Clinical Biomechanics

Progression of muscular co-activation and gait variability in children with Duchenne Muscular Dystrophy: a 2-year follow-up study

--Manuscript Draft--

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Abstract:	Background.Duchenne muscular dystrophy is an X-linked muscle disease caused by dystrophin absence. Muscle weakness is a major determinant of the gait impairments in patients with Duchenne muscular dystrophy and it affects lower limbs more often than upper limbs. Monitoring progression of motor symptoms is key to plan treatments for prolonging ambulation. Methods.The progression of gait impairment in a group of ten patients with Duchenne muscular dystrophy was observed longitudinally three times over a period of 2 years by computerized gait analysis system. Spatio-temporal parameters of gait, and variability indicators were extracted from kinematics, while lower limb muscles coactivation were measured at the baseline and at each follow-up evaluation. The 6-minute walk test was used to evaluate functional capacity at each time session. Findings.We found a significant increase in stride width and in both stride width and stride length variability at the 1-and 2-year follow-up evaluations. Furthermore, significant higher values in proximal muscle coactivation and significant lower values in both distal muscle coactivation and functional capacity were found at the 2-year follow-up evaluation. Significant negative correlations between muscle coactivation at proximal level and functional capacity and between muscle coactivation at distal level and gait variability were observed. Interpretation.Our findings suggest that patients with Duchenne muscular dystrophy exhibit decline in functional capacity after 2 years from the baseline. Moreover, to cope with disease progression, patients try to maintain an effective gait by changing the balance dynamic strategies (i.e. increase in proximal muscle coactivation) during the course of disease.
Suggested Reviewers:	Alberto Ranavolo a.ranavolo@inail.it Competences in movement analysis for clinical applications
	Francesco Di Nardo f.dinardo@univpm.it Competences in surface EMG analysis

Response to Reviewers:	Reviewer #1: General This study investigated the gait change of the Duchenne Muscular Dystrophy children at the 1-and 2-year follow-up. Gait spatio-temporal parameters, variability indicators, muscles co activation indexes, and The 6-minute walk test index were analyzed. The findings implied the gait decline and muscle co-activation strategy after 2 years from the baseline, The topic of the paper is relevant. Gait decline could be caused by several factors including muscle strength and neuromotor function. Quantification of the gait decline pattern is important for understanding of the Duchenne Muscular Dystrophy progression pathology. However, the present results could not clear help to distinguish different factors for gait decline. Thank you for your general comments. As a matter of fact, the reduced sample size, combined with the intrinsic variability of motor manifestations of DMD (and the corresponding mechanisms of compensation) could not help distinguishing factors for gait decline, which, however, was not the main aim of the research study. The aim of our work is to assess the role of lower limb muscle coactivation in a group of patients with Duchenne Muscular Dystrophy; to evaluate its extent and its relationship with the progression of gait impairment at the 1-and 2-year follow-up evaluations and to compare the obtained data with functional capacity. Further study with larger sample sizes could be useful to extend the knowledge of this pathology by investigating other parameters linked to gait decline. These elements were more specifically detailed in the final part of the manuscript. Specific Page 7, 2.2 Gait analysis. Please provide a figure of gait analysis including subject with markers and electrodes. Agreed. A new figure (Figure 1) of gait analysis of a representative subject was added. Page 7, line 160-161. Several muscles have been evaluated, but no results of them have been presented. While, at first, we decided to include results only for those muscles that exhibited significant modifications as time goes by, we concur with this reviewer on the utility of adding the results for all the recorded muscles. We have discarded Gluteus Medius from the subsequent analysis, as we did not record any antagonist muscle acting on the hip. We have added the results regarding all the muscle pairs acting on the same joint, and modified the corresponding figure to include the results. Page 10, 2.5 Statistical analysis. Since the sample size was small, did authors exam the inter-class correlation? The gait changes with children grow up, how did the indexes (Gait spatio-temporal parameters, variability indicators, muscles co activation indexes) change for healthy children of similar age. If you refer to checking linear relations among different evaluation sessions (as classes), thank you and agreed. We added, for each extracted parameter (gait parameters and muscular co-activations) linear relation coefficients, and the corresponding statistical significance. This helped us better clarify the obtained results regarding those gait parameters that exhibited a clear trend with the evaluation time (i.e. gait speed, stride length and stride width variability), and the muscular co-activations that displayed a similar behavior. Regarding the interpretation of results for stride length and width, it is agreed that for healthy children of similar age, gait speed is still somewhat increasing with age (Sutherland), while we did not observe such kind of trend in kids with DMD (and this might be associated with the functional decline in gait). The presence of a decrease in normalized stride length variability (and step width variability) may be linked with an increasing instability of gait ,and this matches with the gait deterioration). We briefly discussed these results in comparison with what the gait

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Rome 9th May 2020

Subject: Submission of a revised manuscript to "Clinical Biomechanics"

Title: Progression of muscular co-activation and gait variability in children with Duchenne Muscular Dystrophy: a 2-year follow-up study

Authors: Martina Rinaldi, Maurizio Petrarca , Alberto Romano, Gessica Vasco, Carmen D'Anna, Daniele Bibbo, Maurizio Schmid , Enrico Castelli, Silvia Conforto.

Dear Editor,
We are re-submitting a revised version of our manuscript "Progression of muscular co-activation and gait variability in children with Duchenne Muscular Dystrophy: a 2-year follow-up study".
We thank the Editor and Reviewers for their comments whose value was fully appreciated by the Authors.

Yours sincerely,

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CLINICAL BIOMECHANICS AUTHOR CHECKLIST

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RESPONSE TO Editor and Reviewers comments:

We would like to thank both the Editor and the Reviewers for their constructive and useful indications and comments, which made us reshape some parts of the manuscript. In particular, we list here the main modifications:

1. *We added the analysis and the results of all the monitored muscle pairs, thus not limiting to the pairs for which we found an effect coming from the evaluation time.*
2. *We slightly modified the statistical analysis to include indicators of interclass correlation, as requested by reviewer #1.*
3. *We added a figure showing the experimental set-up used for the analysis and modified a figure to include results coming from all muscle pairs.*
4. *We modified the discussion section to include comments regarding the relation between all muscle pairs, functional capacity indicators, and gait parameters.*
5. *We modified and reduced the size of the introduction section to maintain the word count approximately within word limits.*

In the following, we will answer to each comment inline in italic format.

Editor:

Avoid abbreviations in abstract (keeping to a maximum of 250 words)

We have removed all the abbreviation, without exceeding 250 word.

Reduce main text word count (max 4000 words, excluding references and legends)

Ok, we reshaped the main text, with specific reference to the introduction, to approximately fit within the word count limits.

Reviewer #1:

<u>General</u>

This study investigated the gait change of the Duchenne Muscular Dystrophy children at the 1-and 2-year follow-up. Gait spatio-temporal parameters, variability indicators, muscles co activation indexes, and The 6-minute walk test index were analyzed. The findings implied the gait decline and muscle co-activation strategy after 2 years from the baseline,

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A typical photo of a walking patient from your lab would enrich conveying of your subject to readers, however, I understand one has to save space. Think about it.

Thank you. A new figure (Figure 1) of gait analysis of a representative subject was added.

Highlights

- Patients with Duchenne muscular dystrophy exhibit gait decline during the years
- Muscle dystrophy at distal level leads to more unstable gait.
- Proximal muscle coactivation is a possible strategy to maintain effective gait

Progression of muscular co-activation and gait variability in children with Duchenne Muscular Dystrophy: a 2-year follow-up study

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Declaration of Interest

Authors declare no commercial or other associations that might pose a conflict of interest in connection with the present article.

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Abstract

Background

Duchenne muscular dystrophy is an X-linked muscle disease caused by dystrophin absence. Muscle weakness is a major determinant of the gait impairments in patients with Duchenne muscular dystrophy and it affects lower limbs more often than upper limbs. Monitoring progression of motor symptoms is key to plan treatments for prolonging ambulation.

Methods

The progression of gait impairment in a group of ten patients with Duchenne muscular dystrophy was observed longitudinally three times over a period of 2 years by computerized gait analysis system. Spatio-temporal parameters of gait, and variability indicators were extracted from kinematics, while lower limb muscles coactivation were measured at the baseline and at each follow-up evaluation. The 6-minute walk test was used to evaluate functional capacity at each time session.

Findings

We found a significant increase in stride width and in both stride width and stride length variability at the 1-and 2-year follow-up evaluations. Furthermore, significant higher values in proximal muscle coactivation and significant lower values in both distal muscle coactivation and functional capacity were found at the 2-year follow-up evaluation. Significant negative correlations between muscle coactivation at proximal level and functional capacity and between muscle coactivation at distal level and gait variability were observed.

Interpretation

Our findings suggest that patients with Duchenne muscular dystrophy exhibit decline in functional capacity after 2 years from the baseline. Moreover, to cope with disease progression, patients try to

maintain an effective gait by changing the balance dynamic strategies (i.e. increase in proximal muscle coactivation) during the course of disease.

Keywords

Duchenne Muscular Dystrophy

Longitudinal study

Muscle coactivation

Gait performance

1. Introduction

Duchenne muscular dystrophy (DMD) is a genetic X-linked muscle disease caused by a dystrophin deficiency damaging and weakening muscular cells. It has an inherited origin, even if 30% of cases are due to new mutations. The absence of dystrophin induces a progressive muscular weakness and compromises locomotion and cardiopulmonary function. The prevalence rate for DMD is around 63 per million. It appears in the first years of life and is generally diagnosed at the age of 3-4 years by physicians' observations and genetic tests **(Armand et al., 2005; Emery 1991; Chakkalakal et al., 2005**). Muscle weakness is more proximal than distal, it usually affects lower more than upper limbs, and extensor more than flexor muscles **(McDonald et al., 1995; Carter et al., 1995).**

When compared with age-matched unaffected children, DMD patients show poorer cognitive, language and motor functions with clear difficulties in running and climbing and descending stairs (**Bakker et al., 2002).** Muscle weakness is a major determinant of the gait impairments in patients with DMD, followed by muscle and tendon retractions and joint deformities. Compensatory movements and biomechanical adaptations are needed to maintain the motor function **(Martini et al., 2014)** and to walk for some period despite limited muscle strength (**Sutherland et al., 1981; Gaudreault et al., 2007; D'Angelo et al., 2009**); to assist in prolonging ambulation, ankle foot orthoses have been proven useful **(de Souza et al., 2016)**.

Nowadays a pharmacological treatment based on corticosteroids is used to postpone spinal deformities and muscle contractures (**Goudriaan et al., 2018**), and to slow down the course of the disease so prolonging the walking autonomy time – most children become wheelchair-dependent when they are about 12 years old (**Gaudreault et al., 2007).** In this scenario, the assessment of mechanisms associated with abnormal gait patterns is important. The six-minute walking test (6MWT) (**McDonald et al., 2010)** is often used to evaluate walking ability in DMD. Being an overall measure of functional capacity, it does not directly allow for a detailed analysis of neuromuscular and biomechanical determinants of walking function.

Kinematics and kinetics recorded during gait of children with DMD outline: i) modifications of trunk and lower limb position during the stance phase; ii) increased anterior pelvic tilt, as the result of a reduced muscular strength for hip and knee muscle extensors (i.e. gluteus maximus and quadriceps); iii) increased hip flexion and abduction in association with an increased ankle plantar flexion, to maintain the pelvic stability and alignment, (**Gaudreault et al., 2007);** iv) prominence of the lumbar curve during the years in relationship with an observed shortening of the plantar flexor muscles (i.e. soleus and gastrocnemius) (**Carvalho et al. 2015)**. Modifications of walking parameters – decrease of gait cadence **(Sutherland et al., 1981),** speed and step length, increase of step width – have been associated with the progression of the disease.

While the biomechanics of gait in DMD children has been investigated also focusing on the analysis of specific joints (**Bakker et al., 2002; Armand et al., 2005**), fewer studies introduced surface EMG (sEMG) for the characterization of this pathology (**Ropars et al., 2016)**.

sEMG seems particularly attractive for the characterization of this pathology, in terms of muscular weakness and deviations in muscular activity that mainly regard activations between muscles acting on the same joints. Moreover, sEMG is a non-invasive technique, which benefits from a variety of algorithms to extract neural correlates from the recorded data: estimation of amplitude (**D'Alessio and Conforto, 2001; Ranaldi et al., 2018)** and timing of the muscular activations (**Vannozzi et al., 2010; Severini et al., 2012)** and co-activation (**Rinaldi et al., 2018; Varrecchia et al., 2018; Rinaldi et al., 2017)**.

In the present study we analysed the co-activation patterns of lower limb muscles in DMD children through sEMG signals recorded at different times from the disease onset during unconstrained gait, and we tried to link these patterns with motor function and gait parameters.

The purposes of this study were therefore: (i) to assess the role of lower limb muscle coactivation in a group of patients with DMD; (ii) to evaluate its extent and its relationship with the progression of gait impairment at the 1-and 2-year follow-up evaluations; (iii) to compare the obtained data with functional capacity indicators.

2. Methods

2.1 Subjects

Ten male children with DMD were recruited (age 5.51 ± 1.35 years, height 1.20 ± 0.09 m, weight 27.16 ± 8.19 kg). All of them were able to walk without assistance or walking aids on a level surface, and no one underwent surgical interventions at the level of the lower limbs. A preliminary visit performed right after the diagnosis included a first evaluation of the severity of the disease, and a battery of motor tests including the 6MWT (**Mylius et al., 2016**) and the North Star Ambulatory Assessment (**Mazzone et al., 2010**). Pharmacological treatment was generally planned at the time of the first visit and comprised glucocorticosteroids therapy administered daily and an alternate daily regimen, according to **(Guglieri et al. 2017**). After the first evaluation, a series of gait sessions was programmed: the first (T0), happened generally two years after the first visit, so to ensure the effect of the pharmacological treatment. The two subsequent gait sessions (T1 and T2) were scheduled with an interval of one year.

2.2 Gait analysis

Kinematics and kinetics were recorded at 200 Hz using a stereo-photogrammetric system (VICON – Nexus motion capture Software) with twelve infrared and three high resolution cameras for video recording, and two force plates (AMTI, or6-6, US). Thirty-five reflective spherical markers were attached on the anatomical landmarks, in accordance with a validated biomechanical model (**Adams et al. 2018; Benedetti at al., 2011**). Anthropometric data were collected for each subject (**Baker 2013; Winter 1979).**

Surface EMG signals were recorded at 1000 Hz using a *Mini Wave* sEMG wireless probe system (Cometa System). After skin preparation, bipolar Ag/AgCl surface electrodes (2 cm diameter) were placed over the muscle belly in the direction of the muscle fibres – according to the European recommendations for surface electromyography (SENIAM) and the atlas of muscle innervation zones (**Barbero et al., 2012**; **Hermens et al., 2000**) – on: rectus femoris (RF); vastus lateralis (VL);

biceps femoris (BF); medial hamstring (HAM); tibialis anterior (TA); medial gastrocnemius (MG), soleus (SOL) (Figure 1).

2.3 Experimental Procedure

For each gait session, children were asked to walk barefoot at comfortable self-selected speeds along a walkway approximately 10 m long while looking forward. Because we were interested in natural locomotion, only general, qualitative instructions were provided. Before the recording session, the subjects practiced for a few minutes to familiarize themselves with the procedure.

Ten trials per patient were recorded, with a 1-minute rest period every three trials, to avoid muscle fatigue.

2.4 Data Analysis

2.4.1 Kinematic parameters

The data were processed using MATLAB (version 8.3.0.532, MathWorks, Natick, MA, USA) software. The anatomical angles of the lower limb in the sagittal plane were computed from motion capture data. For each trial, every gait cycle was defined as the time between two successive foot contacts of the same leg – foot strike and foot-off events were determined according to (**Borghese et al., 1996)** – and then extracted. Since the disease typically affects both lower limbs symmetrically, we focused our analyses on the dominant leg. Gait cycle segmentation was used to calculate the following spatiotemporal parameters of gait: gait speed, gait cadence, step length, stride length, and step width. In addition, the duration of stance phase (expressed as a percentage of the cycle duration) was evaluated.

Following (**Goudriaan et al., 2018**), and to account for possible anthropometry variations associated with growth, stride length, step length and stride width were expressed as a percentage of the limb length. For these parameters, the coefficient of variation CV (expressed as the percent value of the ratio between the standard deviation across multiple steps and its mean value) was computed as a measure of gait variability and instability.

2.4.2 Surface EMG parameters

Segments of sEMG data extracted from each gait cycle were band-pass filtered using a Butterworth filter (20–400 Hz), rectified, and low-pass filtered with a Butterworth filter (cut-off frequency 10 Hz), to obtain the linear envelopes (LE) of each muscle. For each subject and muscle, LE was normalized to its peak value across all trials from the same session. From the processed sEMG signals, the co-activation index was calculated for the pairs of antagonist muscles VL–BF, RF-HAM, VL-HAM, RF-BF, TA–SOL and TA-MG by using the Vector Coding Technique (VCT) (**Yoo et al., 2016**). The advantage of this technique, as compared to amplitude-based indicators of co-activation, is its substantial independence from EMG amplitude, which, in longitudinal studies, where muscle strength and weakness may play a relevant role, reduces confounding effects coming from differences in the overall magnitude of muscle activity.

VCT divides the coordination patterns into 4 classes: *In-phase* or *In-activation* (when amplitudes of the two signals increase or decrease simultanously); *Anti-phase* or *Anti-activation* (when amplitudes change in an opposite direction– when one increases the second decreases or vice versa); *One-only* (when only one muscle is active), and *Other-only* (when only the other muscle is active) (**Chang et al. 2008, Rinaldi et al. 2018).**

To identify the co-activation pattern, a 2-D plot is constructed drawing the envelope of one signal (LE_{M2}) with respect to the other (LE_{M1}). The coupling angle (γ) is then defined as the positive direction angle subtended from a vector adjoining two successive time points relative to the right horizontal:

$$\gamma_i = \begin{cases} \arctan\left(\frac{LE_{M2}(i+1)-LE_{M2}(i)}{LE_{M1}(i+1)-LE_{M1}(i)}\right) if \left(LE_{M1}(i+1)-LE_{M1}(i)\right) \geq 0 \\ 180° + \arctan\left(\frac{LE_{M2}(i+1)-LE_{M2}(i)}{LE_{M1}(i+1)-LE_{M1}(i)}\right) if \left(LE_{M1}(i+1)-LE_{M1}(i)\right) < 0 \end{cases}$$

where i is the current time sample and $0° \leq \gamma \leq 360°$.

When coupling angles are 45° and 225°, the two signals are perfectly in-phase (they increase or decrease of the same relative amount sample by sample). On the other hand, at 135° and 315°, a pure anti-phase coordination is present. When the segment adjoining two successive points is parallel to the horizontal (γ=0° or 180°) or vertical axis (γ = 90° or 270°), there is a pure one-only signal phase (one of the muscles increases or decreases its activity with no change on the other). When coupling angles do not relate to vertical, horizontal and diagonal vectors, the patterns are less pure.

For each time sample, muscle activity pattern is thus classified according to the following: One-only activation if $67.5° \leq \gamma_i < 112.5°$ or $247.5° \leq \gamma_i < 292.5°$; Other-only activation for $0° \leq \gamma_i < 22.5°$, $157.5° \leq \gamma_i < 202.5°$, or $337.5° \leq \gamma_i < 360°$; In-activation if $22.5° \leq \gamma_i < 67.5°$ or $202.5° \leq \gamma_i < 247.5°$; Anti-activation if $112.5° \leq \gamma_i < 157.5°$ or $292.5° \leq \gamma_i < 337.5°$.

CI_{VCT} is then calculated as the total number of temporal indexes classified as either In- or Anti-activation relative to the total duration of the multiplied by 100. Complete co-activation corresponds to a $CI_{VCT} = 100\%$.

2.5 Statistical analysis

To examine the differences among the co-activation, gait parameters, CV and motor test values at the three sessions, a one-way ANOVA test with session times as factor, and analysis of linear correlation with session times was performed using MATLAB (version 8.3.0.532, MathWorks, Natick, MA, USA) software. Furthermore, correlation analysis was performed in order to determine the relationship between the co-activation index of muscle pairs displaying a significant effect driven by evaluation session time, and the level of functional ability (6MWT), parameters of gait performance and gait stability. Descriptive statistics included mean and standard deviation, and significance level was set at $P < 0.05$.

3. Results

Changes in gait variables across evaluation sessions are shown in Figures 2 and 3.

Time had a significant effect on stride width, duration of stance phase, and on coefficients of variability for both stride length and stride width.

Significant differences among the three sessions were observed on both stride width ($F_{(2,27)}$ = 8.37, P = 0.0019) and stance phase duration ($F_{(2,27)}$ = 3.99, P = 0.0326). In the case of stride width, this effect was confirmed by the apparent linear relation with session times (r = 0.65, P = 3E-4). Pairwise comparison between the sessions identified a significant increase of stride width passing from T0 to T2 (P = 0.0015) and a significant decrease of stance phase duration passing from T0 to T1 (P = 0.0262).

Analysis of the CV of stride length at the three sessions showed significant differences ($F_{(2,27)}$=5.57, P = 0.0106), and a positive linear relation with session times resulted (r = 0.52, P = 0.006). Pairwise comparison between the sessions identified a significant increase of CV passing from T0 to T2 (P = 0.0330) and from T1 to T2 (P = 0.0102). Furthermore, a significant effect of time on the CV of stride width was observed ($F_{(2,27)}$=7.72, P = 0.0027), and this was again associated with a positive linear relation with session times (r = 0.56, P = 0.003). Pairwise comparison between the session identified a significant increase of stride width CV values passing from T0 to T2 (T0 vs T1 P = 0.0051; T0 vs T2 P = 0.0134).

Analysis of the CI at the three evaluation sessions, showed significant differences for both the VL–BF ($F_{(2,27)}$=5.44, P = 0.012) and the TA–SOL ($F_{(2,27)}$=7.84, P = 0.002) pairs of antagonist muscles. No significant differences in the analysis of CI at three evaluation sessions for the other antagonist muscles were found. In particular, the remaining pairs of knee antagonist muscles (RF-HAM, VL-HAM, RF-BF) share a substantially common trend for CI among sessions, whereas the remaining pair of antagonist muscles acting on the ankle (TA-MG) displayed a trend similar to that of the TA-SOL couple, though with a reduced effect.

Pairwise comparison between the sessions identified a significant decrease of co-activation from T0 to T1 (P = 0.045) and an increase of co-activation when passing from T1 to T2 for VL-BF pair (P =

0.009), and a significant decrease of co-activation passing from T0 to T1 (P = 0.002) and from T0 to T2 (P = 0.044) for the TA-SOL pair acting on the ankle joint (Figure 4).

Analysis of the 6MWT distance at the three sessions showed significant differences ($F_{(2,27)}$ = 3.56, P = 0.045). In particular, the distance significantly decreased when passing from T1 to T2 (pairwise comparison P = 0.049) (Figure 5).

From the correlation analysis, a slightly negative correlation (r = -0.382, P = 0.049) between CI on VL-BF and 6MWT values was observed. Furthermore, the co-activation of TA-SOL was negatively correlated with the CV of stride width (r = -0.510, P = 0.0077).

4. Discussion

Since preservation of gait autonomy is a priority in the rehabilitation of children with DMD, many authors have studied the gait biomechanics modifications caused by the disease and its progress. It is indeed well-known that patients with DMD show a gradual decline of walking ability due to deterioration of the gait patterns directly caused by both primary (e.g muscle weakness) and secondary deficits (e.g. muscle dystrophy), as well as the result of adaptation processes to diminished muscle strength and coordination **(Sutherland et al., 1981)**.

Muscle weakness has a direct influence on DMD gait (**Goudriaan et al., 2018-I; Sutherland et al., 1981**) with several effects such as a lower power generation at the hip caused by weakness of the hip extensors (e.g. biceps femoris), which might lead to an increased pelvic anterior tilt **(Gaudreaul et al. 2007)**, and then to an increased hip flexion. Even the weakness of the hip flexors (e.g. rectus femoris) could contribute to the power decrease at the hip joint. Regarding this, it is useful to consider that the co-activation of agonist-antagonist muscles is the mechanism that ensures upper body stability during the stance phase. Since muscle weakness elicits the nervous system to exploit the passive properties of the soft tissues, the pelvis anterior tilt could be due to this mechanism put in act by the posterior muscles inserted on the ischium when aiming at the pelvis stabilization by hanging in anterior attitude. Abnormalities at the muscle groups acting on the ankle joint may be

linked to a diminished ability to produce an appropriate dorsiflexion torque at the beginning of stance, when DMD children either place the foot in plantar flexed position or flat on the ground. Also at the ankle level the passive properties of muscles are exploited, but with a different strategy due to the different dynamic joint function: the foot mediates the relationship with the terrain, and it is responsible for energy conservation during gait. Moving on the forefoot at foot strike favours the exploitation of the elastic passive properties of the triceps in energy absorption and release during stance phase.

However, even though lower limb muscle groups are severely involved in DMD children (**McDonald et al., 1995)**, up to now most of the results are qualitative, and no specific muscle parameters have been monitored longitudinally, nor they have been directly linked with gait performance parameters. In the following, by discussing the results obtained in this longitudinal study, we will try to outline some new insights about muscular function and gait performance in DMD.

Gait spatio-temporal characteristics and variability

We found a progressive widening of the stride width that is an adaptive strategy useful to cope with reduced muscles strength and length and aimed at maintaining the dynamic balance on the frontal plane.

We also observed a progressive increase in the variability of both stride length and stride width (Figures 2 and 3) that has been discovered to be linked with locomotion stability (**Sekiya et al., 1997; Hausdorff et al., 2004; Serrao et al., 2017**). The progressive increase in gait variability observed at the 2-year follow-up may thus directly reflect a deterioration of the gait function, which leads to greater instability. Then gait variability seems to be a possible predictor of the loss of walking autonomy, even if further studies are needed to assess its robustness.

While we cannot exclude possible confounding factors associated with growth and with the pharmacological treatment, the normalization of the spatial parameters of gait substantially minimized the direct effect of anthropometry variations on the spatial characteristics of gait.

294 **Muscle co-activation**

295 This study deeply investigated the function of co-activation in patients with DMD, highlighting the
296 relationship among muscle co-activation during gait and disability, gait performance and postural
297 stability. Like spatio-temporal and kinetic parameters, muscle activity indicators may also depend
298 on variability associated with differing processing or recording factors **(Goudriaan et al., 2018)**. In
299 this study, the choice of processing parameters, such as the cut-off frequency or the technique for
300 evaluating muscle co-activation, was based on a preliminary study on a smaller sample (**Rinaldi el**
301 **al. 2018**).

302 During gait, joint stiffness and postural stability **(Boudarham et al. 2015)** are regulated by
303 variations in the forces produced by the simultaneous contraction of antagonistic lower limb
304 muscles.

305 It has been reported that increased co-activation in muscles acting across the knee and ankle in
306 pathologic conditions could help to cope with the loss of selective muscle control, muscle
307 weakness, abnormal muscle tone and fatigue (**Den Otter et al., 2007; Kwon et al., 2003**). For
308 instance, in Multiple Sclerosis it has been found that the neuromuscular system simultaneously
309 increases knee and ankle muscle co-activation to ensure stability during forward progression
310 (**Boudarham 2015**) and in post stroke and hemiplegic patients increased muscle co-activation is an
311 adaptive strategy for enhancing postural stability and locomotor performance (**Lamontagne 2000,**
312 **Kitatani et al. 2016**).

313 Our results revealed changes in lower limb co-activation in relation to disease progression for both
314 proximal and distal segments. Figure 4 showed a significant decrease in co-activation values after a
315 year, which is confirmed even after two years for distal segment muscles. Despite a decreasing
316 trend from T0 to T1 on co-activation values at the proximal level, a significant increase of co-
317 activation was reported at 2-year follow-up, when patients showed the lowest functional capacity
318 (Figures 4 and 5).

319 The absence of a shared behaviour at T2 for proximal and distal muscles may be associated with a
320 change in activation strategies, as the result of an adaptation to the disease progression, which
321 increases and decreases co-activation for the proximal and distal muscles respectively.
322 The reduction of co-contraction activities at the distal level might be related to the direct effect of
323 the muscle dystrophy, which determines a higher reliance on the passive characteristics of soft
324 tissues. Moreover, the co-activation of the ankle joint muscle pair was found inversely proportional
325 to the stride width variability. This might indicate that a decreased co-activation over the time at the
326 ankle and knee joints, due to the relevant presence of muscle dystrophy at the distal level, leads to a
327 more unstable gait. Considering that the latero-lateral stability during stance phase takes advantage
328 of the rotational movement of the foot mediated mainly by the tibialis anterior, tibialis posterior and
329 peroneal muscles, the study of their force and activities could be a future development of this work.
330 The increased co-activation at proximal level may be linked to the decrease in functional capacity.
331 Despite the presence of a large inter-individual variability, this interpretation might be associated
332 with the observed negative correlation between co-activation values at the muscle pair acting at the
333 proximal level, and the gait functional outcomes during the years (Figure 6). We indeed observed a
334 decrease in the 6MWT values from the 1-year follow-up to the 2-year follow-up. These results are
335 well aligned with previous studies as this functional decline reflects the progressive nature of
336 degenerative DMD over time (**Manzur et al., 2008; Ropars et al., 2016).** However, while the
337 functional capacity decreases, increase in co-activation at the proximal level at the 2-year follow-up
338 suggests that patients try to maintain an effective gait despite disease progression, by increasing
339 muscle co-activation, especially when the muscular manifestations of the pathology are more
340 evident. Thus, since walking speed during gait analysis tasks remained approximately unchanged
341 over time (Figures 2 and 3), increased muscle co-activation at proximal level represents the most
342 important strategy to preserve gait instability in patients with DMD.

In healthy subjects also, it has been reported that the central nervous system co-activates many muscles to increase the whole lower limb stiffness to maintain gait stability at lower speed (**Fan et al., 2016**; **Varrecchia et al., 2018**). Conversely, a low level of co-activation is required during natural self-selected speed to likely minimize the energy expenditure and guarantee an effective gait, indeed the co-activation is positively correlated with some balance-related gait temporal parameters, i.e. durations of first and second double support and overall stance. In older individuals, high muscle co-activation was a sign of low postural control ability. Increased muscle co-activation could thus be a necessary change in order to freeze not otherwise controllable degrees of freedom for a deterioration in postural control also in the sample observed in this study, in a similar way to what observed in healthy aging. (**Nagai et al., 2011**).

5. Conclusions

We evaluated gait patterns over a period of 2 years in a group of patients affected by DMD. This longitudinal study was the first to investigate muscle behaviour through the analysis of coactivation, and to link possible changes of these parameters in relation with gait alterations and functional capacity modifications. The main findings of this study suggest that patients with Duchenne muscular dystrophy exhibit a decline in functional capacity and gait instability after 2 years from the baseline, even if this has not determined a substantial variation in gait functional capacity. However, to maintain this capacity, we hypothesized that patients try to maintain an effective gait by increasing stiffness mainly at proximal level (i.e. increase in proximal muscle co-activation) to counteract the main direct effects coming from disease progression.

The variability of the obtained results, associated with the relatively small sample size of patients, might be considered as a whole as the main limitation of the current study: the first aspect may be associate both with the agreed variability in clinical manifestations of DMD (**Desguerre et al., 2019**), and with the presence of diverse compensation strategies put forward to cope with the exacerbation of motor symptoms; moreover, Duchenne Muscular Dystrophy affects a few children

per 10.000 births, and the rather rapid disease progression makes it hard to gather large sample sizes, to be followed longitudinally, unless multicentre trials are specifically designed for this. The limitation of the small sample size was partly offset by the adoption of sensitive quantitative measures of motion. Studies with larger sample sizes could be useful to further validate our results.

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Figure captions

Fig 1. Patient set-up for gait analysis.

Fig 2. Time-distance parameters and CV values at baseline (T0), at 1-year follow-up (T1) and at 2-year follow-up (T2). Asterisks indicate significant differences among evaluation sessions. Results of analysis of linear correlation with evaluation session times was also reported (significant correlations are indicated in bold).

Fig 3. Radar plot illustrating the pattern of the time-distance parameters at the baseline (T0, light gray line), at 1-year follow-up (T1, dark gray line), and at 2-year follow-up (T2, black line). Group mean values for each parameter are shown.

Fig 4. CI values at the baseline (T0), at 1-year follow-up (T1) and at 2-year follow-up (T2) for both knee and ankle muscle pairs. Asterisks indicate significant differences among evaluation sessions. Results of analysis of linear correlation with evaluation session times was also reported.

Fig 5. 6MWT values at baseline (T0), at 1-year follow-up (T1) and at 2-year follow-up (T2). Asterisks indicate significant differences among evaluation sessions.

Fig 6. CI values for both VL-BF and TA-SOL pairs of antagonist muscles and CV Stride Width values at the baseline (T0, circles), 1-year follow-up (T1, triangles), and 2-year follow-up (T2, squares). Each smaller element represents the results of one session for one patient, with different shades of gray according to the 6MWT values (see bar on the right). With the same shape coding, the larger elements represent the group mean values at baseline, 1-year follow-up, and 2-year follow-up.

Figure 1

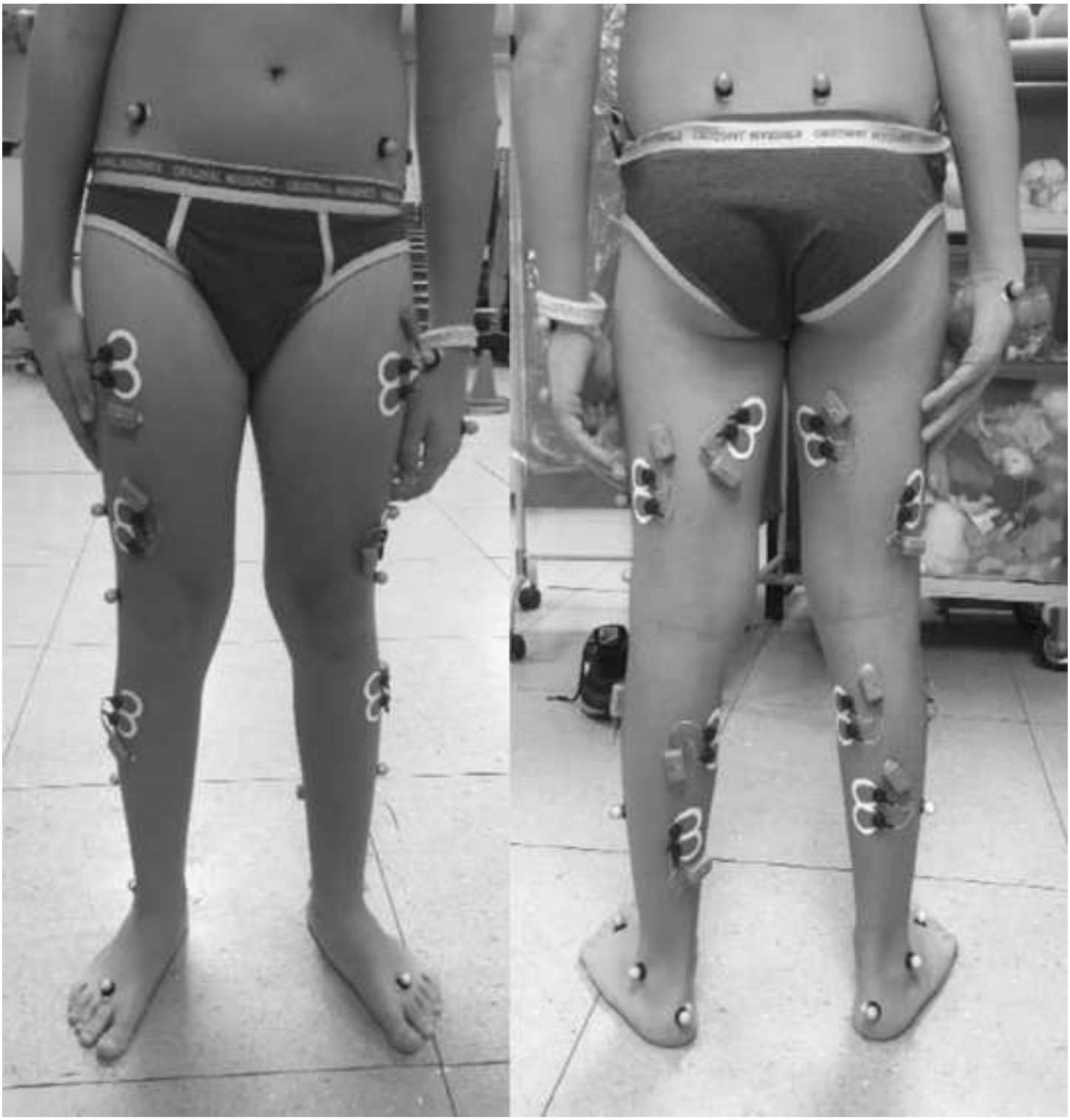


Figure 2

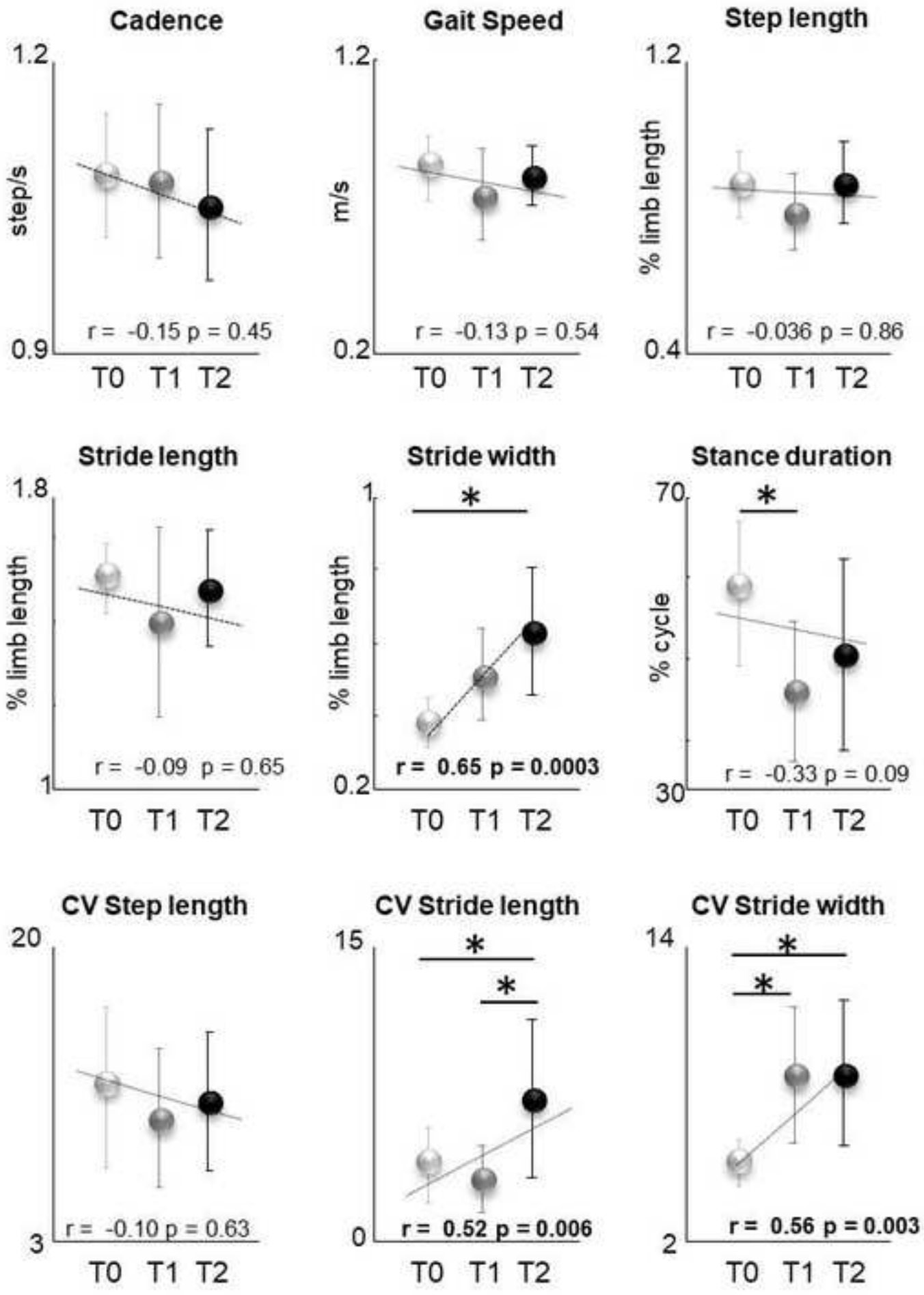

Cadence
1.2
step/s
0.9
r = -0.15 p = 0.45
T0 T1 T2
Gait Speed
1.2
m/s
0.2
r = -0.13 p = 0.54
T0 T1 T2
Step length
1.2
% limb length
0.4
r = -0.036 p = 0.86
T0 T1 T2
Stride length
1.8
% limb length
1
r = -0.09 p = 0.65
T0 T1 T2
Stride width
1
% limb length
0.2
*
r = 0.65 p = 0.0003
T0 T1 T2
Stance duration
70
% cycle
30
*
r = -0.33 p = 0.09
T0 T1 T2
CV Step length
20
3
r = -0.10 p = 0.63
T0 T1 T2
CV Stride length
15
0
*
*
r = 0.52 p = 0.006
T0 T1 T2
CV Stride width
14
2
*
*
r = 0.56 p = 0.003
T0 T1 T2

Figure 3

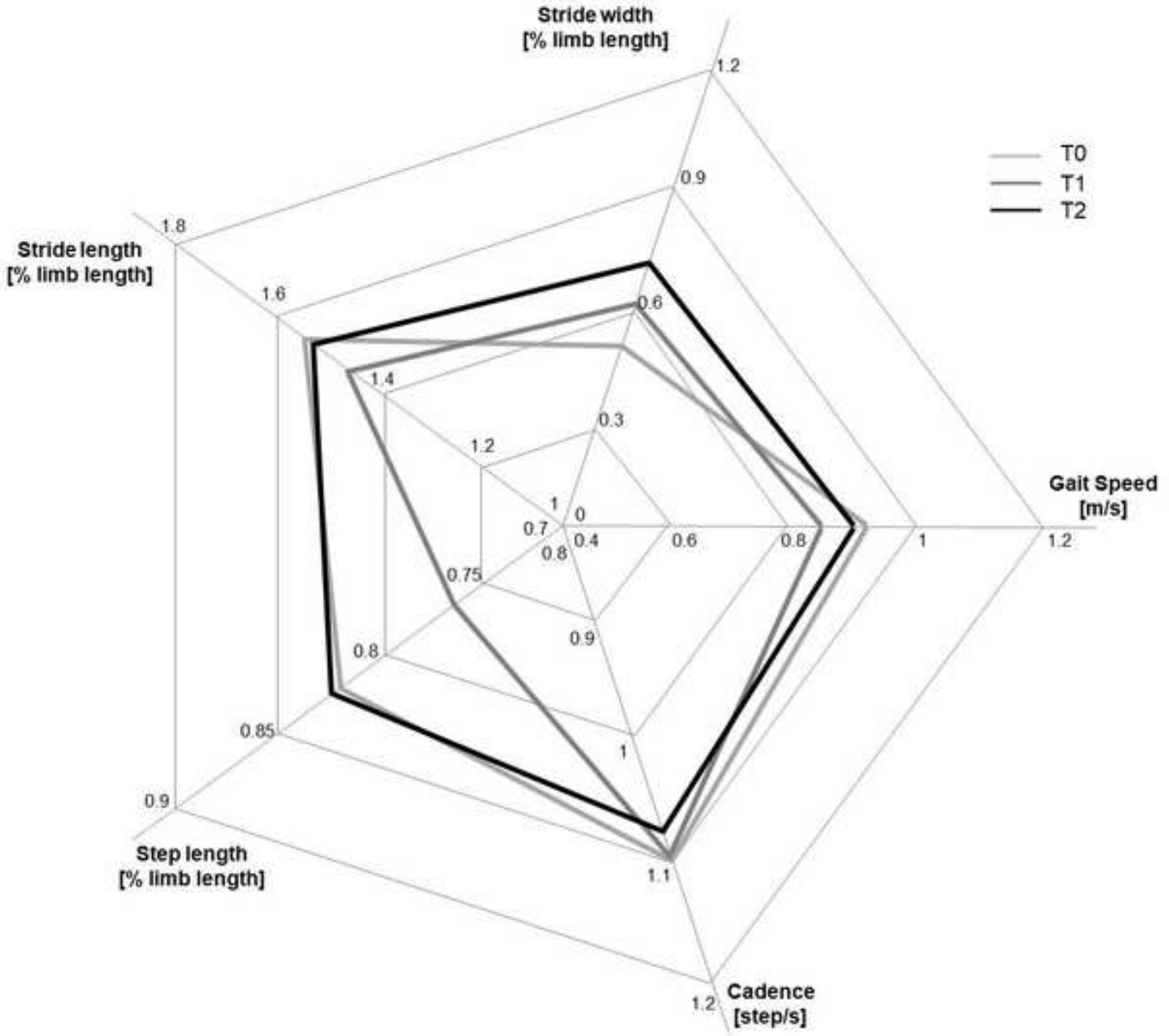

Stride width
[% limb length]
1.2
0.9
0.6
0.3
0
0.4
0.6
0.8
1
1.2
Gait Speed
[m/s]
1.1
1.2
Cadence
[step/s]
1
0.9
0.8
0.7
0.75
0.8
0.85
0.9
Step length
[% limb length]
1.2
1.4
1.6
1.8
Stride length
[% limb length]
T0
T1
T2

Figure 4

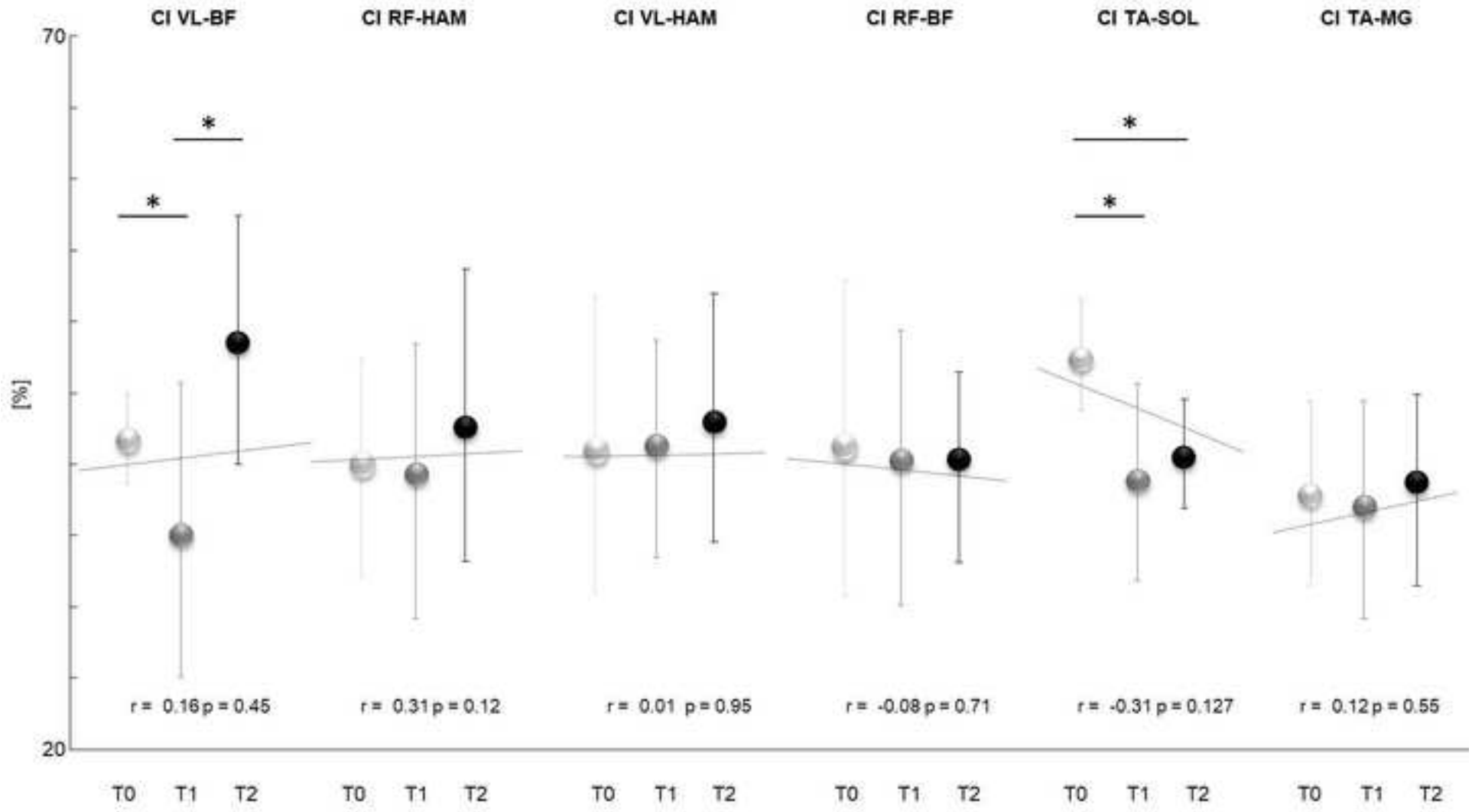

CI VL-BF
CI RF-HAM
CI VL-HAM
CI RF-BF
CI TA-SOL
CI TA-MG
70
20
[%]
*
*
*
*
r = 0.16 p = 0.45
r = 0.31 p = 0.12
r = 0.01 p = 0.95
r = -0.08 p = 0.71
r = -0.31 p = 0.127
r = 0.12 p = 0.55
T0 T1 T2
T0 T1 T2
T0 T1 T2
T0 T1 T2
T0 T1 T2
T0 T1 T2

Figure 5

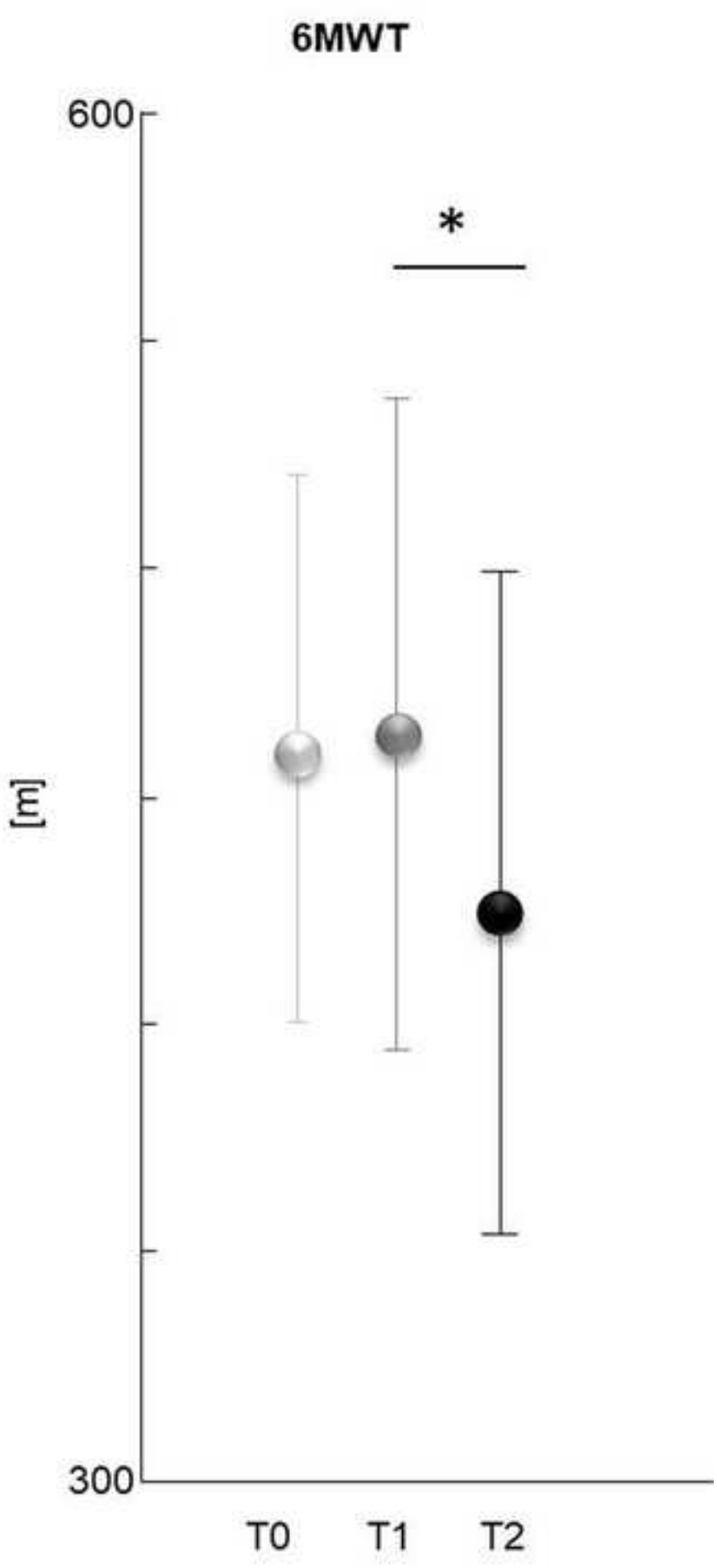
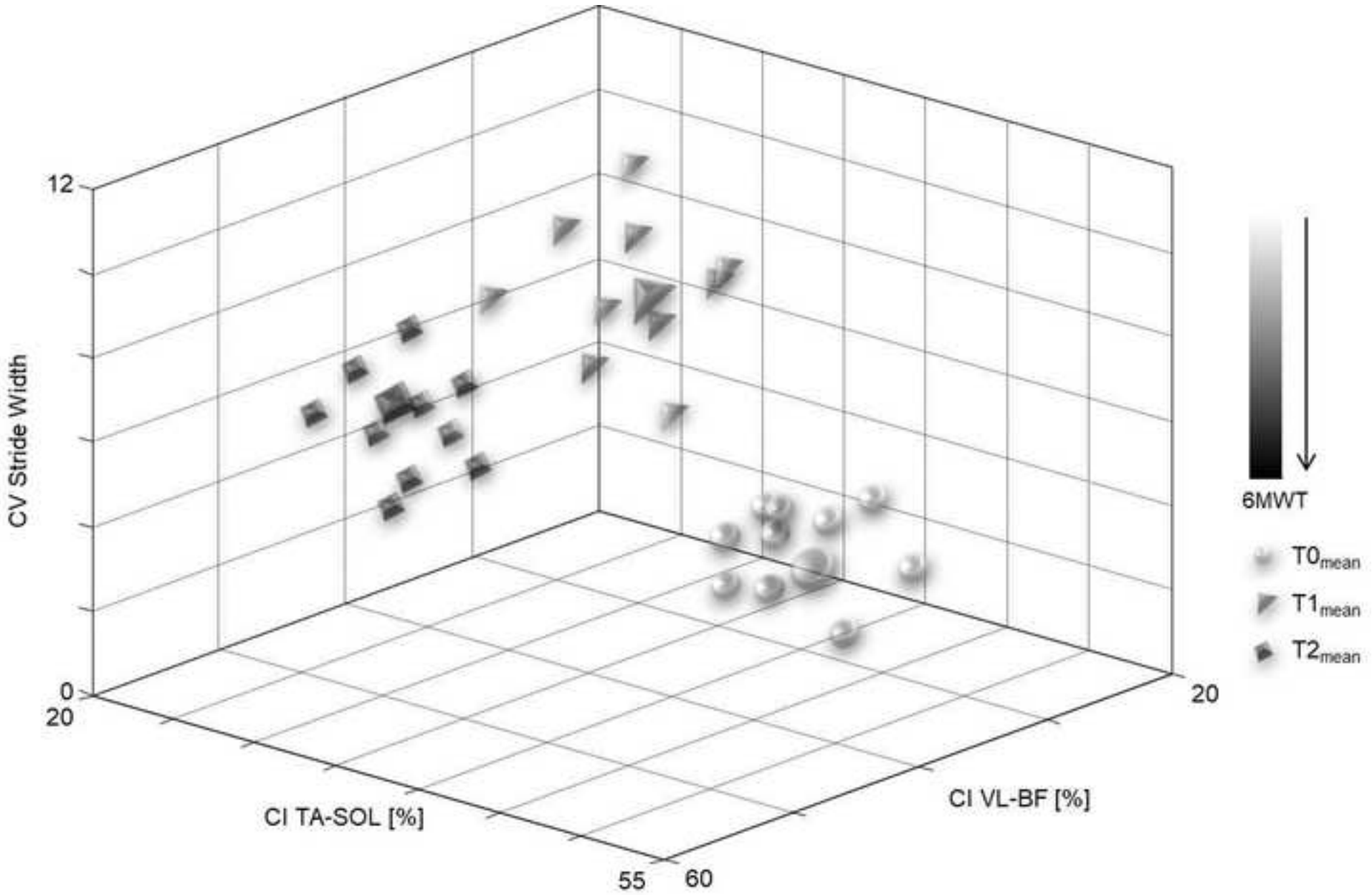


Figure 6


12
0
CV Stride Width
20
CI TA-SOL [%]
55
60
CI VL-BF [%]
20
6MWT
T0mean
T1mean
T2mean

Declaration of interest

Authors declare no commercial or other associations that might pose a conflict of interest in connection with the present article.

Author contributions

MR: Conceptualization; Methodology; Formal Analysis; Software; Validation; visualization; Writing – Original draft

MP: Conceptualization; Software; Supervision; Writing – Review & editing

AR: Formal analysis; Investigation; Writing – Review & editing

GV: Investigation; Data curation

CDA: Formal Analysis; Validation; Writing – Review & editing

DB: Software; Writing – Review & editing

MS: Methodology; Supervision; Writing – Review & editing

EC: Funding acquisition; Investigation; Project administration

SC: Conceptualization; Project administration; Supervision; Writing – Review & editing