PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

Title (Provisional)

Unravelling age-related gait decline in Cerebral Palsy: Insights into physiological changes and functional implications through an observational study.

Authors

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VERSION 1 - REVIEW

Reviewer	**1**
Name	**Faccioli, Silvia**
Affiliation	**Azienda Unità Sanitaria Locale - IRCCS Tecnologie Avanzate e Modelli Assistenziali in Oncologia di Reggio Emilia, Children Rehabilitation Unit**
Date	**23-Jul-2024**
COI	**No competing interests**

The study is appropriate for the journal selected.
The research is important for the field. While there are different studies demonstrating gait decline in adults with cerebral palsy, none of them includes this comprehensive evaluation of gait performances. This could permit the authors to investigate the potential pathophysiological mechanisms of gait decline due to aging.
Nonetheless, management of confounders and the outcomes should be better detailed and there is no explicit reference to the statistical analysis. For this reason, I suggest a major review of the protocol.

Introduction
Pag 6 line 13: They make a list of associated issues: they include cognitive impairments and decreased motor control that are part of the definition of the condition and other problems that are actually secondary problems, can you formulate this part better?

Methods and analysis

It is a well-structured assessment program, using a wide range of instruments they can have a comprehensive understanding of the walking performances. Nonetheless, there are several aspects that need to be addressed.

It seems like there are a lot of possible confounders that are not taken into account and the sample size might be too limited to enquire them statistically.
As the authors write CP is associated with a range of issues. They all might influence the decline in mobility. How is this type of confounders managed?
Authors declare that TD subjects are comparable to individuals with CP in terms of BMI, age and gender. What about the two CP groups? Are they comparable concerning these aspects?
The period of recovery after botulinum (3 months) and moreover after surgery (6 months) might be two short. I would suggest to consider 6 months for botulinum and 12 months for surgery.

Can you better define the primary and secondary objectives? The primary outcome should be reported as first. Several measures are described in the “fatigability protocol” and it is not clear to which one the effect size is referred.

What about the statistical analysis?

Which will be the recruiting setting?

Pag 8 line 25: Why not include GMFCS III? this population could be more affected by gait decline.

Reviewer	**2**
Name	**Ozden, Fatih**
Affiliation	**St Luke’s Wood River Medical Center**
Date	**10-Aug-2024**
COI	**None.**

The paper has a well-designed protocol. I do not have any contribution to the available methodology. I also do not have any concerns about writing. This protocol should be published as it stands.

VERSION 1 - AUTHOR RESPONSE

Reviwer 1 – Dr. Faccioli of University of Modena and Reggio Emilia

The study is appropriate for the journal selected.

The research is important for the field. While there are different studies demonstrating gait decline in adults with cerebral palsy, none of them includes this comprehensive evaluation of gait performances. This could permit the authors to investigate the potential pathophysiological mechanisms of gait decline due to aging.

Nonetheless, management of confounders and the outcomes should be better detailed and there is no explicit reference to the statistical analysis. For this reason, I suggest a major review of the protocol.

Dear Dr. Faccioli, thank you for you time and effort in providing feedback. We hope that we addressed them in an appropriate manner. With regards to management of confounders, outcomes and the statistical analysis, we have added a section that can be retained if the editor wishes so.

Introduction

Pag 6 line 13: They make a list of associated issues: they include cognitive impairments and decreased motor control that are part of the definition of the condition and other problems that are actually secondary problems, can you formulate this part better?

Thank you for pointing out inconsistencies, we have reformulated the part (page 4, lines 6-8):

"Alongside this heterogeneity, CP is associated with a range of issues, including cognitive impairments (2) and decreased motor control (3, 4) as main CP-related issues, as well as musculoskeletal morbidities (5, 6), pain (11) and/or fatigue (12, 13) as secondary issues, alongside co-existing issues such as comorbid diseases (7-10).

Methods and analysis

It is a well-structured assessment program, using a wide range of instruments they can have a comprehensive understanding of the walking performances. Nonetheless, there are several aspects that need to be addressed.

It seems like there are a lot of possible confounders that are not taken into account and the sample size might be too limited to enquire them statistically. As the authors write CP is associated with a range of issues. They all might influence the decline in mobility. How is this type of confounders managed?

Thank you for this comment. Although the reviewer is right, it is a difficult issue to address. With our study being an observational one, we are of course at risk of bias through confounders, effect modifiers and random effects not controlled for. However, in our case, we submit our participants to the same assessments on the same day of the protocol, which helps minimize bias. As such, this protocol provides a picture in time of the current state of mobility amongst these participants, with no differences between groups.

We are interested in the effects of ageing amongst adults with CP, which, *per se*, include possible confounding effects (comorbid diagnoses, fat infiltration of musculature, increased extracellular matrix) on measures such as strength, cardiopulmonary exercise test, and fatigability.

The medical history of each participant is known, with medical interventions available for publishing alongside results allowing the reader to understand the heterogeneity among adults with CP (GMFCS I/II at age 18). As we only have one timepoint (four visits within two months), it is impossible to account for individual differences in evolution over time, however, this could be possible with a longitudinal approach. A longitudinal approach with a similar protocol, although extremely difficult to conduct, would be of great interest and would allow for understanding the effects of issues faced on the evolution of gait performance. Similarly, a much larger study population could investigate the effect of known issues by stratifying the analysis into people with issues such as cognitive impairments. The current study has a minimum of 3 degrees of freedom in each statistical analysis (4 groups, no time evolution), further, we expect 3 (group) + 3 (stages completed + baseline) + 2 (interaction term) = 8 degrees of freedom for the analysis of the fatigability protocol. Adding another strata, e.g. for cognitive impairment, would increase the degrees of freedom in each group by 1, requiring an exponentially larger population.

We agree that we will not have the statistical power to account for interpersonal differences with a one-time observational study, we do however believe that the protocol offers a thorough understanding of individuals with CP who were at GMFCS level I/II at age 18 stratified in age-groups of 18-25 years old and 35-50 years old. We envision presenting results in such a way that it is possible to follow each participant in all measures (through a numbering or letter/sign system), helping to show the individual alongside their medical history. If *per se*, motor control is considered an important aspect, one would be able to see the voluntary activation as tested through the fatigability protocol and see that participants' performance in strength testing. We do however, not have the number of participants to infer performance in one test based off of other measures obtained. We have added a line in "Ethics and dissemination" (p.., line…)

"Therefore, we anticipate multiple papers, with collaborators contributing to each based on the Vancouver protocol (ICMJE). We envision consistent first and last authors across all manuscripts. In order to facilitate a comparison across papers, we will code every participant with either a number or a letter, allowing the reader to follow a participant, not only in the individual paper, but across all papers. "

Further, we intend on mentioning confounders and the lack of control thereof as a limitation in the articles that will come from this study.

Authors declare that TD subjects are comparable to individuals with CP in terms of BMI, age and gender. What about the two CP groups? Are they comparable concerning these aspects?

This is another important question. We opted to have comparable groups with regards to GMFCS level at age 18 (I/II), without restrictions regarding current BMI, age and gender. With the age as an important factor, there is no chance of having a comparable young and older group regarding this measure. Gender could be a potentially important comparator; however, we wish to include as many participants as possible. We did that in order not to limit ourselves in terms of inclusion of participants from an already limited pool. Further, with age-related decline in mobility as the subject of interest, the groups should be comparable at the same age, meaning we would have had to know the BMI at age 18, not their current BMI. We will aim to include all available participants, this with the risk of an uneven inclusion. We have clarified this in the section on participants (page 6, lines 9-10):

"For TD counterparts, inclusion criteria involve being comparable to a participant in the CP group in terms of BMI, age, and gender. Due to the expected challenges in finding suitable participants with CP, no matching will be attempted between the two age-groups."

The period of recovery after botulinum (3 months) and moreover after surgery (6 months) might be two short. I would suggest to consider 6 months for botulinum and 12 months for surgery.

Thank you for your valid concern. We debated this while writing the ethical committee application. You are indeed correct, some protocols use 6 months, some 3, with no official guidelines available. We perused the literature and found the half-life estimated at 8.6 weeks for the toxins typically administered in France (Ojardias et al. 2022). Moreover, we know that for selective injections (i.e. specifically single muscles like rectus femoris, semitendinosus etc.), the non-affected muscle volume will be able to compensate for the partial loss in strength for global movements (Brunner et al. 2023) and that the differences in muscle forces before and following treatment are limited (Wesseling et al. 2020). Regarding surgery, similar arguments could be made.

Your concerns are very valid; however, we find that changing the inclusion criteria after starting inclusion would be deemed worse than maintaining contestable criteria. To reassure readers we propose highlighting the limitation in the articles written from the data and supplying readers with a table detailing toxins and surgical procedures.

Can you better define the primary and secondary objectives? The primary outcome should be reported as first. Several measures are described in the "fatigability protocol" and it is not clear to which one the effect size is referred.

Thank you for the remark, we have added clarification and now reported the fatigability protocol as first within the methods:

Changes made:

Moved the section on Fatigability up as the first.

Introduction last paragraph (page 4-5 lines: 32-2):

"To achieve this objective, we will investigate differences in gait performance, self-reported outcomes, muscle strength, metabolic cost and quantitative gait analysis (QGA) of walking, and fatigability between young (18-25-year-old) and older (35-50-year-old) groups of individuals with CP and TD peers. In the present study, our primary objective is specifically to compare exercise-induced fatigability (i.e. the decline in force production capacities after a standardized fatiguing task) among young and older CP and TD groups. Our secondary objectives are to compare all the other aforementioned parameters between groups."

Methods section on the fatigability protocol first line (page: 6-7, lines: 31-1):

"Exercise-induced fatigability will be investigated on a recumbent bike (Figure 2), as in previous studies (Varesco et al. 2023; Doyle-Baker et al. 2018; Brownstein et al. 2022), with the aim to quantify the decline in voluntary force production during exercise and at exhaustion."

Methods section on the fatigability protocol penultimate sentence (page: 8, lines: 5-6):

"The fatiguing protocol's outcomes will include number of completed stages, EMG activity during stages, as well as data on maximal torque (N or N/kg), voluntary activation (%), and twitch amplitude (N or N/kg) across

stages and at task failure. Decrease in maximal torque across stages (i.e. fatigability) will be our primary outcome."

What about the statistical analysis?

We did not include a paragraph on statistical approaches as it was not mentioned in the submission guidelines. We are however happy to include a section of statistical analyses envisioned if necessary. In short, we will treat data using either one-way ANOVA's or Kruskal-Wallis tests with Bonferroni post hoc corrected Wilcoxon Rank sum tests for between group comparisons in non-repeated measures. For repeated measures (Fatigability protocol), we expect to use the same analysis as Varesco et al., i.e. a generalized estimating equation, to test the time x group effects until the latest common stage – Before and at task failure we will use either ANOVA's or Kruskal-Wallis, depending on normality of data. This has also been included in a section called "Data analysis" in the methods section following "Cost of walking" (page 13, lines: 5-13):

"Data analysis

In all statistical analyses, significance will be set at $p \leq 0.05$. For our primary objective, data will be treated as a time series and evaluated using Generalized Estimating Equations, a two-way ANOVA-like non-parametric test that was also used in the paper by Varesco, Luneau (28). A time, group and interaction term will be used to investigate the evolution of neuromuscular parameters. All *non-time series data (baseline data and all other tests) will either be treated using one-way ANOVA's or non-parametric tests such as the Kruskal-Wallis test depending on normality of data. Post hoc testing will in either case be Bonferroni corrected. To test for matching of participants, paired t-tests will be used. Data analysis will be conducted using R (R Development Core Team 2022) and the code will be available in an online repository."*

Which will be the recruiting setting?

Sorry, it is an oversight on our part. We have changed wording in the "Design of the study" and "Participants" parts.

In "Design of the study" (page 5, line 12) - *"Participants will attend four sessions at our university laboratory, spaced at least one week apart (**Error! Reference source not found.**)."*

In "Participants" (page 6, lines: 14-15) – *"Participants with CP will be sourced through medical treatment centres by their respective doctors who will be responsible for the first point of contact and recruitment."*

Pag 8 line 25: Why not include GMFCS III? this population could be more affected by gait decline.

It was discussed in the group prior to formulation of the protocol. We opted to include individuals aged 35-50 at GMFCS level III if they were at level I or II at the age of 18. We however decided not to include young participants at GMFCS level III. This was chosen as a large part of the protocol requires gait (clinical outcomes, cost of walking on the treadmill) or an ability to pedal (fatigability protocol). As such, we have allowed people in the older age-group to partake if they have experienced a gait-decline. You are completely right about the risk of gait decline in the population of adults with GMFCS III, we suspect that would be towards a wheelchair making comparisons very difficult.

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Wesseling, M, H Kainz, T Hoekstra, S Van Rossom, K Desloovere, F De Groote, and I Jonkers. 2020. 'Botulinum toxin injections minimally affect modelled muscle forces during gait in children with cerebral palsy', Gait & posture, 82: 54-60.

Reviewer 2 – Dr. Ozden of St. Luke's Wood River medical Center

The paper has a well-designed protocol. I do not have any contribution to the available methodology. I also do not have any concerns about writing. This protocol should be published as it stands.

Thank you for your time and effort in reviewing our protocol. We are happy to hear of your acceptance of it.