Santos, Adriana N.; Pavão, Silvia L.; Avila, Mariana A.; Salvini, Tania F.; Rocha, Nelci A. C. F. Reliability of isokinetic evaluation in passive mode for knee flexors and extensors in healthy children
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São Carlos, Brasil

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ABSTRACT | Background: The isokinetic dynamometer has been considered the gold-standard measurement of muscle performance. However, the reliability for the passive mode in children has not been reported to date. Objectives: The purpose was to evaluate the reliability of the isokinetic dynamometer in passive mode in children. Method: Twenty-one healthy children (ten girls, eleven boys), aged 5 to 12 years (age: 8.5±2.2 years), were evaluated using an isokinetic dynamometer. Each participant was tested twice with a one-week interval and performed five consecutive cycles of knee extension and flexion. The test was performed at 60°/s in the concentric passive mode and the children performed maximum contractions. The measured variables were peak torque, average peak torque, total work, and average power, time to peak torque and angle of peak torque for dominant and non-dominant lower limbs. Reliabilities were determined using intraclass correlation coefficient (ICC \(_{3,1}\)), standard error of measurement (SEM and SEM%), and coefficient of variation (CV). Results: We found good reliability in both lower limbs for peak torque, average peak torque, total work and average power of knee flexors and extensors, with ICC\(_{3,1}\) values greater than 0.80; SEM ranging from 6.7 to 79.2; SEM% ranging from 10.4% to 16.8%; CV lower than 15%. Bland-Altman analysis showed that the bias was low than 10% and limits of agreement (LOAs) ranging from 33.9% to 59.2%, and -28.8% and -52.8%, showing that measures tended to disagree. However, time to peak torque (ICC\(_{3,1}\) <0.68; SEM > 0.34; SEM% >37.4%; CV>15.3%; bias >11.0%; LOA>61.0%) and angle of peak torque (ICC\(_{3,1}\) <0.76; SEM>9.3; SEM%>27.6%; CV>15.3%; bias>11.0%; LOA>61.0%) were not reliable. Conclusions: The findings indicate that isokinetic evaluation in passive mode for knee extensors and flexors of dominant and non-dominant lower limbs of children without disabilities was reliable for peak torque, average peak torque, work, and power. However, average time to peak torque and angle of peak torque were not reliable. Keywords: isokinetic evaluation; children; knee; reliability; rehabilitation; movement.

Introduction

Previous studies have shown that muscle strength is important for activities of daily living given that deficits in the ability to produce torque are related to impairments in functional activities performance and decreased mobility\(^1,2\). Muscle strength has been evaluated with different methods, such as manual tests\(^3\), hand-held dynamometer\(^4,5\), and isokinetic dynamometer\(^6,7\). The isokinetic dynamometer has been considered the gold-standard evaluation\(^7,8\) because it allows a quantitative evaluation of muscle function, throughout variables such as torque, power, and endurance\(^6\).

However, before the isokinetic dynamometer can be used for research or clinical rehabilitation, its reliability must be tested\(^9\). Reliability is defined as the consistency between different measures performed under similar conditions\(^10\), and it shows the degree to which test scores are free from errors of measurement\(^11\). Therefore, when the test is reliable, device, clinician or testing errors can be excluded\(^9\) and measures obtained during isokinetic evaluation over a period of time can be compared\(^9,12\). Previous studies have reported that isokinetic evaluation has good reliability in healthy children\(^13-16\), elderly people\(^17,18\), and healthy adults\(^19,20\).

These studies verified the reliability of isokinetic evaluation in active mode. However, individuals with muscle weakness usually do not have enough strength to move the lever arm against gravity or complete full range of motion required to perform isokinetic evaluation in active mode. Therefore, previous studies used the passive mode for subjects with muscle weakness, such as children with cerebral palsy\(^21\) and patients with neuromuscular disorders\(^22\). In the
Reliability of isokinetic evaluation in children

During the passive mode test, the individual performs a maximal effort and, at the same time, the dynamometer produces movement at a constant preset velocity. Therefore this test is called an active-assisted test.

We found one study that applied isokinetic evaluation in passive mode in children with disabilities, however we did not find any studies that reported how isokinetic variables are characterized in children without disabilities during active-assisted test. Studies have reported the reliability for healthy children in the active test but, given the differences in neuromuscular properties required to perform active and active-assisted tests, it is necessary to determine baseline measures of muscle performance during isokinetic evaluation in passive mode in healthy children.

Before describing how isokinetic parameters are presented in healthy children, the reliability of passive mode protocol must be shown. After that, a normative database in active-assisted test can be developed and future studies aiming to compare children with and without disabilities can be conducted. Therefore, the purpose of this study was to evaluate the reliability of isokinetic dynamometer evaluation in passive mode of knee flexors and extensors in healthy children.

### Method

#### Study design

A test-retest design was used to assess the reliability of isokinetic variables during knee flexor and extensor evaluation. Each subject was assessed twice at the same time of day by the same rater. A seven-day interval between evaluations was chosen.

#### Participants

Twenty-six children were recruited for the study. Three were excluded because they missed the second evaluation, and two were excluded due to equipment problems. Therefore, twenty-one children, 10 girls and 11 boys, aged 5 to 12 (5 years = 2 children; 6 years = 3 children; 7 years = 3 children; 8 years = 2 children; 9 years = 4 children; 10 years = 2 children; 11 years = 3 children; 12 years = 2 children) participated in the study. Inclusion criteria were children without neuromuscular, orthopedic or cardiovascular diseases who did not participate in sports activities more than three days per week. A sample size of 21 participants was recruited to detect a difference in reliability of 0.9 and 0.7 at 80% power and a 5% level of significance using two ratings.

The Research Ethics Committee of Universidade Federal de São Carlos (UFSCar), São Carlos, SP, Brazil (Protocol number: 479/2010, process number: 23112.003678/2010-57) approved the study, which is in agreement with the Declaration of Helsinki and Resolution 196/96 of the National Health Council.

### Test protocol

Isokinetic data was collected with the Biodex Multi-Joint System 3 isokinetic dynamometer (Biodex Medical System, Shirley, NY). Each child underwent two identical test sessions, with a seven-day interval. All tests were conducted by the same investigator. Before the start of each testing session, the system was calibrated according to the manufacturer specifications. Testing was performed on the dominant and non-dominant lower limbs. The dominant limb was defined as the limb used to kick a ball. In the present study, eighteen children were right-footed and three children were left-footed.

Children were seated in the chair, with hip and knee in 90° of flexion, stabilized with thigh and trunk straps. The rotational axis of the knee joint (lateral femoral epicondyle) was aligned with the rotational axis of the lever arm. The resistance pad was attached approximately 3cm above the medial malleolus. The total range of motion was set as 70°, from 90° of knee flexion to 20° of knee extension (0° being full extension). Additional back support was provided when necessary to ensure the biomechanical alignment between the rotational axis of both knee and dynamometer.

After the children were positioned at the chair, they were given a demonstration of the test through pictures and verbal instructions about the movement to be performed. Verbal instructions were the same for all children and were as follows: a) knee extensors: “you must extend your leg as if you were kicking a ball, producing the greatest strength that you can”; b) knee flexors: “you must bend your leg, producing the greatest strength that you can”. Then, their lower limbs were moved passively by the examiner through the desired range of motion, while the examiner explained the test.

Three submaximal contractions were performed for familiarization with test procedures. After a 2-min rest and additional brief description of the contraction, the children performed the test, which was composed of five maximal voluntary contractions in the concentric passive mode. During each test,
visual feedback and verbal encouragement were given.

The 60°/s velocity was chosen because the muscle’s capacity to generate torque is better at low velocities (30°/s and 60°/s) and activities of daily living are likely to be performed with torques generated at these velocities. Additionally, 60°/s was chosen over 30°/s because a pilot study detected that this velocity was the most comfortable for children.

Data analysis and statistics

The following variables were measured: a) peak torque: defined as the highest force output at any moment during a repetition; b) average peak torque: defined as the average of the peak torque values obtained during a series of repetitions (may be considered a better estimate of overall function than peak torque given that function is dependent on repetition of movement); c) total work: defined as force multiplied by distance (indicates the ability of the muscle group to maintain torque through the test); d) average power: defined as the total work divided by the time it takes to perform the work; e) time to peak torque: defined as the average measure of the time from the start of the muscular contraction to the peak torque point of each repetition (ability of a muscle group to produce strength rapidly); f) angle of peak torque: defined as the point in the ROM where peak torque is produced. Data were normalized by body weight and multiplied by 100 (N/Kg×100) since body mass seems to contribute to muscle strength.

The variables considered in the study were normalized by subtracting the torque curve produced during isokinetic passive mode from a torque curve measured when the child was relaxed. This curve was obtained by asking the child to relax as much as possible while a passive mobilization of the knee occurred.

The between-session reliability was calculated for all variables (evaluation 1 versus evaluation 2). Relative reliability was assessed by intraclass correlation coefficient (ICC). ICC is a measure that determines the consistency between different tests executed by the same examiner in a test-retest design study. An ICC was selected because a single rater collected the measurements, and it is based on a repeated-measures analysis of variance. For ICC, a 95% confidence interval was calculated. ICC was classified as follows: over 0.90 as excellent; between 0.80 and 0.89 as good; between 0.70 and 0.79 as acceptable; below 0.70 as unacceptable.

Moreover, absolute reliability was determined with the coefficient of variation, the standard error of the measurement (SEM and SEM%), and the Bland-Altman plot analysis. The coefficient of variation (CV) was used to express intra-subject variation between two measurements. It was considered that CV should be 15% or below. The CV was calculated, for each subject, as:

\[ CV = \frac{SD \text{ two measurements}}{\text{Mean two measurements}} \times 100 \]

SEM was considered to analyze method errors. It determines how much a score is likely to vary with repeated measurements of the same subject. The SEM is defined by:

\[ \text{SEM} = \frac{\sqrt{(1-\text{ICC}_{\text{cv}})}}{2 \times \text{SEM}} \]

where SD is the standard deviation of all the measurements from the two sessions.

Moreover, SEM% was considered, as it is more easily interpreted. The SEM% indicates the measurement errors in relative values. The SEM% is defined by:

\[ \text{SEM\%} = \left( \frac{\text{SEM}}{\text{mean}} \right) \times 100 \]

where mean is the mean for all the observations from test sessions one and two.

The Bland-Altman analysis determines the agreement between the measures of sessions 1 and 2. In the test, the mean of differences between the two measures and the confidence limits are calculated. The mean of differences is considered the estimated bias and the confidence limits, or limits of agreement (LOAs), provide information about how much random variation may be influencing the ratings. When there is a tendency that agreement between two measures occurs, the mean will be near zero and the range between these two limits will be small. When a measure tends to be higher than the other one, the mean will be far from zero, but the confidence intervals will be short. If the measurements tend to disagree the mean will be near zero and the confidence intervals will be wide.

In the present study, the LOAs were determined. First, the absolute differences against the individual means of the two measurements were plotted in order to verify homoscedasticity. We performed a linear regression; when p was greater than 0.05, classic 95% LOAs were calculated as follows: the standard deviation of the differences between evaluation 1 and 2 was calculated and multiplied by +1.96 and –1.96 to obtain, respectively, the upper and lower 95% LOAs. When p was less than 0.05, indicating that the difference increases as the average increases (heteroscedasticity), the variables were log-transformed (natural logarithm, ln). Then, the limits of these variables were back-transformed (antilogarithm). We determined the LOAs and the mean of differences in percentage.
The SPSS software program, version 17.0, was used for statistical analysis.

● Results

Table 1 shows the ICC, CV, SEM, SEM%, LOAs, and mean of differences for all variables of isokinetic evaluation methods for knee extensors and flexors of both lower limbs. Regarding ICC, moderate test-retest reliability was found for peak torque, average peak torque, total work, and average power of knee flexors and extensors of both lower limbs. However, insufficient relative reliability was found for time to peak torque and angle of peak torque. For all variables, except time to peak torque and angle of peak torque, the CVs were lower than 15%, as recommended.

Moreover SEM% values ranged from 10.4 to 16.8% for peak torque, average peak torque, total work, and average power of knee flexors and extensors of both lower limbs. However, time to peak torque and angle of peak torque showed high SEM and SEM% values, indicating that the measures of evaluations 1 and 2 did not agree.

According to the Bland-Altman analysis, peak torque, average peak torque, total work and average power showed mean of differences of less than 10%. However, the LOAs were high (LOAs>28%), showing that these variables tend to disagree between the two measurements. Regarding angle to peak torque and time to peak torque, the mean difference showed high bias (mean of differences>11.0%), and the limits of agreement were very high (LOAs>61.0%), showing that between two evaluations these measurements disagreed. Moreover, according to our findings, the mean difference for time to peak torque and angle to peak torque of knee flexors of both lower limbs were biased positively, indicating that these measurements were higher in evaluation 1 than in evaluation 2 in most cases. Regarding angle to peak torque of knee extensors of both lower limbs, the mean of differences was biased negatively indicating that these measurements were higher in evaluation 2 than in evaluation 1 in most cases.

● Discussion

In the present study, the reliability of peak torque, average peak torque, average power, total work, time to peak torque and angle of peak torque of knee flexors and extensors from dominant and non-dominant lower limb were assessed in the passive mode of the isokinetic dynamometer. The results suggested that there is a good reliability concerning peak torque, average peak torque, average power and total work. However time to peak torque and angle to peak torque were not reliable in healthy children.

According to our findings, peak torque and average peak torque were reliable since ICC was moderate and CV, SEM, and SEM% were low. Our findings are consistent with previous studies. Good reliability (ICC>0.80) of peak torque of knee extensors and flexors was reported in children with CP and without disabilities in isokinetic active mode evaluation15-16; as well as in healthy adults37 and aged people18. Moreover, previous studies reported low SEM% values, ranging from 8% to 14% for knee extensors and from 10% to 11% for knee flexors in older adults18, persons with late effects of polio13 and subjects after stroke58. Also, previous studies reported low CVs, ranging from 7 to 20%, for peak torque of knee flexors and extensors17,18.

Besides peak torque and average peak torque; we found moderate reliability for total work and average power in healthy children. Previous studies generally have reported good reliability for total work and power of knee flexors and extensors22,39. Feiring et al.39 demonstrated, in healthy adults, that ICC values for total work were 0.96 for knee extensors and 0.94 for flexors. Tiffreau et al.22 reported excellent relative reliability (ICCs>0.90) for power and work of knee flexors and extensors in individuals with neuromuscular disorders.

Moreover, concerning peak torque, average peak torque, total work and average power, the Bland-Altman analysis showed that the mean of differences values were lower than 10% and the LOAs ranged from 28.0 to 63.6%. Previous studies with older adults showed that LOAs were moderate to high ranging from 21 and 54% for peak torque, average peak torque, work, and power of knee flexors and extensors in older adults17,18. These findings indicate that the measurements tended to disagree between the two evaluations and a low precision of the test is presented.

These findings could be related to the fact that we evaluated 21 children. In order to precisely estimate the 95% interval limits of agreements a sample size greater than 50 is necessary. Therefore, we can conclude that, with the test protocol used in the present study, measurements tend to disagree. However, it is possible to determine a range that could indicate how much a measure needs to improve to detect any change caused by training or treatment.

In addition to the variables already reported, time to peak torque and angle of peak torque were not
Table 1. Reliability of isokinetic evaluation in passive mode for knee flexors and extensors in healthy children.

<table>
<thead>
<tr>
<th>Knee Extensors</th>
<th>Mean ± SD</th>
<th>ICC (3)</th>
<th>CV</th>
<th>SEM</th>
<th>SEM%</th>
<th>Limits of Agreement (%)</th>
<th>Mean of differences (%) - Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak Torque (Nm/Kg×100)</strong></td>
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<tr>
<td>Dominant</td>
<td>174.0±44.5</td>
<td>170.39±51.7</td>
<td>0.84</td>
<td>10.1</td>
<td>18.2</td>
<td>10.4</td>
<td>Upper limit:36.5 Lower limit:-28.8</td>
</tr>
<tr>
<td>Non-Dominant</td>
<td>167.5±38.1</td>
<td>172.8±54.1</td>
<td>0.83</td>
<td>12.2</td>
<td>20.3</td>
<td>11.9</td>
<td>Upper limit:40.7 Lower limit:-49.2</td>
</tr>
<tr>
<td><strong>Average Peak Torque (Nm/Kg×100)</strong></td>
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</tr>
<tr>
<td>Dominant*</td>
<td>139.5±36.8</td>
<td>139.8±44.2</td>
<td>0.87</td>
<td>12.9</td>
<td>15.8</td>
<td>11.2</td>
<td>Upper limit:46.2 Lower limit:-39.0</td>
</tr>
<tr>
<td>Non-Dominant*</td>
<td>137.5±34.8</td>
<td>138.3±49.3</td>
<td>0.81</td>
<td>11.4</td>
<td>16.3</td>
<td>11.8</td>
<td>Upper limit:49.5 Lower limit:-43.8</td>
</tr>
<tr>
<td><strong>Average Power (W/Kg×100)</strong></td>
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</tr>
<tr>
<td>Dominant</td>
<td>73.1±24.0</td>
<td>69.3±31.0</td>
<td>0.80</td>
<td>14.9</td>
<td>12.0</td>
<td>16.8</td>
<td>Upper limit:47.7 Lower limit:-37.8</td>
</tr>
<tr>
<td>Non-Dominant*</td>
<td>68.6±21.1</td>
<td>67.8±27.8</td>
<td>0.81</td>
<td>15.0</td>
<td>10.3</td>
<td>15.1</td>
<td>Upper limit:52.2 Lower limit:-48.0</td>
</tr>
<tr>
<td><strong>Total work (J/Kg×100)</strong></td>
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<tr>
<td>Dominant</td>
<td>550.3±173.6</td>
<td>509.2±198.4</td>
<td>0.86</td>
<td>13.9</td>
<td>77.0</td>
<td>12.6</td>
<td>Upper limit:58.0 Lower limit:-38.4</td>
</tr>
<tr>
<td>Non-Dominant*</td>
<td>524.0±161.6</td>
<td>516.6±196.5</td>
<td>0.80</td>
<td>15.0</td>
<td>79.2</td>
<td>15.2</td>
<td>Upper limit:63.6 Lower limit:-40.2</td>
</tr>
<tr>
<td><strong>Time to Peak Torque (s)</strong></td>
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</tr>
<tr>
<td>Dominant</td>
<td>0.54±0.36</td>
<td>0.39±0.31</td>
<td>0.68</td>
<td>41.7</td>
<td>0.54</td>
<td>40.0</td>
<td>Upper limit:170.0 Lower limit:-101.1</td>
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<tr>
<td>Non-Dominant</td>
<td>0.66±0.45</td>
<td>0.63±0.50</td>
<td>0.27</td>
<td>49.8</td>
<td>0.34</td>
<td>52.0</td>
<td>Upper limit:204.3 Lower limit:-155.9</td>
</tr>
<tr>
<td><strong>Angle to Peak Torque (°)</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Dominant</td>
<td>64.3±21.4</td>
<td>73.5±18.9</td>
<td>0.76</td>
<td>15.3</td>
<td>9.3</td>
<td>13.4</td>
<td>Upper limit:32.2 Lower limit:-61.6</td>
</tr>
<tr>
<td>Non-Dominant</td>
<td>67.2±27.1</td>
<td>69.9±31.1</td>
<td>0.33</td>
<td>29.0</td>
<td>19.4</td>
<td>30.1</td>
<td>Upper limit:104.2 Lower limit:-107.7</td>
</tr>
</tbody>
</table>

SD: standard deviation; ICC: intraclass correlation coefficient; CV: coefficient of variation; SEM: standard error of the measurement; SEM%: standard error of measurement %. *Data that required log transformation (non-normality and heteroscedasticity).
Table 1. Continued...

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Test-Retest Reliability</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Evaluation 1</td>
<td>Evaluation 2</td>
</tr>
<tr>
<td>Knee Flexors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Torque (Nm/Kg×100)</td>
<td>Dominant</td>
<td>133.2±39.5</td>
</tr>
<tr>
<td></td>
<td>Non-Dominant</td>
<td>134.4±34.9</td>
</tr>
<tr>
<td>Average Peak Torque</td>
<td>Dominant*</td>
<td>106.9±21.6</td>
</tr>
<tr>
<td></td>
<td>Non-Dominant</td>
<td>113.9±30.2</td>
</tr>
<tr>
<td>Average Power (W/Kg×100)</td>
<td>Dominant</td>
<td>59.7±13.2</td>
</tr>
<tr>
<td></td>
<td>Non-Dominant*</td>
<td>59.0±14.7</td>
</tr>
<tr>
<td>Total work (J/Kg×100)</td>
<td>Dominant</td>
<td>463.0±101.5</td>
</tr>
<tr>
<td></td>
<td>Non-Dominant*</td>
<td>458.0±113.2</td>
</tr>
<tr>
<td>Time to Peak Torque (s)</td>
<td>Dominant</td>
<td>0.92±0.53</td>
</tr>
<tr>
<td></td>
<td>Non-Dominant*</td>
<td>1.07±0.51</td>
</tr>
<tr>
<td>Angle to Peak Torque (°)</td>
<td>Dominant</td>
<td>57.7±25.1</td>
</tr>
<tr>
<td></td>
<td>Non-Dominant</td>
<td>67.2±27.1</td>
</tr>
</tbody>
</table>

SD: standard deviation; ICC: intraclass correlation coefficient; CV: coefficient of variation; SEM: standard error of the measurement; SEM%: standard error of measurement %. *Data that required log transformation (non-normality and heteroscedasticity).
consistent between the two evaluations. Moreover, according to Bland-Altman analysis, time to peak torque tends to decrease as does the angle of peak torque for knee extensors. Regarding knee flexors, angle of peak torque tended to decrease between evaluations. This means that, children tended to produce peak torque at a low angle in evaluation 2.

One possible explanation for these findings could be the effects of the learning process that occurs when an individual is not familiarized with a procedure. In the first evaluation, children were concerned with understanding the demands of the task, which is called the cognitive phase of the learning process. In the second session, after familiarization, children could concentrate more on the test performance itself. Therefore, it is possible to suggest that, between the two evaluations, children learned how to activate the muscles, which reflected on the capacity to produce maximal contraction faster. The fact that a lower angle of peak torque was also found for knee flexors and extensors reinforces the suggestion that in second evaluation children were able to contract with maximal effort faster. In passive mode, the shaft velocity does not change unless torque limits are exceeded, which did not occur in the present study. Therefore, a lower angle of peak torque can be directly related with a lower time to peak torque, which could be related to the ability to produce maximal contraction faster.

Brown and Whitehurst also reported that variables related with the velocity of the test changed between short intervals of tests. It was reported that, after short training session (one and two days), the average time to peak torque decreased and the rate of velocity development increased in healthy adults. The rate of velocity development is defined as the change in joint angle while the limb is accelerating to the preset angular velocity and depends on the rate of muscle activation. These findings were related to changes in neural mechanisms, such as increases in motor unit recruitment and firing rates.

This possible learning effect could be minimized if familiarization sessions were conducted before test sessions. Ploutz-Snyder and Giamis found that 8 to 9 familiarization sessions are required to achieve consistent one-repetition maximum strength measurements in untrained older subjects, while 3 to 4 sessions were required for healthy adults. However, no studies were found that evaluated the effects of familiarization sessions in children. We suggest that future studies investigate if familiarization sessions are required during isokinetic evaluation with the intention of minimizing the learning effects that can influence qualitative isokinetic measures, such as time to peak torque and angle of peak torque.

This study had some limitations. First, a familiarization procedure should be applied in order to avoid the learning effect, especially regarding variables related with time of contraction. Second, LOAs could be determined in a large sample in order to be used to determine the range in which one significant difference between evaluations should occur.

Despite these limitations, we believe that this study is relevant because it provides information about the reliability of isokinetic evaluation for knee flexors and extensors in healthy children considering the active-assisted test (passive mode). Future studies should be done in order to provide normative values of active-assisted protocol in healthy children, allowing future comparison with populations with neuromuscular deficits who are not able to perform an active test. Furthermore, the normative data can be used to assess the effectiveness of treatments for developing muscle strength.

In conclusion, the reliability of the isokinetic dynamometer (Biodex) in the passive mode was good for peak torque, average peak torque, average power, and total work of knee flexors and extensors of the dominant and non-dominant lower limbs in healthy children. Thus, isokinetic evaluation in the passive mode can be used to evaluate muscle performance in this population. However, time to peak torque and angle to peak torque were not reliable. Thus, it is not recommended to consider these variables when the protocol used in the present study is applied to children.

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### References


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