Acute and Long-Term Changes in Blood-Borne Biomarkers in Response to Dynamic Standing in Nonambulant Children With Cerebral Palsy

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Purpose: To investigate acute and long-term changes in hormonal and inflammatory biomarkers in nonambulant children with cerebral palsy in response to dynamic standing exercise. Methods: Fourteen children with severe cerebral palsy were recruited. Anthropometrics and body composition measures were obtained. Physical activity levels before the study were assessed using hip-worn accelerometry. All children underwent a 30-minute dynamic standing exercise using the Innowalk standing aid. Respiratory data during exercise were collected using indirect calorimetry. Blood samples were collected before and after exercise. Blood samples were also obtained after two 16-week exercise protocols, in a resting state. Hormonal and inflammatory metabolites were measured from blood serum/plasma, and acute and long-term changes in biomarker levels were assessed using Wilcoxon signed-rank tests. Results: Of the 14 children at baseline, all had slightly/moderately/severely elevated C-reactive protein and cortisol levels. C-reactive protein levels were decreased following a 30-minute bout of dynamic standing (before exercise: 53 mg/L [interquartile range: 40–201]; after exercise: 39 mg/L [interquartile range: 20–107]; P = .04). Conclusions: We show that several hormonal and inflammatory biomarkers are dysregulated in children with cerebral palsy. Our preliminary results from a small, but deep-phenotyped prospective cohort indicate acute and long-term alterations of several biomarkers in response to exercise.

Keywords: exercise physiology, hormones, inflammation, rehabilitation

Cerebral palsy is the most common physical disability in childhood, affecting approximately 1.6 out of every 1000 live births (23). In addition to a movement disorder and affected muscle tone, many children experience problems with pain, osteoporosis, epilepsy, intellectual, communicational, and behavioral impairments (25,33), and a higher risk of developing health problems related to decreased physical activity in adulthood, such as cardiovascular disease. The degree of motor impairment in cerebral palsy is extremely variable and classified in the 5-level Gross Motor Function Classification System—Expanded & Revised (GMFCS–E&R) scale (28). Children with severe cerebral palsy (GMFCS–E&R IV–V) who cannot stand or walk independently have been shown to have low physical activity levels (18,20). Standard care in Sweden includes daily static supported standing for 30 to 90 minutes to improve bone density, range of motion, and reduce spasticity (27). Compared with sitting and laying down, children with cerebral palsy have increased energy expenditure and muscle activity when they perform static supported standing (39). Robotic walking aids have been used to facilitate walking among ambulant children with cerebral palsy, demonstrating higher walking speed and distance (3). The effects of robotic walking (defined as dynamic standing exercise) on blood-borne biomarkers for nonambulant children with cerebral palsy are largely unknown. Innowalk, a motorized assistive device made by Made for Movement in Norway, offers whole-body exercise through assisted and repetitive movements of the lower extremities in an upright weight-bearing position. In a previous study, we compared static and dynamic standing for 20 children with cerebral palsy (17,36). The results showed that dynamic standing exercise improved passive range of motion and spasticity in the hip, and overall health-related quality of life. Another study involving 46 patients showed that dynamic standing improved passive assisted motion, intestinal function, body stability, joint mobility, and secure means of supine positioning (29).

Physical inactivity is a known modifiable risk factor for early death (8), and exercise has been shown to prevent a range of diseases in the general population (6,7), as well as improve cognitive functions (5). Individuals with cerebral palsy reaching adulthood face an increased risk of getting diseases related to physical inactivity due to their physical disability (4,30). However, there is limited research on the effect of exercise on cognitive function in this population. One study has demonstrated that intense exercise had a positive impact on cognitive function among ambulant children with cerebral palsy (22), and another cross-sectional study showed lower levels of brain-derived neurotrophic factor (BDNF) levels in children with severe cerebral palsy, who also exercised less compared with other children with milder forms

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of cerebral palsy and those without the disease (13). An animal study showed elevated BDNF levels in the brain of rats with induced cerebral palsy after treadmill exercise (26).

The heterogeneity of nonambulant children with cerebral palsy challenges the traditional structure of treatment and rehabilitation and warrants innovative and effective prevention and treatment strategies. In the Long-term Exercise Effects from Robotic Walking (LEER) project, we established a comprehensive, complex, mixed-method study investigating the impact of exercise using the Innowalk in nonambulant children with cerebral palsy. In this project, we describe baseline characteristics of the LEER cohort using deep phenotyping and generate novel hypotheses by investigating acute and long-term changes in laboratory biomarkers in response to exercise in children with cerebral palsy.

Materials and Methods

Study Population

Children with cerebral palsy were recruited to the LEER Study via their parents through the National Association for Disabled Children in Sweden, and through the Child and Youth Habilitation Services during the fall/winter of 2019. A mixed-method exercise intervention study with a cross-over design was performed, using the Innowalk for dynamic standing exercise with several exercise possibilities and different velocities. In total, 24 children were invited to participate, and 14 children completed at least one study visit. Families were invited to be interviewed during the study period about facilitating and hindering factors with regard to the children performing physical activity. Ethical approval was obtained by the Regional Ethical Committee (Lund, dnr. 2019-00106), and informed consent was obtained from the parents of all participants. The study has been registered at ClinicalTrials.gov (LU2019LEER). The current project was undertaken according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (Supplementary Material S1 [available online]).

Measurements and Exercise Testing

The children were tested at an exercise research lab (Health Sciences Center, Lund University) following a protocol shown in Figure 1. The test started with measurements of weight, height, and body composition measured by bioimpedance (InBody S10). The acute effects of the exercise programs were assessed through indirect calorimetry during a 30-minute exercise test. To mimic an incremental exercise test in nonambulant children, the participants exercised at 5 different velocities (30, 40, 50, 60, and 70 steps per minute) for 6 minutes at each velocity (in total, 30 min) using the Innowalk. An airtight mask covering the mouth and nose was worn by the participants to measure breath-by-breath oxygen consumption (VO2), carbon dioxide production (VCO2), minute ventilation (VE), and other related metrics (Oxycon Mobile). Heart rate was monitored with a chest strap continuously throughout the test (Polar Coded). Nonfasting venous blood samples were collected before (baseline) and immediately after 30 minutes of dynamic standing.

Exercise Intervention

Each participant was asked to exercise 3 times per week for two 16-week periods starting with either high-intensity interval training or medium-intensity continuous training with a washout period of 16 weeks between the 2 exercise intervention periods. The exercise programs are described in detail in Supplementary Material S2 (available online). In response to difficulties in study management caused by the COVID-19 pandemic, several participants could not be instructed on the second type of exercise; these children continued to exercise as instructed in their first period.

Daily physical activity was assessed through accelerometry. The accelerometer was worn for 7 consecutive days before and after each intervention period. The accelerometer was placed above the iliac crest in line with the current recommendations of accelerometer device placement in pediatric populations (1,10,24). The ActiLife software (version 6.13.4, ActiGraph LLC) was used to categorize wear-time into (1) sedentary time, (2) low-intensity physical activity, and (3) moderate to vigorous physical activity. The vector magnitude was analyzed and a nonwear time of 90 constitutive minutes was set. Assessments were considered as valid based on 500 minutes of recording per day, with at least 3 valid days (15). Evenson et al’s cut-points were used to classify activity counts. Sedentary time was defined as ≤100 activity counts per minute (≤16.67 per 10 s), low-intensity physical activity was defined as 101 to 2295 activity counts per minute (16.83–382.5 per 10 s) and moderate to vigorous physical activity was defined as ≥2296 activity counts per minute (≥382.67 per 10 s) (10).

Biomarker Measurements

Serum-separating tubes were rested at room temperature for 30 minutes, centrifuged for 10 minutes, and the supernatant was aliquoted and stored at −80°C. Tubes for plasma collection were kept on ice, centrifuged at 4°C and after the separation of the

Figure 1 — Study flowchart. HIIT indicates high-intensity interval training; MICT, medium-intensity continuous training.

(Ahead of Print)
fractions, plasma aliquots were stored at −80°C. Multiplex analyses of serum analytes were performed on a Luminex MAGPIX instrument (Luminex Corporation) according to the manufacturers’ protocol (Merck Millipore) and protein concentrations were calculated with the Belysma Immunoassay curve fitting software (Merck KGaA). Plasma cortisol was analyzed with the Cortisol Parameter Assay Kit (R&D Systems, Bio-Techne).

Statistical Analysis

Statistical analyses were performed using R 4.1.2 (32).

All measures are presented as medians and interquartile ranges (IQR). There was no missing data.

Due to the low sample size, the Wilcoxon signed-rank test was used to compare levels before and after exercise. The Wilcoxon signed-rank test is a nonparametric statistical test to assess whether the locations of 2 sets of observations (eg, matched pairs) are different. First, baseline (resting) biomarker levels were compared with biomarker levels after one bout of dynamic standing exercise. Following, baseline (resting) biomarker levels were compared with resting biomarker levels measured after the 16 to 48 weeks exercise regime. For some participants, blood samples were obtained after 16 weeks, for others, samples were obtained after 48 weeks, while for a few participants, samples were obtained at both 16 and 48 weeks. Therefore, to mitigate the issue of low sample size, for each individual, long-term resting biomarker levels were averaged, and these were used as follow-up values. Due to the exploratory, hypothesis-generating nature of the statistical analysis, α = .05 type I error rates were used. Density plots were drawn to illustrate marker distributions in the total populations before and after exercise regimes.

During exercise, VO₂, VCO₂, VE, and related metrics were available at resting state, and at 5, 10, 15, 20, 25, and 30 minutes. All values were standardized to each participant’s resting values and averaged standardized values at 15 and 20 minutes were compared with resting values using Wilcoxon signed-rank tests. The averaged 15 and 20 minute values were chosen to be compared with baseline values as by this time during the training the children have reached steady state, and the values generally varied the least during these 2 timepoints during the 30 minutes.

Results

The median age of the 14 participants was 13.5 years (IQR: 12–15.5). The sex distribution of participants was 50% to 50% (7 male and 7 female participants). Four participants scored the most severe, 5, on the GMFCS-E&R scale, while the remaining 10 participants scored 4. Table 1 shows medians, IQRs, and full ranges of anthropometric and body composition measures for the population. All 14 children completed the 30-minute exercise and provided blood samples before and after dynamic standing. In total, 9 children completed at least one 16-week period exercise program, with 3 children completing both 16-week programs with a 16-week washout period in between, thus completing the full 48-week exercise program. The observed dropout was observed due to the COVID-19 pandemic, which made adherence and study visits challenging or impossible for the vulnerable study population.

Exercise During the Study

Results from the accelerometry show consistent levels of physical activity during the study period, with a median of 93% (IQR: 89%–95%), 90% (IQR: 85%–93%), 96% (IQR: 94%–97%), and 94% (IQR: 93%–95%) percentage of time being sedentary during the 4 measurement periods, respectively, while the rest of the time was generally spent with low-intensity physical activity. The percentage of time spent with moderate to vigorous physical activity was negligible. In total, 10 and 4 families filled in the children’s exercise diaries for the first, and subsequent exercise periods, respectively. The children were expected to spend a total of 22.5 to 33 hours with dynamic standing exercise during the 16-week exercise programs (Supplementary Material S2 [available online]). For the first period (average duration: 10 wk), the average number of exercise sessions performed by the children was 26 (range: 7–48 sessions), and the total average time spent exercising per session was 30 minutes (range: 26–40 min). The average total time spent exercising during the first period was thus ~13.5 hours. During the second period (average duration: 9 wk), the average number of exercise sessions performed by the children was 26 (range: 12–43 sessions), and the total average time spent exercising per session was 34 minutes (range: 29–39 min). The average total time spent exercising during the second period was thus ~16 hours.

Baseline Biomarker Levels and Their Acute Changes

For selected biomarkers, we compared the ranges of measured values versus standard classifications of ranges. C-reactive protein (CRP) levels are considered normal below 3 mg/L, slightly elevated between 3 and 10 mg/L, moderately elevated between 10 and 100 mg/L, elevated between 100 and 500 mg/L, and severely elevated above 500 mg/L. Of the 14 children at baseline, none had normal, 1 had slightly elevated, 6 had moderately elevated, 5 had elevated, and 4 had severely elevated CRP levels (one missing). The normal range of cortisol is 10 to 20 ng/mL. All children had elevated cortisol levels. The normal range of adrenocorticotropic hormone (ACTH) is 6 to 76 pg/mL. Only one participant was classified as having normal levels, while 12 children had low levels (one missing).

Median levels of laboratory biomarkers at baseline and their acute changes in response to one bout of dynamic standing exercise are presented in Table 2.

Using Wilcoxon ranked-sum tests, CRP levels demonstrated a statistically significant decrease following the exercise session (before exercise: 53 mg/L [IQR: 40–201]; after exercise: 39 mg/L [IQR: 20–107]; P = .04) (Figure 2), while for the rest of the

Table 1 Anthropometric and Body Composition Measures of Children With Cerebral Palsy at Baseline (n = 14)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Median value</th>
<th>IQR</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height, cm</td>
<td>148</td>
<td>135–161</td>
<td>119–165</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>43</td>
<td>34–53</td>
<td>24–68</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>19.5</td>
<td>17.7–20.7</td>
<td>13.6–33.3</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>5.5</td>
<td>3.4–7.3</td>
<td>0.5–16.4</td>
</tr>
<tr>
<td>Soft lean mass, kg</td>
<td>13.7</td>
<td>10.3–15.6</td>
<td>6.2–17.2</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>14.6</td>
<td>11.4–16.6</td>
<td>7–18.2</td>
</tr>
<tr>
<td>Skeletal muscle mass, kg</td>
<td>7.5</td>
<td>5.3–8.8</td>
<td>2.8–10.3</td>
</tr>
<tr>
<td>Percent body fat, %</td>
<td>28.2</td>
<td>20.6–34.5</td>
<td>3.0–53.2</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; IQR, interquartile range. Note: Range represents the minimum and maximum values.
biomarkers, we were not able to reject the null hypothesis of no difference before and after exercise. However, visual inspection of biomarker distributions before and after exercise indicates a suggestive decrease similar to that of CRP for tumor necrosis factor alpha (TNFα), lactic acid, and insulin levels. For insulin-like growth factor 1 (IGF-1), we observe a suggestive increase based on the density plots (before exercise: 32.4 ng/mL [IQR: 25.8–46.7]; after exercise: 37.7 ng/mL [IQR: 29.3–50.1]; \(P = .07\)).

No suggestive distributional shifts were observed for the rest of the biomarkers (Supplementary Material S3 [available online]).

We had access to VO2, VCO2, VE, and related respiratory data during the 30-minute dynamic standing from 11 children (Table 3). Multiple metrics, such as blood flow, minute ventilation, oxygen consumption, and metabolic equivalents show suggestive elevations during the exercise. Visual inspection of the trajectories of changes in these metrics confirms these findings. Spaghetti plots of

Table 2  Acute and Long-Term Changes in Laboratory Biomarkers of Children With Cerebral Palsy Before and After a Dynamic Standing Exercise Regime (n = 14)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Median value before exercise (IQR), n = 14</th>
<th>Median value after one bout exercise (IQR), n = 14</th>
<th>(P) acute*</th>
<th>Median value after 16–48 weeks of exercise (IQR), n = 9</th>
<th>(P) long term*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH, pg/mL</td>
<td>1.2 (0.4–1.7)</td>
<td>1.5 (0.5–2.9)</td>
<td>.88</td>
<td>1.6 (1.2–4.3)</td>
<td>.01</td>
</tr>
<tr>
<td>BDNF, ng/mL</td>
<td>9.3 (8.0–10.5)</td>
<td>8.9 (7.7–10.3)</td>
<td>.63</td>
<td>10.1 (8.8–14.2)</td>
<td>.30</td>
</tr>
<tr>
<td>Cortisol, ng/mL</td>
<td>174 (116–259)</td>
<td>178 (116–239)</td>
<td>.97</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>53 (40–201)</td>
<td>39 (20–107)</td>
<td>.04</td>
<td>65 (51–87)</td>
<td>.50</td>
</tr>
<tr>
<td>GH, pg/mL</td>
<td>124 (64–636)</td>
<td>374 (62–848)</td>
<td>.68</td>
<td>79 (31–731)</td>
<td>.43</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.3 (4.2–4.9)</td>
<td>4.6 (4.0–5.1)</td>
<td>.78</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>IGF-1, ng/mL</td>
<td>32.4 (25.8–46.7)</td>
<td>37.7 (29.3–50.1)</td>
<td>.07</td>
<td>46 (31.7–52.9)</td>
<td>.65</td>
</tr>
<tr>
<td>IGFBP-3, μg/mL</td>
<td>3.9 (3.6–4.4)</td>
<td>4.0 (3.4–4.3)</td>
<td>.47</td>
<td>3.9 (3.7–4.1)</td>
<td>.13</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>0.3 (0–3.0)</td>
<td>1.7 (0–3.2)</td>
<td>.45</td>
<td>0.7 (0–4.4)</td>
<td>.68</td>
</tr>
<tr>
<td>Insulin, pg/mL</td>
<td>474 (163–795)</td>
<td>397 (98–639)</td>
<td>1</td>
<td>632 (519–1543)</td>
<td>.10</td>
</tr>
<tr>
<td>Lactic acid, mmol/L</td>
<td>1.4 (1.3–2.2)</td>
<td>1.9 (1.8–2.3)</td>
<td>.33</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>TNFα, pg/mL</td>
<td>5.1 (4.3–6.5)</td>
<td>5.3 (4.2–6.1)</td>
<td>.29</td>
<td>4.8 (4.2–5.7)</td>
<td>.14</td>
</tr>
<tr>
<td>VEGF, pg/mL</td>
<td>246 (159–360)</td>
<td>262 (160–342)</td>
<td>.85</td>
<td>297 (194–344)</td>
<td>.13</td>
</tr>
</tbody>
</table>

Abbreviations: ACTH, adrenocorticotropic hormone; BDNF, brain-derived neurotrophic factor; CRP, C-reactive protein; GH, growth hormone; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor-binding protein 3; IL-6, interleukine-6; IQR, interquartile range; TNFα, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

*\(P\) values are calculated using Wilcoxon ranked-sum tests.

Figure 2 — CRP and TNFα density plots at baseline, after 30 minutes of dynamic standing, and after a 16–48 weeks of exercise program in children with cerebral palsy (N = 14). CRP indicates C-reactive protein; TNFα, tumor necrosis factor alpha.
for instance, BDNF and vascular endothelial growth factor are shown in Supplementary Material S3 (available online).

1.6 pg/mL [IQR: 1.2 – 4.3] median values were observed among the children at baseline, suggesting systemic inflammation in most participants. This finding is consistent with previous research, which has consistently demonstrated elevated levels of inflammatory markers, such as CRP and transforming growth factor beta 1, in children with cerebral palsy compared to adults with or without the condition (31). The results of previous studies, such as a comparison of muscle biopsies from children with and without cerebral palsy, have also revealed higher levels of gene expression of the inflammatory markers interleukine-1β, interleukine-6, and TNF in children with cerebral palsy (40). In addition, studies that have compared cellular responses to endotoxin stimulation ex vivo have indicated altered inflammatory responses in cells of children with cerebral palsy compared with healthy controls, with one study showing elevated TNFα response (19), while another showing hyperresponsiveness to lipopolysaccharide stimulus regarding interleukine-1, interleukine-2, and interleukine-6 levels compared with controls (41). A recent systematic review supports these findings, indicating that the majority of published studies demonstrate a statistical association between elevated inflammatory markers and neurodevelopmental outcomes in children with cerebral palsy (21). It is reasonable to assume that the observed high levels of CRP are likely because the recruited participants were classified as the most severe levels on the GMFCS-E&R scale, reflecting high levels of neurodevelopmental damage. Another hypothesis is that elevated inflammatory markers might be the consequence of latent infections that are more prevalent in this population. While Hansen et al (13) found lower average BDNF levels (~4 ng/mL) in children with severe cerebral palsy (GMFCS-E&R IV-V) compared with children with milder forms of cerebral palsy (GMFCS-E&R I-III) and children with typical developmental (>6 ng/mL), somewhat surprisingly, children in the LEER study had higher BDNF levels (median = 9.3 ng/mL) at baseline.

We observed a statistically significant decrease in CRP levels after 30 minutes of dynamic standing, and visual observation of biomarker distributions showed similar decreasing trends for TNFα, lactic acid, and insulin levels, and an increase in IGF-1 levels. In addition, we observed a statistically significant increase in ACTH levels following a long-term exercise program. Physical

### Table 3  Acute Changes in Oxygen Consumption and Other Metrics in Children With Cerebral Palsy During Dynamic Standing (n = 11)

<table>
<thead>
<tr>
<th>Metric</th>
<th>Median value before exercise (IQR)</th>
<th>Median value during exercise (IQR)</th>
<th>Median standardized value during exercise (IQR); resting value = 1</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2</td>
<td>179 (109–246)</td>
<td>170 (131–261)</td>
<td>1.11 (1.06–1.40)</td>
<td>.08</td>
</tr>
<tr>
<td>VO2/kg</td>
<td>4.4 (3.3–4.7)</td>
<td>4.9 (3.9–5.6)</td>
<td>1.11 (1.06–1.40)</td>
<td>.08</td>
</tr>
<tr>
<td>MET</td>
<td>1.26 (0.95–1.36)</td>
<td>1.41 (1.11–1.59)</td>
<td>1.12 (1.05–1.40)</td>
<td>.08</td>
</tr>
<tr>
<td>RER</td>
<td>0.83 (0.78–0.85)</td>
<td>0.81 (0.79–0.85)</td>
<td>1.01 (0.98–1.06)</td>
<td>.58</td>
</tr>
<tr>
<td>VCO2</td>
<td>135 (90–207)</td>
<td>127 (104–229)</td>
<td>1.09 (1.05–1.40)</td>
<td>.04</td>
</tr>
<tr>
<td>BF</td>
<td>21.8 (18.5–28.1)</td>
<td>24.7 (19.4–29.5)</td>
<td>1.10 (1.01–1.17)</td>
<td>.02</td>
</tr>
<tr>
<td>VE</td>
<td>5.3 (4.5–8.8)</td>
<td>6.9 (5.1–9.7)</td>
<td>1.13 (1.08–1.22)</td>
<td>.02</td>
</tr>
<tr>
<td>HR</td>
<td>97 (95–102)</td>
<td>103 (99–113)</td>
<td>1.05 (1.02–1.08)</td>
<td>.20</td>
</tr>
</tbody>
</table>

Abbreviations: BF, blood flow; HR, heart rate; IQR, interquartile range; MET, metabolic equivalent; RER, respiratory exchange ratio; VE, minute ventilation; VCO2, carbon dioxide production; VO2, oxygen consumption, VO2/kg, oxygen consumption per kilogram body weight. Note: During exercise, metrics were available at resting state, and at 5, 10, 15, 20, 25, and 30 minutes. Metrics at 15 and 20 minutes were averaged and reported in the table. Subsequently, all values were standardized to each participant’s resting values and averaged standardized values at 15 and 20 minutes were compared to resting values.

*P values are calculated using Wilcoxon ranked-sum tests.
activity has been demonstrated to increase ACTH in other populations (14); and increases in ACTH are associated with better anti-inflammatory and anti-stress responses (11). We observe that growth factors BDNF and vascular endothelial growth factor, previously linked to improvements in neurological deficit and promoting recovery processes (16), gradually increase during the full study period, albeit not to a statistically significant degree. These findings are in line with prior research showing elevated growth factors and anti-inflammatory hormones in response to exercise (14,34). While inflammatory markers CRP and TNFα did not show a statistically significant difference between baseline and after the long-term exercise program, they do show a gradual decrease across the 3 timepoints based on the density plots indicating that long-term exercise programs have promising potential in decreasing systemic inflammation. We believe that the lack of statistically significant findings for CRP and TNFα are related to lack of statistical power due to the small sample size, and thus, replication in larger cohorts is warranted to confirm these changes.

We were able to identify only a few studies published to date that concern themselves with biomarker changes in response to exercise in children with cerebral palsy, one showing altered blood immunological biomarker concentrations following rehabilitation (35), while another one showing gene expression profile changes following aerobic exercise (38). However, none of them investigated the hormonal or inflammatory biomarkers for which we observed changes. In our previous project, we tracked blood glucose and lactate levels before and after 30 minutes of dynamic standing in 24 children with cerebral palsy and observed a higher decrease in lactate following exercise in those children with higher baseline lactate levels compared to others (20). Elsewhere, changes in inflammatory biomarkers, salivary cortisol, and alpha-amylase were evaluated in response to a physical therapy session between children with and without cerebral palsy (9). Similar to our findings, higher baseline cortisol levels were observed, but they also noted a marked acute reduction of cortisol levels in response to exercise in children with cerebral palsy, which we did not see in our study (9).

Strengths and Limitations
The study design allowed us to collect comprehensive and detailed information from the participants, allowing us to understand the impact of exercise in nonambulant children with cerebral palsy. The collected data on physical activity levels, anthropometrics, respiratory markers, and blood samples provided insight into the acute and long-term effects of exercise in this population. While not all children completed the full study period, and the study was simplified due to the COVID-19 pandemic, that proved to be especially risky for those with cerebral palsy (2), we still managed to collect data from a considerable proportion of our population for up to 48 weeks.

The small sample size limited our statistical power and even though we showed a small number of statistically significant metabolic changes, we consider all our results suggestive and to be treated with caution. All our results warrant replication in independent cohorts. We mitigated the sample size limitation by conducting nonparametric statistical testing. The standardization of the respiratory data with the participants’ body weight and their resting values made it difficult to interpret the changes during the 30-minute dynamic standing exercise. We aimed to mitigate this limitation by plotting individual trajectories of these metrics. The small sample size also limited our abilities in performing interesting subgroup comparisons; for instance, differences between boys and girls, and by age groups (before and after puberty) warrant further investigation in larger samples. A further limitation is that some biomarkers were only available at baseline and after the first exercise session, so we could not test long-term changes across all metabolites.

Clinical and Research Implications
The effects of cerebral palsy on the muscular system have been well documented; while cerebral palsy is associated with shorter muscles that are drastically reduced in volume, it has also been associated with altered cellular composition, muscular regulation, and gene expression profiles (12). As those living with cerebral palsy often have reduced opportunities for regular exercise, cerebral palsy can perpetuate further muscular deterioration, which, in turn, can lead to a cascade of cardiometabolic consequences, particularly for children with severe cerebral palsy (GMFCS-E&R IV-V). Results from the LEER study suggest that a number of immunological and hormonal biomarkers are dysregulated in these children. The long-term impact of chronic inflammation indicated by our results in this population is unknown and future studies should address this knowledge gap. Regular exercise, using a dynamic standing device, offers a feasible alternative for families to adopt regular exercise for children with severe cerebral palsy. Although our results are preliminary, we show promising alterations in blood-borne immunological, hormonal, and other biomarkers in response to as short as 30 minutes of exercise. Although a challenging undertaking, larger, prospective studies utilizing similar deep-phenotyping as LEER (eg, by Valadão et al 37), are warranted to confirm our findings and gain a more thorough understanding of physiological changes in response to physical activity. In addition, we find it crucial to assess the long-term impact of regular exercise on patient-reported outcome measures, such as participation in regular activities and quality of life, and clinical outcomes, such as the development of type 2 diabetes and cardiovascular diseases.

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