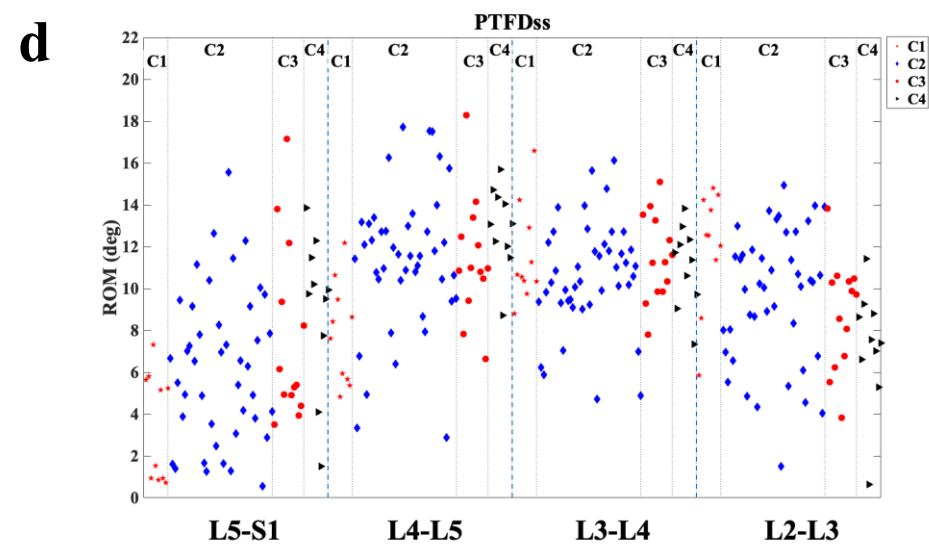
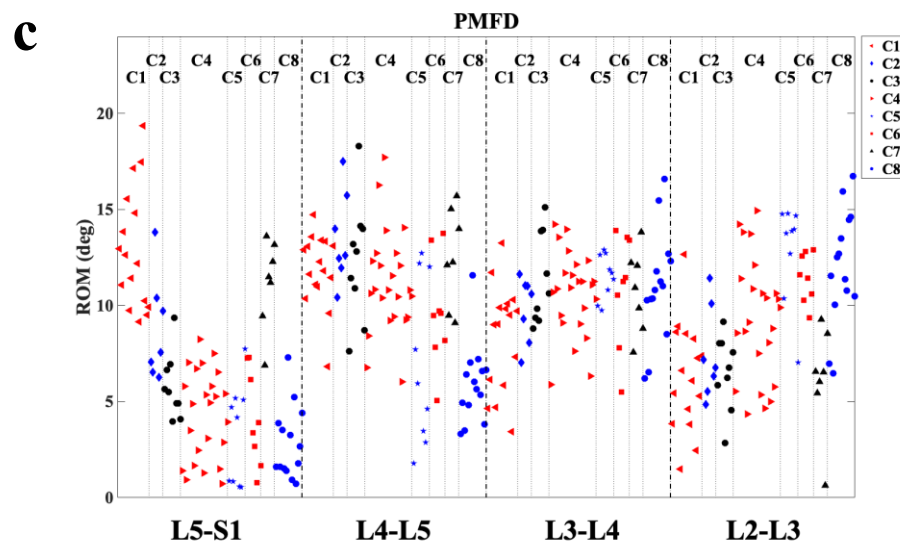
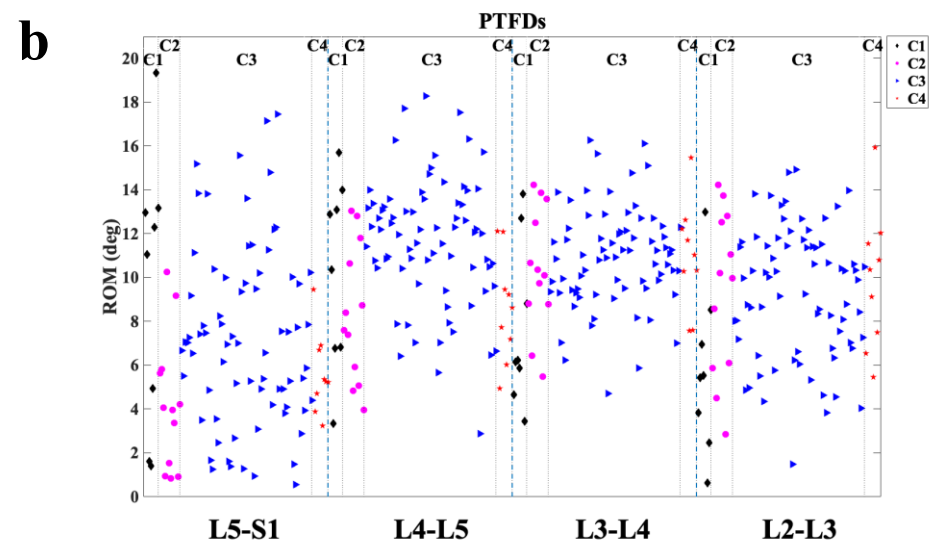
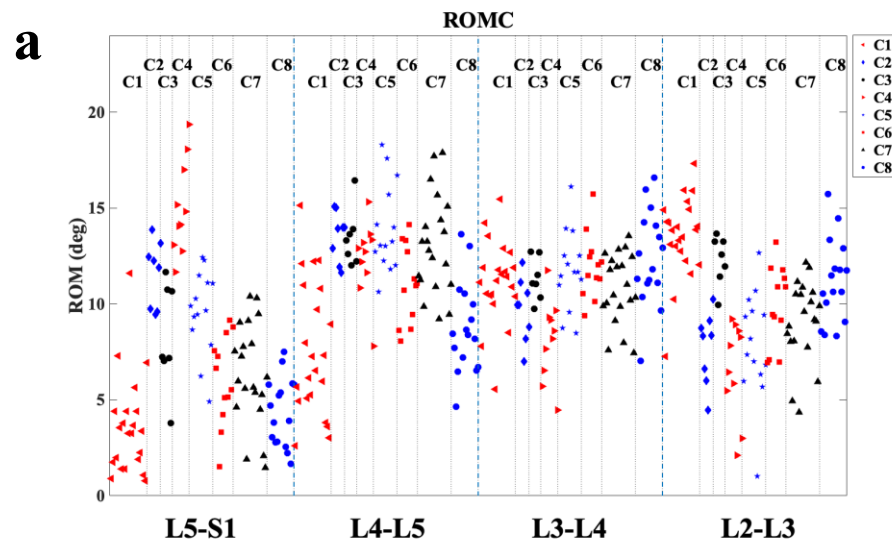
**Fig. 6.** Ensemble mean of intervertebral angle for each clustering variable: a) range of motion clustering (ROMC); C1 (black), C2 (red), C3 (blue), C4 (magenta), C5 (orange), C6 (light green), C7 (cyan), C8 (dark green). b) Peaking time of first derivative with separate segmentation (PTFDs); C1 (black), C2 (red), C3 (blue), C4 (green). c) peaking magnitude of first derivative (PMFD); C1 (black), C2 (red), C3 (blue), C4 (magenta), C5 (orange), C6 (light green), C7 (cyan), C8 (dark green). d) Peaking time of first derivative with stepwise segmentation (PTFDss); C1 (black), C2 (red), C3 (blue), and C4 (green). Sig: significant (magenta patch area).

**Fig. 7.** Segmental ensemble means of first derivatives (velocity): a) clustering (C1-C4) of participants by peaking time of first derivative with separate segmentation (PTFDs); L2-L3 (green), L3-L4 (blue), L4-L5 (orange), L5-S1 (red). b) clustering (C1-C4) of the participants by peaking time of first derivative with stepwise segmentation (PTFDss); L2-S1 (green), L3-S1 (blue), L4-S1 (orange), L5-S1 (red).

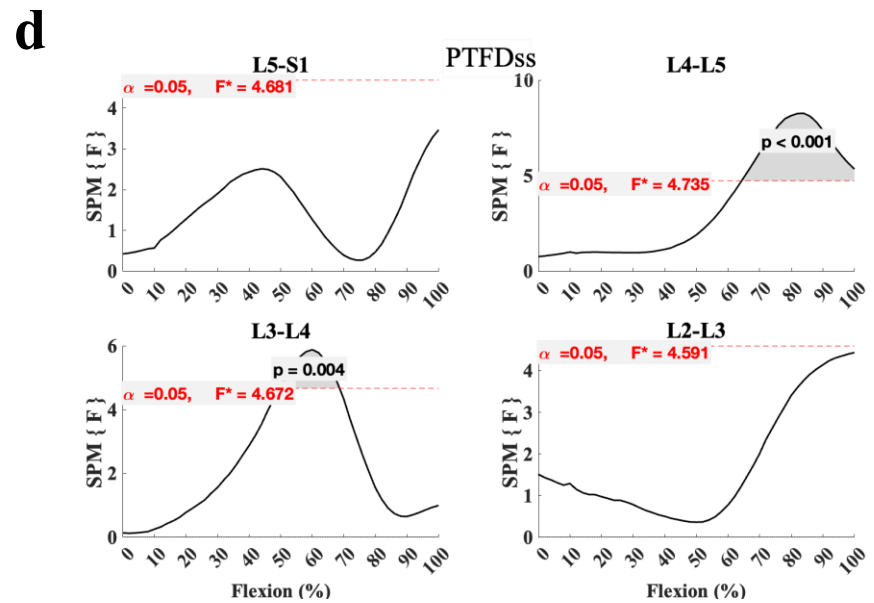
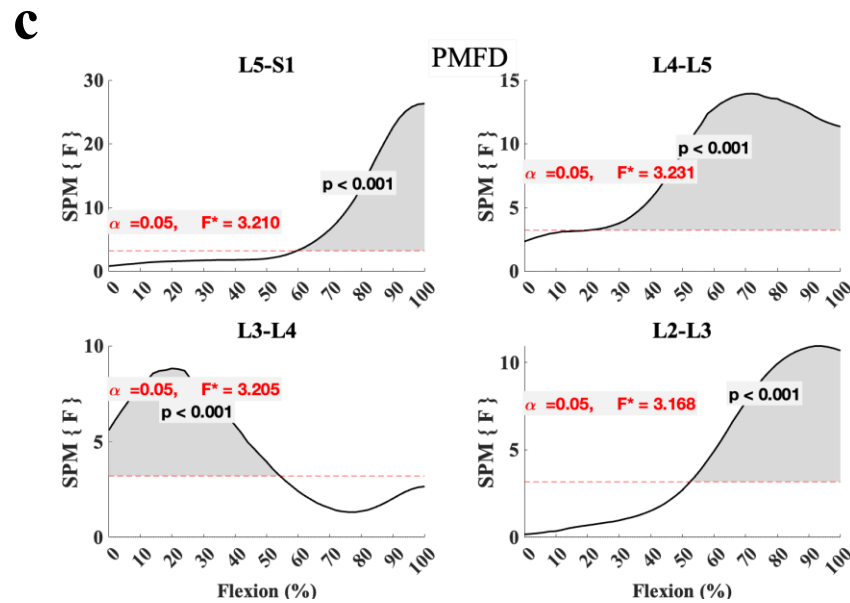
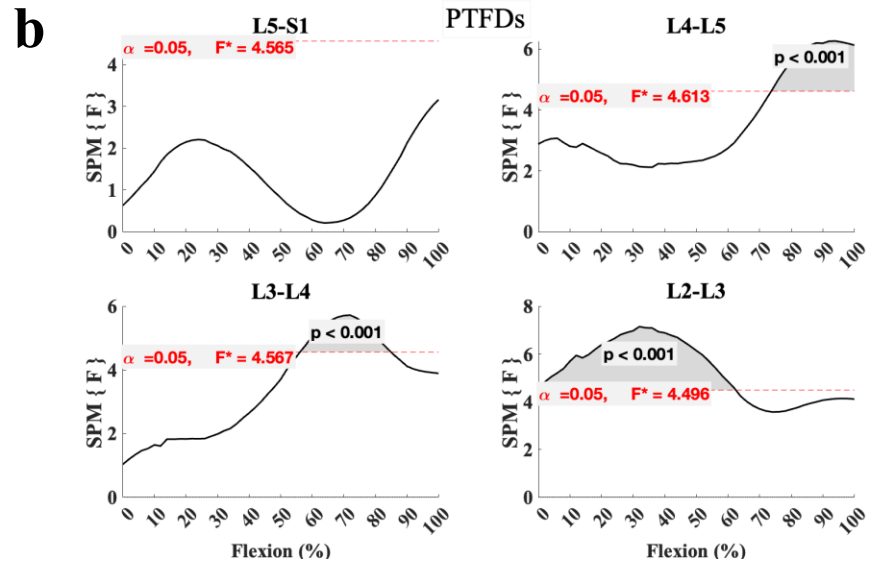
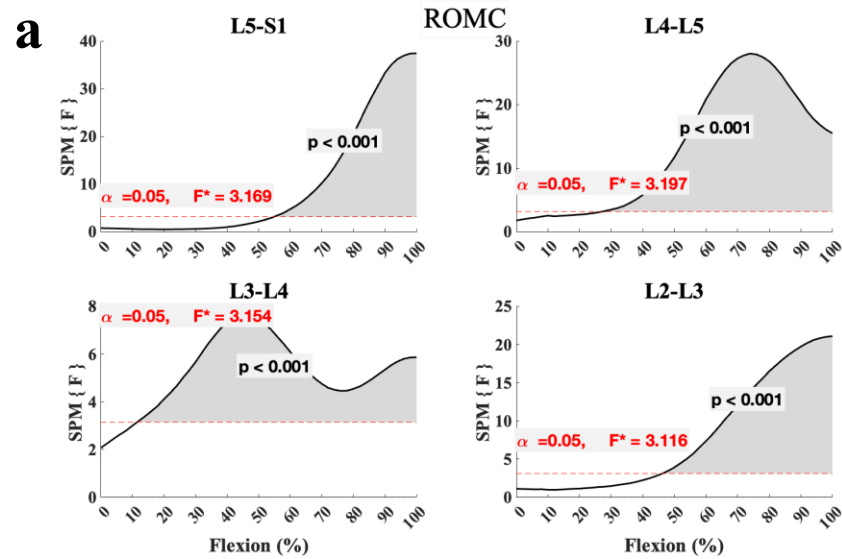


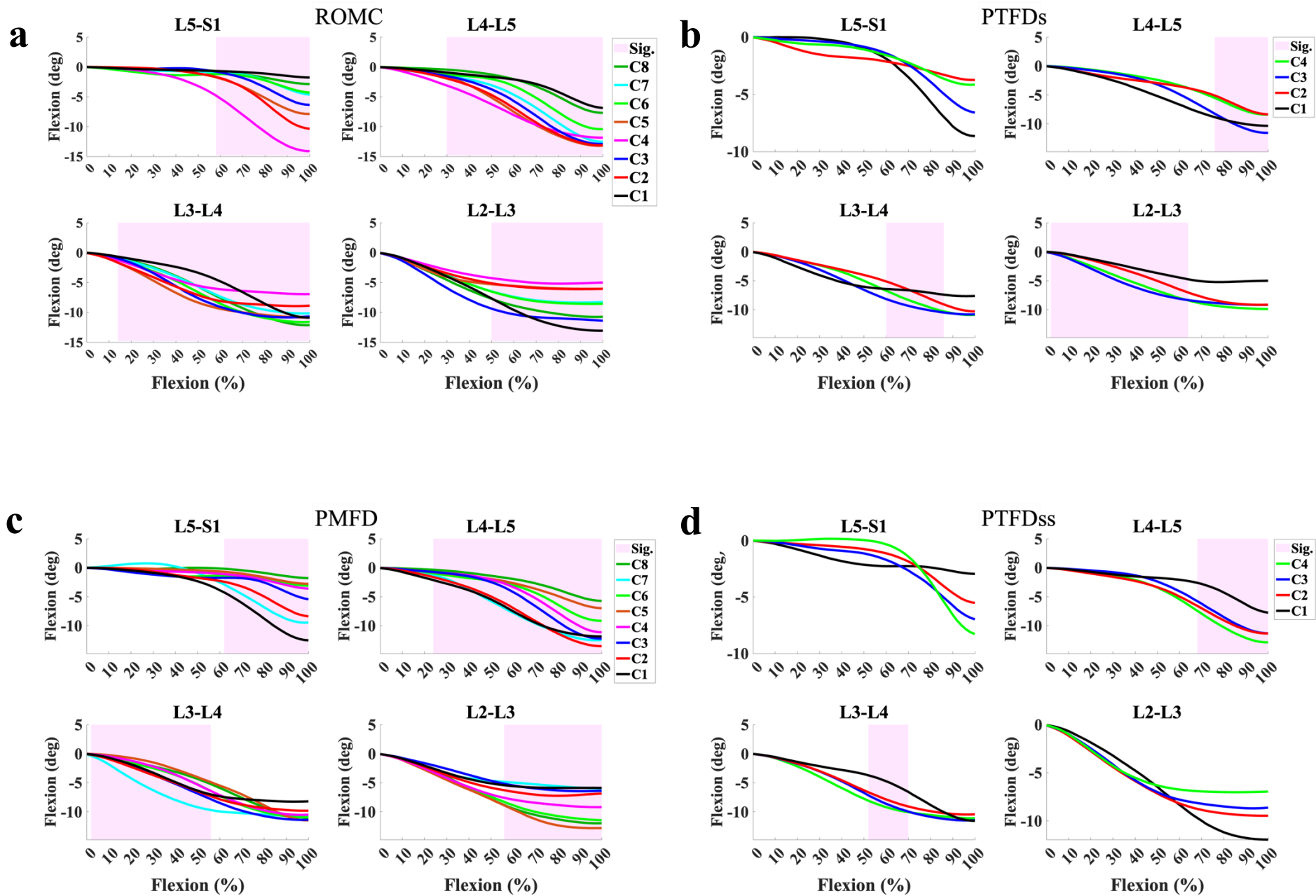














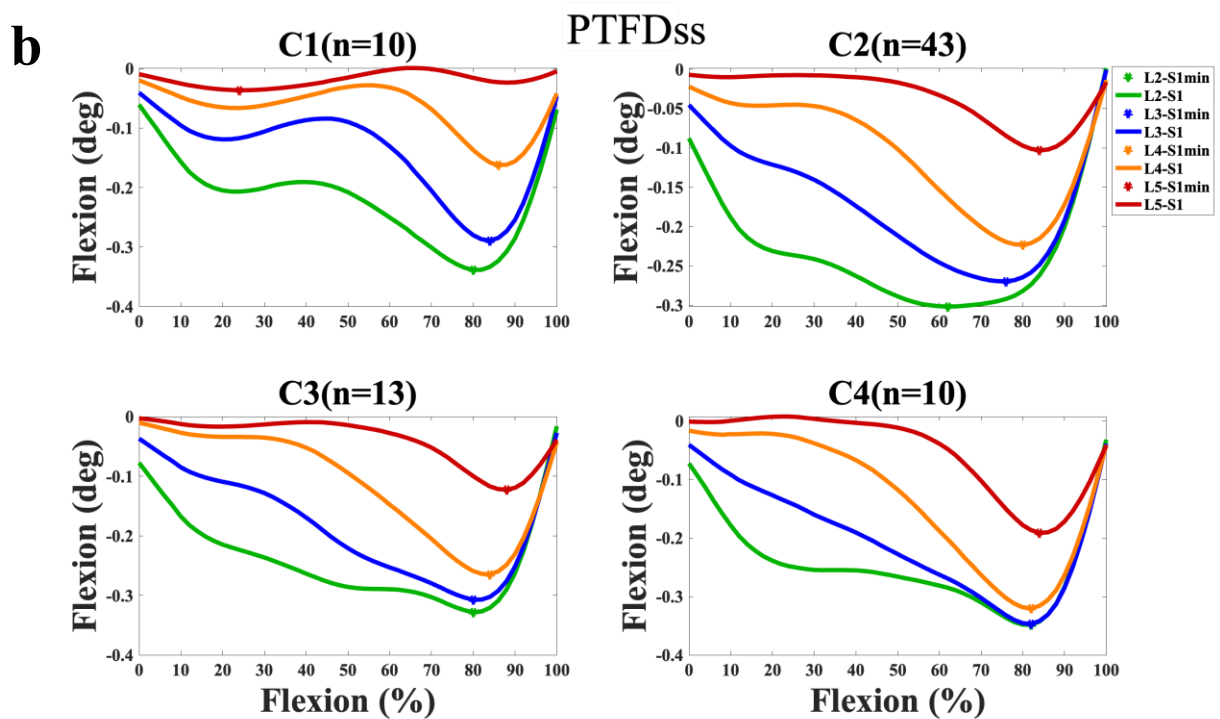
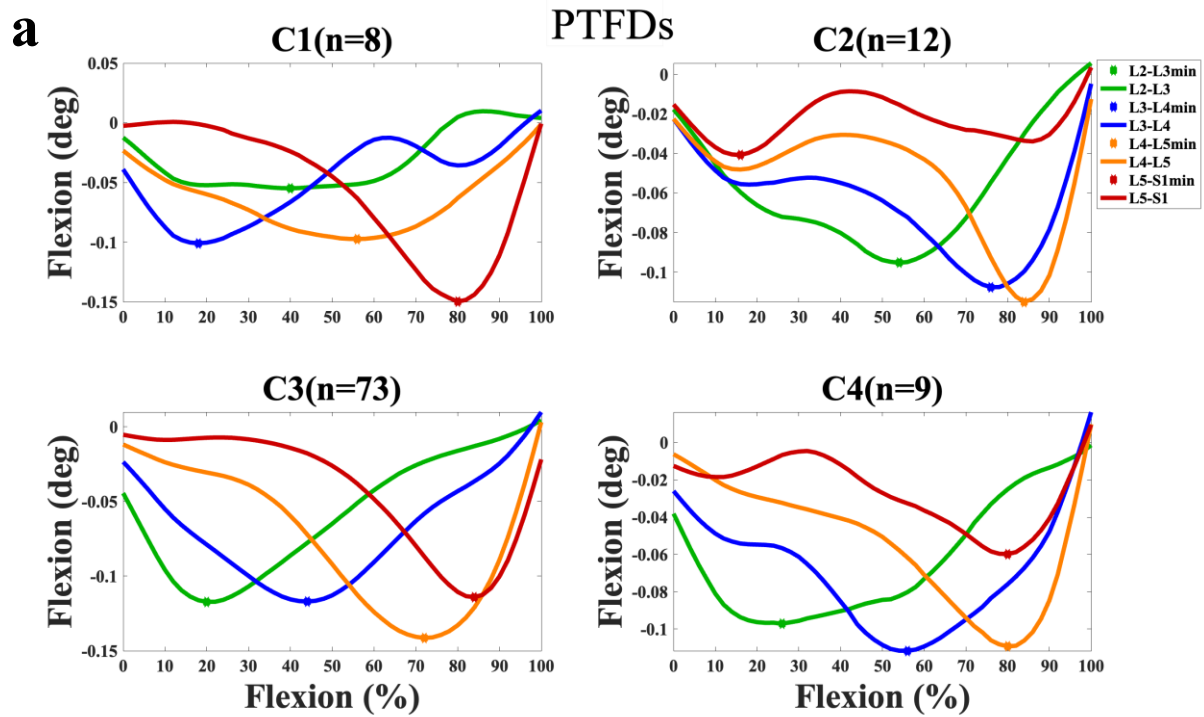


Table 1. Demographic information of the subjects with and without considering gender.


| Gender | Number | Age (year) |       |             | Height (m) |      |           | Weight (kg) |       |              | BMI (kg/m <sup>2</sup> ) |      |             |
|--------|--------|------------|-------|-------------|------------|------|-----------|-------------|-------|--------------|--------------------------|------|-------------|
|        |        | Mean       | SD    | Range       | Mean       | SD   | Range     | Mean        | SD    | Range        | Mean                     | SD   | Range       |
| Male   | 68     | 34.34      | 13.09 | 22.00-70.00 | 1.79       | 0.06 | 1.65-1.94 | 80.69       | 9.38  | 63.00-112.40 | 24.91                    | 2.35 | 18.99-31.80 |
| Female | 59     | 43.27      | 12.89 | 21.00-69.00 | 1.66       | 0.06 | 1.53-1.78 | 62.91       | 7.96  | 47.60-79.00  | 22.74                    | 2.90 | 16.03-29.01 |
| All    | 127    | 38.56      | 13.70 | 21.00-70.00 | 1.73       | 0.09 | 1.53-1.94 | 72.30       | 12.45 | 47.60-112.40 | 23.89                    | 2.83 | 16.03-31.80 |

Table 2. Clustering variables, patterns and ROM's means and standard deviations for each cluster and variable

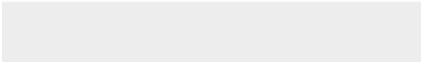

Clustering asymptomatic participants


| Clustering asymptomatic participants |                       |              |               |             |               |   |     |       |      |       |      |       |      |       |      |       |       |  |
|--------------------------------------|-----------------------|--------------|---------------|-------------|---------------|---|-----|-------|------|-------|------|-------|------|-------|------|-------|-------|--|
| Demographic                          |                       |              |               |             |               |   |     |       |      |       |      |       |      |       |      |       |       |  |
| Segments ROM                         |                       |              |               |             |               |   |     |       |      |       |      |       |      |       |      |       |       |  |
| C                                    | Pattern               | Gender (m/f) | Age (SD)      | Hight (SD)  | Weight (SD)   | D | Frq | L5-S1 | SD   | L4-L5 | SD   | L3-L4 | SD   | L2-L3 | SD   | L2-S1 | SD    |  |
| ROMC                                 | 1 L5S1<L4L5<L3L4<L2L3 | 15/7         | 33.86 (11.26) | 1.74 (0.07) | 73.10 (10.82) | U | 22  | 3.60  | 2.55 | 7.60  | 3.47 | 11.18 | 2.11 | 13.53 | 2.11 | 35.91 | 6.83  |  |
|                                      | 2 L4L5>L5S1>L2L3>L3L4 | 7/1          | 31.00 (10.29) | 1.78 (0.06) | 80.12 (9.76)  | L | 8   | 11.55 | 1.73 | 13.55 | 1.29 | 9.71  | 1.66 | 7.72  | 1.88 | 42.55 | 4.65  |  |
|                                      | 3 L4L5>L2L3>L3L4>L5S1 | 3/4          | 44.14 (16.33) | 1.71 (0.09) | 69.78 (10.12) | - | 7   | 8.33  | 2.80 | 13.42 | 1.44 | 11.30 | 1.11 | 12.30 | 1.29 | 45.37 | 0.74  |  |
|                                      | 4 L5S1>L4L5>L3L4>L2L3 | 4/6          | 40.00 (13.03) | 1.70 (0.06) | 63.37 (9.72)  | L | 10  | 15.01 | 2.44 | 12.35 | 2.00 | 8.09  | 2.11 | 6.56  | 2.48 | 42.02 | 5.02  |  |
|                                      | 5 L4L5>L3L4>L5S1>L2L3 | 8/6          | 36.42 (13.18) | 1.74 (0.07) | 73.45 (12.81) | M | 14  | 9.62  | 2.16 | 13.93 | 2.30 | 11.77 | 2.05 | 7.88  | 2.82 | 43.20 | 5.83  |  |
|                                      | 6 L3L4>L4L5>L2L3>L5S1 | 6/6          | 38.58 (10.41) | 1.71 (0.11) | 69.47 (11.08) | M | 12  | 6.07  | 2.34 | 11.03 | 2.05 | 11.93 | 1.69 | 9.91  | 2.10 | 38.94 | 5.40  |  |
|                                      | 7 L4L5>L3L4>L2L3>L5S1 | 8/12         | 40.36 (12.52) | 1.71 (0.07) | 71.57 (14.73) | M | 20  | 6.37  | 2.66 | 13.09 | 2.56 | 10.81 | 1.83 | 9.09  | 2.14 | 39.37 | 6.82  |  |
|                                      | 8 L3L4>L2L3>L4L5>L5S1 | 7/9          | 40.00 (15.40) | 1.71 (0.09) | 73.28 (12.65) | U | 16  | 4.38  | 1.77 | 8.75  | 2.39 | 12.40 | 2.47 | 11.22 | 2.15 | 36.76 | 5.92  |  |
| PTFDs                                | N                     | 58/51        |               |             |               |   | 109 |       |      |       |      |       |      |       |      |       |       |  |
|                                      | ANOVA                 | F            | 1.11          | 1.04        | 1.40          |   |     | 33.15 |      | 14.61 |      | 5.34  |      | 16.74 |      | 3.98  |       |  |
|                                      | Sig.                  |              | 0.363         | 0.407       | 0.214         |   |     | 0.000 |      | 0.000 |      | 0.000 |      | 0.000 |      | 0.001 |       |  |
|                                      | 1 L5S1>L4L5>L3L4>L2L3 | 2/6          | 42.57 (13.48) | 1.67 (0.10) | 64.00 (15.89) | L | 8   | 9.60  | 6.34 | 10.37 | 4.32 | 7.71  | 3.77 | 5.80  | 3.82 | 33.47 | 18.25 |  |
|                                      | 2 L4L5>L3L4>L2L3>L5S1 | 8/4          | 31.41 (8.29)  | 1.76 (0.10) | 78.72 (11.83) | U | 12  | 4.23  | 3.11 | 8.35  | 3.15 | 10.38 | 2.81 | 9.37  | 3.80 | 32.33 | 12.87 |  |
|                                      | 3 L5S1>L4L5>L3L4>L2L3 | 40/33        | 38.64 (14.00) | 1.73 (0.08) | 71.32 (10.93) | - | 73  | 7.31  | 4.08 | 11.66 | 2.94 | 10.89 | 2.28 | 9.32  | 3.00 | 39.17 | 12.30 |  |
|                                      | 4 L4L5>L5S1>L3L4>L2L3 | 5/4          | 48.66 (17.54) | 1.74 (0.12) | 78.06 (18.64) | U | 9   | 5.63  | 1.85 | 8.60  | 2.46 | 10.98 | 2.47 | 9.92  | 3.20 | 35.13 | 9.98  |  |
|                                      | N                     | 55/47        |               |             |               |   | 102 |       |      |       |      |       |      |       |      |       |       |  |
| PMFD                                 | ANOVA                 | F            | 2.85          | 1.41        | 3.01          |   |     | 3.44  |      | 6.10  |      | 4.00  |      | 3.218 |      | 5.92  |       |  |
|                                      | Sig.                  |              | 0.041         | 0.244       | 0.034         |   |     | 0.020 |      | 0.001 |      | 0.010 |      | 0.026 |      | 0.001 |       |  |
|                                      | 1 L5S1>L4L5>L3L4>L2L3 | 8/8          | 34.66 (12.69) | 1.72 (0.07) | 66.96 (11.96) | L | 16  | 12.94 | 3.16 | 11.89 | 1.90 | 8.41  | 2.76 | 6.34  | 2.81 | 39.57 | 10.63 |  |
|                                      | 2 L4L5>L5S1>L3L4>L2L3 | 4/3          | 37.42 (18.01) | 1.71 (0.09) | 72.76 (15.25) | M | 7   | 8.76  | 2.72 | 13.53 | 2.41 | 9.81  | 1.73 | 7.46  | 2.42 | 39.55 | 9.28  |  |
|                                      | 3 L4L5>L3L4>L5S1>L2L3 | 4/5          | 37.77 (9.56)  | 1.73 (0.09) | 73.70 (14.97) | M | 9   | 5.77  | 1.69 | 12.34 | 3.18 | 11.38 | 2.37 | 6.56  | 1.95 | 36.04 | 9.19  |  |
|                                      | 4 L4L5>L3L4>L2L3>L5S1 | 11/13        | 42.34 (13.74) | 1.72 (0.08) | 74.12 (12.25) | M | 24  | 4.32  | 2.32 | 11.18 | 2.65 | 10.64 | 2.17 | 9.35  | 3.16 | 35.49 | 10.31 |  |
|                                      | 5 L3L4>L2L3>L4L5>L5S1 | 7/2          | 32.33 (10.97) | 1.75 (0.10) | 74.33 (12.88) | U | 9   | 3.30  | 2.65 | 7.03  | 4.31 | 11.52 | 1.16 | 12.87 | 2.60 | 34.72 | 10.72 |  |
|                                      | 6 L2L3>L4L5>L3L4>L5S1 | 6/2          | 32.75 (10.52) | 1.77 (0.09) | 74.15 (12.63) | U | 8   | 4.14  | 2.51 | 9.63  | 2.86 | 10.93 | 2.97 | 11.45 | 1.29 | 36.14 | 9.63  |  |
| PTFDss                               | 7 L5S1>L3L4>L4L5>L2L3 | 4/3          | 38.57 (18.33) | 1.76 (0.11) | 79.10 (12.74) | L | 7   | 11.15 | 2.33 | 12.52 | 2.57 | 10.75 | 2.16 | 6.14  | 2.78 | 40.57 | 9.85  |  |
|                                      | 8 L2L3>L3L4>L4L5>L5S1 | 7/7          | 36.57 (14.78) | 1.70 (0.06) | 67.90 (9.55)  | U | 14  | 2.85  | 1.88 | 5.92  | 2.07 | 11.01 | 2.87 | 12.00 | 3.01 | 31.78 | 9.82  |  |
|                                      | N                     | 51/43        |               |             |               |   | 94  |       |      |       |      |       |      |       |      |       |       |  |
|                                      | ANOVA                 | F            | 0.84          | 0.66        | 1.08          |   |     | 30.57 |      | 11.08 |      | 2.36  |      | 10.53 |      | 2.46  |       |  |
|                                      | Sig.                  |              | 0.557         | 0.700       | 0.382         |   |     | 0.000 |      | 0.000 |      | 0.029 |      | 0.000 |      | 0.024 |       |  |
|                                      | 1 L4S1>L3S1>L2S1>L5S1 | 5/5          | 32.00 (8.76)  | 1.73 (0.08) | 73.81 (9.46)  | U | 10  | 3.41  | 2.62 | 7.87  | 2.45 | 11.54 | 2.36 | 12.02 | 2.83 | 34.84 | 10.26 |  |
|                                      | 2 L5S1>L4S1>L3S1>L2S1 | 21/22        | 40.57 (14.62) | 1.72 (0.10) | 70.42 (10.50) | M | 43  | 6.28  | 3.58 | 11.40 | 3.39 | 10.59 | 2.56 | 9.62  | 3.22 | 37.89 | 12.76 |  |
|                                      | 3 L5S1>L4S1>L3S1>L2S1 | 6/7          | 35.00 (11.52) | 1.71 (0.07) | 66.66 (12.57) | M | 13  | 7.63  | 4.29 | 11.41 | 2.91 | 11.49 | 2.09 | 8.78  | 2.66 | 39.31 | 11.95 |  |
|                                      | 4 L5S1>L4S1>L3S1>L2S1 | 6/4          | 33.50 (12.08) | 1.74 (0.08) | 71.86 (12.48) | M | 10  | 9.03  | 3.74 | 12.94 | 1.98 | 11.10 | 1.95 | 7.26  | 2.87 | 40.34 | 10.54 |  |
|                                      | N                     | 38/38        |               |             |               |   | 76  |       |      |       |      |       |      |       |      |       |       |  |
|                                      | ANOVA                 | F            | 1.80          | 0.32        | 0.87          |   |     | 4.51  |      | 5.09  |      | 0.76  |      | 4.34  |      | 1.30  |       |  |
|                                      | Sig.                  |              | 0.155         | 0.810       | 0.458         |   |     | 0.006 |      | 0.003 |      | 0.520 |      | 0.007 |      | 0.279 |       |  |

ROMC: range of motion clustering; PTFDs: peaking time of first derivative for separate segmentation; PMFD: peaking magnitude of first derivative; PTFDss: peaking time of first derivative for stepwise segmentation; C: cluster's number; m: male; f: female; SD: standard deviation; D: segmental mobility domain; U: upper; M: middle; L: lower; Frq: number of participants to each clustering variable; N: total number of participants that distributed by each variable in clusters; F: f value for one way ANOVA; Sig: Significant; bolded number: main significant difference. L5S1 (red), L4L5 (orange), L3L4 (blue), L2L3 (green).

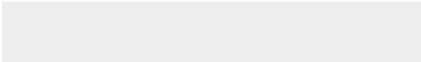




Click here to access/download  
**Supplementary Material**  
Fig.2a.png



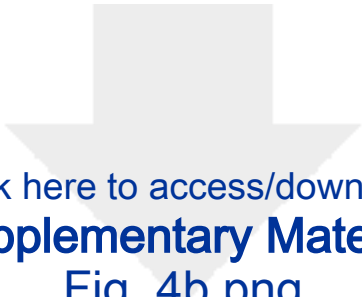


Click here to access/download  
**Supplementary Material**  
Fig.2b.png

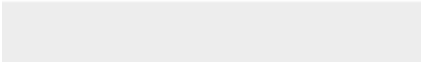






Click here to access/download  
**Supplementary Material**  
Fig. 4a.png

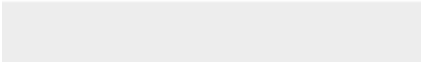



Click here to access/download  
**Supplementary Material**  
Fig. 4b.png







Click here to access/download  
**Supplementary Material**  
Fig. 4c.png



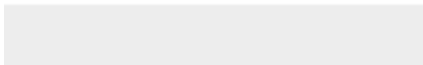



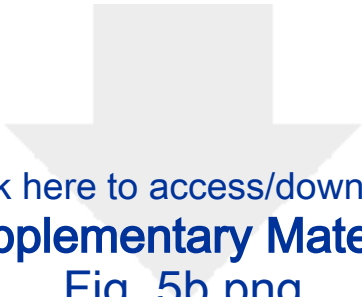


Click here to access/download  
**Supplementary Material**  
Fig. 4d.png

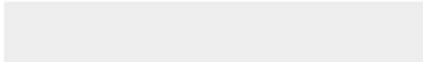




Click here to access/download  
**Supplementary Material**  
Fig. 5a.png



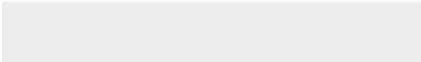




Click here to access/download  
**Supplementary Material**  
Fig. 5b.png



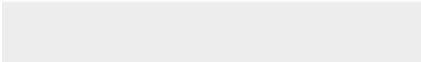




Click here to access/download  
**Supplementary Material**  
Fig. 5c.png



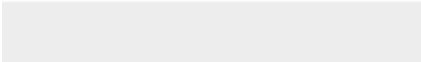
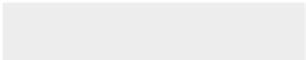


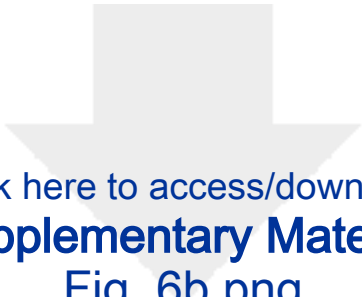
Click here to access/download  
**Supplementary Material**  
Fig. 5d.png



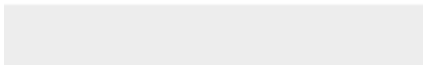
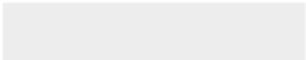



Click here to access/download  
**Supplementary Material**  
Fig. 6a.png



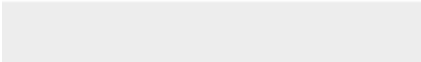



Click here to access/download  
**Supplementary Material**  
Fig. 6b.png






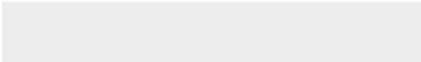

Click here to access/download  
**Supplementary Material**  
Fig. 6c.png







Click here to access/download  
**Supplementary Material**  
Fig. 6d.png



**Conflict of interest statement**

The authors declare no conflicts of interest.