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Difference in the Motor Unit Firing Behavior During Submaximal Isometric Ramp Contraction Between Healthy Children Aged 6–12 Years and Young Adults

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ABSTRACT

Introduction/Aims: Although nervous system maturation is a key factor underlying the difference in muscle strength between children and adults, specific neural strategies for modulating motor unit (MU) firing during graded force production remain largely unexplored. This study aimed to clarify the differences in MU firing behavior between children and young adults during submaximal isometric ramp contractions.

Methods: Eighteen healthy children (aged 6–12 years) and 18 healthy young adults performed maximal voluntary contractions (MVCs) and submaximal ramp contractions to 50% of MVC for isometric knee extension. High-density surface electromyography data were collected from the vastus lateralis muscle and were decomposed to identify individual MU firing. MUs were analyzed according to their recruitment thresholds (RTs) to compare their firing rates (FRs) and the change in FR (ΔMU FR) at various force intervals.

Results: Children consistently exhibited significantly higher MU FRs than adults across almost all RT groups and force levels. ΔMU FR was higher in children only during the initial 10%–20% MVC interval for the lowest-threshold MUs but was significantly lower for higher threshold MUs at higher force levels.

Discussion: Higher MU FRs in children likely represent a functional adaptation to compensate for immature muscle contractile properties, thereby ensuring effective force generation. This distinct neural control strategy, which combines high initial rates with subsequent firing saturation, may reflect the ongoing maturation of spinal motor control. These findings provide a valuable reference for assessing pediatric neuromuscular disorders and can inform the design of more effective exercise and rehabilitation programs.

Abbreviations: ATT, adipose tissue thickness; BW, body weight; FR, firing rate; HDsEMG, high-density surface electromyography; MU, motor unit; MVC, maximal voluntary contraction; RT, recruitment threshold; VL, vastus lateralis.

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1 | Introduction

Muscle force generation is a complex process that is initiated and controlled by the central nervous system (CNS). The final common pathway for this neural command is the α-motor neuron and the muscle fibers it innervates, known as a motor unit (MU). To produce progressively greater force, the nervous system employs two primary strategies: the orderly recruitment of additional MUs, typically from smallest to largest, according to Henneman's size principle, and the increase in the firing rate (FR) of already active MUs, a process termed rate coding [1, 2].

The difference in muscle strength between children and adults cannot be solely explained by muscle size but is also attributed to the ongoing maturation of the nervous system [3–5]. Much of the research investigating these neural factors has focused on the maximal voluntary contraction (MVC). These studies suggest that, during maximal effort, children's voluntary activation is not fully developed, potentially limiting their ability to recruit higher threshold MUs and achieve higher FRs [6–8]. This view is supported by evidence of the ongoing maturation of corticospinal pathways throughout adolescence [9, 10]. However, although crucial for understanding the limits of strength production, many functional movements and motor skills depend on the precise regulation of force at submaximal levels. It remains largely unknown how the developmental characteristics of the CNS are reflected in the firing behavior of individual MUs during these more common, controlled contractions.

High-density surface electromyography (HDsEMG) decomposition allows for the noninvasive study of individual MU firing patterns [11–13]. Previous studies comparing MU FR between children and adults have primarily focused on the sustained steady-state phase of a contraction, often without considering the recruitment threshold (RT) [14–16]. However, this approach offers limited insight into the critical phase of progressive force generation. Consequently, how children modulate MU FR to increase force output—a key aspect of rate coding—remains largely unexplored. Furthermore, motor neuron firing is profoundly shaped by that cell's intrinsic properties, which amplify synaptic drive [17]. Recent findings suggest that these properties are expressed differently during maturation, which is thought to influence the nonlinear characteristics of firing behavior during the ascending phase of a ramp contraction [18]. Understanding how these age-related intrinsic properties influence firing behavior during graded force production is essential for fully comprehending the maturation of motor control.

Therefore, this study aimed to investigate MU firing behavior in healthy children during a submaximal ramp contraction in relation to RT and to compare these characteristics with those of young adults. We hypothesized that children would exhibit FRs in low-threshold MUs comparable to those of adults, whereas they would demonstrate lower FRs in higher threshold MUs. Additionally, we hypothesized that the change in MU FR during the ascending phase of a ramp contraction would differ between children and adults.

2 | Methods

2.1 | Participants and Ethical Approval

Primary school-aged children and young adults were recruited from the local community and Chukyo University via flyers and word of mouth. All participants were healthy and free from any known neuromuscular or orthopedic disorders that could have affected the experimental results. The data collected during the single experimental session described herein were also utilized for a separate analysis addressing a different research question that has been reported elsewhere [19, 20]. The inclusion and exclusion criteria were identical for both analyses, as they were derived from the same participant cohort and data acquisition sessions. Written informed consent was obtained from all the young adults. For the children, their parents or legal guardians provided written informed consent. The study protocol was approved by the Research Ethics Committee of Chukyo University (approval number: 2021-101) and was consistent with the standards of the Declaration of Helsinki.

2.2 | Ultrasound Measurements

Two longitudinal views of the ultrasound images were captured using an ultrasound B-mode system (MicrUS EXT-1H; TELEMED, Milan, Italy) and a multifrequency linear array probe (L12-5L40S-3). Participants were instructed to sit on a table with their hip and knee joints at 90°. A standardized reference line from the greater trochanter to the upper lateral edge of the patella was used to control the measurement area. An experienced investigator adjusted the ultrasound probe perpendicular to the center of the reference line. The distance from the edge of the superficial aponeurosis of the vastus lateralis (VL) muscle to the boundary between the VL and the vastus intermedius muscle was calculated using imaging software (ImageJ, National Institute of Health, MD, USA), and was referred to as the muscle thickness (MT) of the VL muscle. Adipose tissue thickness (ATT) was measured from the same images as the distance between the skin surface and the superficial aponeurosis of the VL muscle. For each participant, two values for both MT and ATT were calculated and averaged for further analyses.

2.3 | Force Recording

The experimental setup is illustrated in Figure S1. The exerted force signal was corrected from a force transducer (LU-100KSE; Kyowa Electronic Instruments, Tokyo, Japan) attached to a custom-made knee extension chair (Takei Scientific Instruments Co. Ltd., Niigata, Japan). The participants performed every knee extension trial with their hips and knees flexed to 90° while gripping the bottom of the chair. The force transducer was firmly connected to the participant's ankle (5 cm above the lateral malleolus) without slack. The force signal was measured at 2000 Hz and converted into digital data using a 16-bit multichannel amplifier (Sessantaquattro, OT Bioelettronica, Torino, Italy).

2.4 | HDsEMG Recordings

A 64-channel HDsEMG electrode grid (GR08MM1305; 13 rows × 5 columns; gold-coated; 1-mm diameter; 8-mm inter-electrode distance; OT Bioelettronica, Torino, Italy) was placed along the reference line. The skin was shaved with a razor if necessary and cleaned with clean water. A disposable bi-adhesive form (KIT08MM1305; OT Bioelettronica, Torino, Italy) with a conductive paste (Elefix V; NIHON KOHDEN, Tokyo, Japan) was used to attach the grid to the skin. A wet electrode strap was secured to the proximal end of the shin. The HDsEMG signals were recorded in monopolar mode and converted to digital data using the same 16-bit multichannel amplifier as the force signal (Sessantaquattro, OT Bioelettronica, Torino, Italy). These signals were amplified (256×), sampled at 2000 Hz, and bandpass-filtered (10–500 Hz). HDsEMG signals were synchronized with the force signal using processing software (OTBioLab+, OT Bioelettronica, Torino, Italy).

2.5 | Experimental Design

Standardized familiarization protocols, which consisted of submaximal isometric contractions of 2% × 50% and 1% × 70% of perceived maximal effort (separated by 15–30 s of rest), were provided before experimental measurements. The participants then performed MVC during isometric knee extension using their right leg twice. A 90-s rest period was provided between each MVC trial. Higher values were used for further analyses. After MVC measurement, the submaximal contraction test, which consisted of a 17-s increasing ramp phase from baseline to 50% of MVC and a 10-s sustained holding phase, was conducted with HDsEMG measurement from the VL muscle on the right leg. This contraction intensity was chosen because the accuracy of HDsEMG decomposition is known to decrease at higher contraction levels and to ensure that young participants could perform the task with a high degree of quality and reliability. To ensure the quality of force tracing, two or three practice trials were administered before the measurements. A 90-s rest period was provided between each of these submaximal contractions. The MT of the VL was measured to determine the muscle strength relative to muscle size before any knee extension test.

2.6 | Force and HDsEMG Analysis

Muscle strength relative to muscle size was calculated using the quotient of MVC and MT (MVC/MT). Signal processing and analysis were performed using MATLAB software (MATLAB R2019a, MathWorks Inc., Natick, Massachusetts, US). All monopolar HDsEMG signals were differentiated between adjacent electrodes along the longitudinal axis of the muscle. The 59 bipolar HDsEMG signals were then decomposed into individual MUs using the Convulsion Kernel Compensation technique with the Decomposition of Motor Unit Surface EMG (DEMUSE) software (ver. 5.0.1; University of Maribor, Slovenia) [11, 21]. All detected MUs were carefully inspected by an experienced single investigator (MO) based on a previous suggestion [22], and only MUs with a pulse-to-noise ratio > 30 dB were used for further analysis. For each MU, the FR during submaximal ramp contraction was calculated from the reciprocal value of the interspike interval. The RT was defined as the relative force (% MVC) exerted at the time of the first firing from each MU. The MU FRs during the 10%, 20%, 30%, and 40% MVC were defined as the median MU FR for the MVC intervals 7.5%–12.5%, 17.5%–22.5%, 27.5%–32.5%, and 37.5%–42.5%, respectively (Figure S2). To assess the modulation of MU FRs with increasing force, we calculated the change in MU FR (ΔMU FR) across three 10% MVC intervals (10%–20%, 20%–30%, and 30%–40% MVC). For example, the ΔMU FR for the 10%–20% MVC interval was calculated as the MU FR at 20% MVC minus the MU FR at 10% MVC.

TABLE 1 | Anthropometric characteristics and maximal voluntary isometric contraction of the knee extensor for children and adult groups.

Variables	Children ($n = 18$)	Adults ($n = 18$)	p
Age (years)	9.46 ± 1.62	22.1 ± 3.40	
Height (m)	1.35 ± 0.09	1.71 ± 0.57	
BW (kg)	28.8 ± 5.1	65.2 ± 8.80	
MVC (Nm)	57.4 ± 19.7	210.8 ± 57.9	< 0.001
MVC/BW (Nm/kg)	2.0 ± 0.5	3.2 ± 0.7	< 0.001
MT (cm)	1.73 ± 0.28	2.86 ± 0.35	< 0.001
MVC/MT (Nm/cm)	33.7 ± 12.6	73.4 ± 18.2	< 0.001
ATT (cm)	0.47 ± 0.18	0.29 ± 0.26	0.022*

Note: Data are presented as mean ± standard deviation.
Abbreviations: ATT, adipose tissue thickness; BW, body weight; MT, muscle thickness; MVC, maximal voluntary isometric contraction.
* $p < 0.05$.

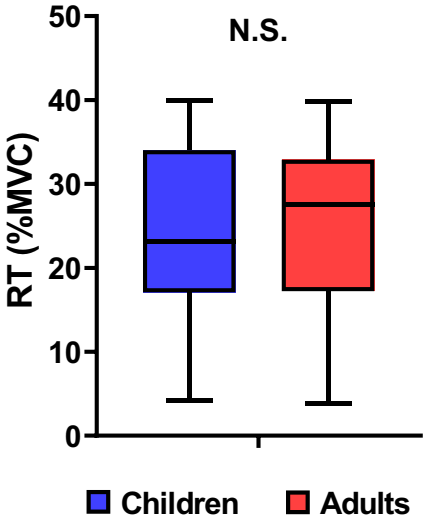


FIGURE 1 | Recruitment threshold (RT) during a submaximal ramp contraction (50% of maximal voluntary contraction) in children aged 6–12 and adults. The box plots compare the RTs of all motor units identified in the children (blue) and adult (red) groups. There was no significant difference in RT between groups. N.S. indicates no significant difference between the groups.

Since RT influences MU firing behavior, all identified MUs were subsequently categorized for analysis into four distinct groups according to their RTs: (1) MU recruited at < 10% MVC, (2) MU recruited at ≥ 10% and < 20% MVC (10%–20% MVC), (3) MU recruited at ≥ 20% and < 30% MVC (20%–30% MVC), and (4) MU recruited at ≥ 30% and < 40% MVC (30%–40% MVC).

2.7 | Statistical Analysis

Descriptive data are presented as the mean ± standard deviation. All statistical analyses were conducted using the RStudio software (version 4.3.1, RStudio Inc., Boston, MA, US). An independent-samples t-test was used to compare MVC, MVC

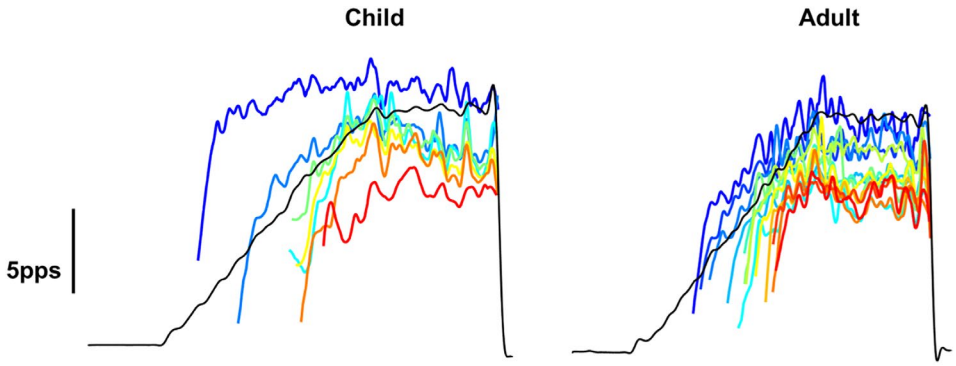


FIGURE 2 | Typical examples of individual motor unit (MU) firing rate (FR) in a child (aged 9.6 years) and an adult (aged 23.5 years). The black line indicates the exerted contraction force, and each colored line indicates an individual MU FR.

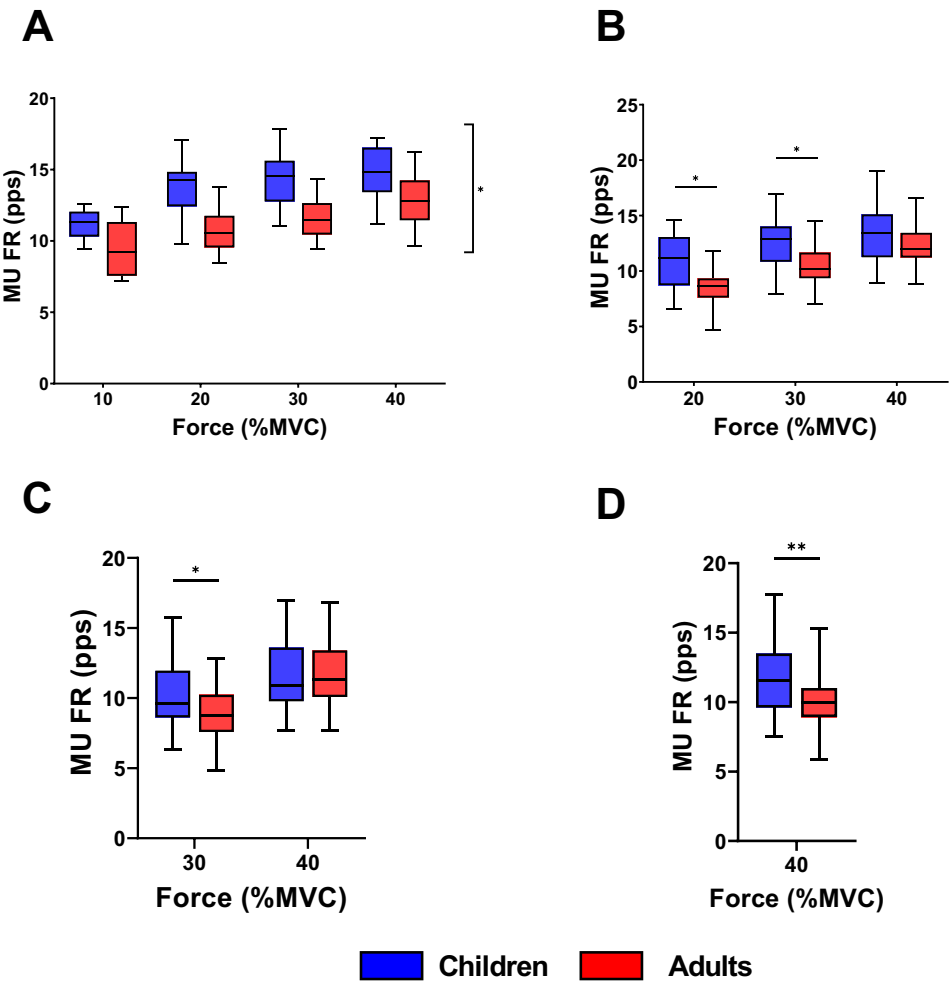


FIGURE 3 | Comparison of motor unit (MU) firing rate (FR) categorized by recruitment threshold in the children and the adults during submaximal ramp contraction (50% of maximal voluntary contraction [MVC]). MUs are grouped by their recruitment thresholds as follows: (A) < 10% MVC, (B) 10%–20% MVC, (C) 20%–30% MVC, and (D) 30%–40% MVC. The vertical bracket in panel (A) indicates a significant main effect of group. Horizontal lines indicate a significant difference between groups at a specific force level, as determined by post hoc analysis. Asterisks indicate the level of significance: *$p < 0.05$; **$p < 0.01$.

relative to body weight (BW) (MVC/BW), MT, and MVC/MT between the groups. A linear mixed effects model was constructed using the *lmerTest package* (version 3.1.3) in R. The model included group (children vs. adults), force (e.g., 10%, 20%, 30%, and 40% MVC), and their two-way interactions as fixed effects. For random effects, we included random intercepts for participants and for MUs nested within participants. The statistical significance of the fixed effects was determined using a type III ANOVA with Kenward–Roger's degrees of freedom approximations. Post hoc pairwise comparisons were performed using the *emmeans* package (version 1.10.0). The *p* values for these comparisons were adjusted using Tukey's method. For all tests, the significance level was set at 0.05.

3 | Results

3.1 | Clinical Parameters

A total of 18 primary school-aged children (age: 9.46 ± 1.62 years; height: 1.35 ± 0.09 m; and body mass: 28.8 ± 5.1 kg) and 18 young adults (age: 22.1 ± 3.4 years; height: 1.71 ± 0.57 m; and body mass: 65.2 ± 8.8 kg) participated in the study. Both groups consisted of 16 males and 2 females. The children exhibited lower MVC, MVC/BW, MVC/MT, and smaller MT than the adults (Table 1).

3.2 | Motor Unit Firing Rate

The RTs of the detected MUs are shown in Figure 1. No significant difference in the distribution of RTs was observed between the groups. Typical examples of individual MU FR in a child (aged 9.6 years) and adult (aged 23.5 years) are shown in Figure 2.

Figure 3 illustrates the MU FRs at different force levels categorized by RT. A key finding of this study was that children consistently exhibited higher MU FRs than adults across nearly all RT groups and force levels. Particularly, for MUs with RTs < 10% MVC (Figure 3A), a significant main effect of group confirmed that children had higher FRs. For MUs recruited between 10% and 30% MVC (Figure 3B,C), significant group × force interactions were observed. Post hoc tests revealed that children's FRs were significantly higher at 20% and 30% MVC (for 10%–20% RT MUs) and at 30% MVC (for 20%–30% RT MUs) than adults. Similarly, for the highest threshold MUs (30%–40% RT, Figure 3D), children showed a significantly higher FR at 40% MVC. The detailed statistical results are provided in Table 2.

Figure 4 illustrates the changes in MU FRs at different force levels categorized by RT. For the lowest threshold MUs (< 10% RT), children showed a significantly greater change than adults only in the 10%–20% MVC range (Figure 4A). Interestingly, this pattern was reversed for the higher threshold MUs. Children demonstrated a significantly smaller change in MU FR in the 30%–40% MVC range for MUs recruited at both 10%–20% MVC (Figure 4B) and 20%–30% MVC (Figure 4C). Please refer to Table 3 for detailed statistical analysis.

4 | Discussion

The primary finding of this study was that children aged 6–12 years exhibit higher MU FRs. Furthermore, the modulation of MU FR with increasing force revealed that, except for the initial recruitment phase of low-threshold MUs, children exhibited similar or smaller increases in MU FR than adults.

TABLE 2 | Statistical summary of the motor unit firing rates (MU FR) between children and adults.

	ANOVA				Post hoc	
MU group		***F***	**df**	***p***	**Group comparison**	***p***
Recruitment Threshold < 10% MVC	Group Main Effect	5.46	1, 13	0.036*		
	Interaction	2.56	3, 48	0.066	—	—
Recruitment Threshold 10%–20% MVC	Group Main Effect	4.43	1, 23	0.046*		
	Interaction	5.63	2104	0.005**	At 20% MVC	0.015*
					At 30% MVC	0.029*
					At 40% MVC	0.245
Recruitment Threshold 20%–30% MVC	Group Main Effect	1.82	1, 22	0.191		
	Interaction	24.4	1, 59	< 0.001***	At 30% MVC	0.046*
					At 40% MVC	0.583
Recruitment Threshold 30%–40% MVC	Group Main Effect	5.17	1, 24	0.032*		
	Interaction	—	—	—	—	—

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

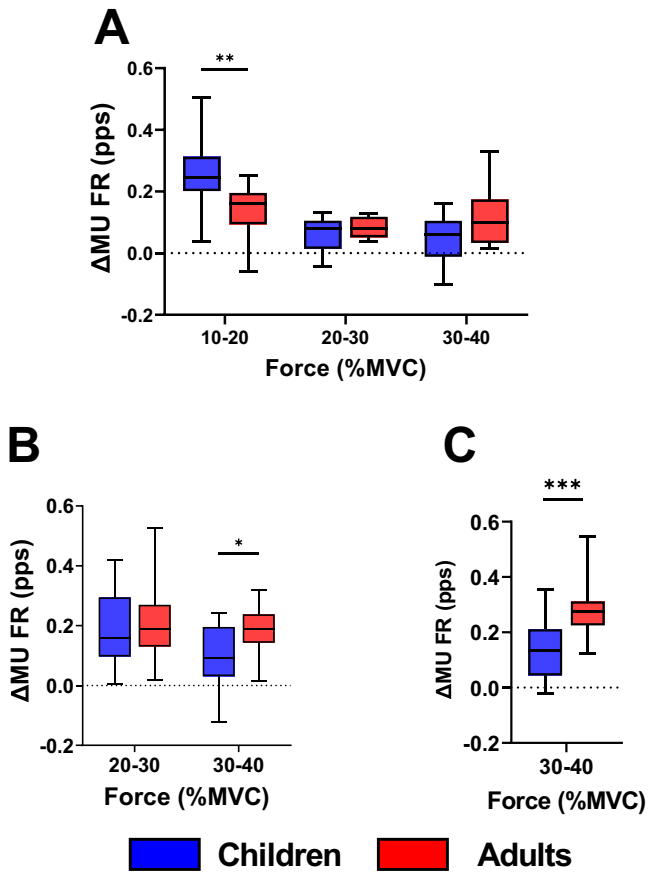


FIGURE 4 | Comparison of the changes in motor unit firing rate (ΔMU FR) categorized by recruitment threshold in the children and the adults during submaximal ramp contraction (50% of maximal voluntary contraction [MVC]). MUs are grouped by their recruitment thresholds as follows: (A) < 10% MVC, (B) 10%–20% MVC, and (C) 20%–30% MVC. Horizontal lines with asterisks denote a significant difference between groups for a specific force interval, as determined by post hoc analysis. Asterisks indicate the level of significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

In previous studies examining the sustained steady-state phase of submaximal isometric contractions, no consistent findings regarding differences in MU FR between children and adults were observed [14–16], which may be due to the normalization method or different target muscles [23]. Therefore, we applied grouping by RT for each MU and identified higher MU FR values in children in almost all RT groups and force intervals. These findings may be explained by the contractile properties of peripheral muscle fibers in children [24, 25]. Previous studies have reported that the duration of after-hyperpolarization is shorter in the muscle fibers of children [24]. Such physiological properties suggest that muscle fibers in children do not fully contract (i.e., tetanic contraction) unless higher stimulation frequencies are applied. Indeed, another study examining electrically elicited contractions in children indicated that younger children (9 years old) demonstrated lower elicited contraction force at a subtetanic stimulation frequency (10 Hz) than older children (15 years old) [25]. This supports the idea that higher stimulation frequencies are necessary for effective tetanic contractions in the developing muscles. Therefore, the elevated MU FRs observed in children in our study likely represent a functional adaptation by the nervous system to ensure that their muscle fibers contract effectively.

This observation of higher MU FRs may also have implications for the neural factors limiting maximal strength in children [3–5]. Although our study focused on submaximal contractions, we also observed that children had lower strength relative to muscle size. Given that voluntary force depends on both MU recruitment and rate coding [1, 2], if children already utilize high FRs at submaximal levels, it is plausible that the primary restriction for achieving maximal force is not an inability to excite MUs but rather a more limited capacity to recruit a sufficient number of them. Future studies should examine MU behavior at higher contraction intensities to explore this possibility.

The observed firing behavior in children, featuring a rapid escalation of FR that subsequently decelerates relative to adults,

TABLE 3 | Statistical summary of the change in motor unit firing rates (ΔMU FR) between children and adults.

		ANOVA			Post hoc	
MU group		**F**	**df**	***p***	**Group comparison**	***p***
Recruitment Threshold < 10% MVC	Group Main Effect	0.30	1, 11	0.590		
	Interaction	5.61	2, 32	0.008**	At 10%–20%	0.005**
					At 20%–30%	0.630
					At 30%–40%	0.155
Recruitment Threshold 10%–20% MVC	Group Main Effect	3.21	1, 23	0.086		
	Interaction	4.77	1, 52	0.034*	At 20%–30%	0.472
					At 30%–40%	0.016*
Recruitment Threshold 20%–30% MVC	Group Main Effect	16.7	1, 21	< 0.001***		
	Interaction	—	—	—	—	—

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

points toward the underlying developmental differences in spinal motor neuron control. This phenomenon may be explained by the combination of immature inhibitory circuits and distinct intrinsic motor neuron properties [26, 27]. For instance, key inhibitory pathways such as presynaptic inhibition of Ia afferents and recurrent inhibition are known to be underdeveloped in early life in animal studies [26, 27]. A lack of inhibitory braking could facilitate the initial sharp increase in the FR. The subsequent saturation of FRs may be attributable to the intrinsic properties of the motor neurons, particularly the behavior of persistent inward currents (PICs). Recent evidence suggests that PICs are larger and more active at lower thresholds in children than in adults [18]. The powerful and early activation of dendritic PICs leads to sustained depolarization, which, in turn, diminishes the electrochemical driving force for additional synaptic currents [28]. This would effectively render the neuron relatively insensitive to further excitatory inputs, resulting in a ceiling effect on the FR. Taken together, this suggests that the unique firing behavior in children can be attributed to the functional state of a system in which powerful excitatory mechanisms are not yet fully modulated by mature inhibitory control.

The findings of this study provide insights for clinicians and researchers engaged in assessing pediatric neuromuscular function. Specifically, our data can serve as a reference for healthy developing children, allowing for comparisons of MU behavior with those of children with neurodevelopmental or neuromuscular disorders such as cerebral palsy, muscular dystrophy, or developmental coordination disorder [29]. From a physiological standpoint, distinct neural control strategies in children have direct implications for exercise prescription and rehabilitation. The heavy reliance on rate coding and the observed saturation of firing gain suggest that children may be less neurally efficient and more susceptible to neuromuscular fatigue during sustained contraction [30]. Therefore, exercise programs aimed at improving motor function in children, whether for rehabilitation or general fitness, can be optimized by incorporating various skill-based tasks that challenge the nervous system to develop more efficient control patterns.

4.1 | Experimental Limitations

The present study has some experimental limitations that warrant further discussion. First, the neuromuscular activity of the antagonist muscle was not assessed during contraction; thus, the effect of coactivation might have affected the present findings. There is still debate whether a greater degree of coactivation could be observed in children or not [6, 31, 32]. Furthermore, the target of the submaximal contraction task for the MU analysis was only 50% of MVC, and the MU firing activities may differ at higher contraction intensities [3]. Additionally, our sample was predominantly male, and the potential influence of sex on our results should be considered [33]. Finally, fewer MUs were identified in children than in adults. This is likely attributable to the significantly greater ATT observed in our child participants (Table 1), which can attenuate surface EMG signals and complicate the accurate decomposition of individual MUs [34, 35]. Furthermore, the higher elasticity of musculotendinous tissue in children could increase the variability of the MU action potential waveform during contraction, making it more difficult to track each unit reliably during the task [36, 37].

5 | Conclusions

In conclusion, this study provides evidence that children aged 6–12 years exhibit higher MU FRs than young adults during submaximal isometric ramp contractions. A key characteristic of this firing behavior is a steep initial rise followed by saturation, resulting in a smaller overall increase in firing gain during force modulation, except in the initial recruitment phase of low-threshold MUs. This distinct neural strategy may be attributed to functional adaptation to compensate for immature muscle contractile properties and may reflect the ongoing maturation of spinal motor control. These findings provide a reference for assessing pediatric neuromuscular disorders and can help inform the design of more effective exercise and rehabilitation programs.

Author Contributions

M.O. and K.W. contributed to the conception and design of the study. M.O., R.T., T.H., T.N., and S.K. conducted the experiment and acquired the data. M.O. performed analysis of the results. M.O., R.T., T.H., T.N., S.K., and K.W. contributed to the interpretation of the results and revision of the manuscript. All authors have approved the final version of the submitted manuscript for publication. All those designated as authors qualify for authorship.

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Ethics Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data that support the findings of the present study are presented in the text, figures and table, and are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Illustration of the experimental setup. A participant was seated on a custom-made knee extension chair with the hip and knee joints flexed at 90°. A high-density electromyography (HDsEMG) electrode grid was attached to the lateral thigh, and a force sensor was firmly attached to the ankle. Visual feedback of the exerted knee extension force during the submaximal ramp contraction (50% of maximal voluntary contraction) was displayed on a screen in front of the participant. **Figure S2:** An example of the individual motor unit (MU) firing rate (FR) during submaximal (50% of MVC) ramp contraction. The black line indicates the exerted contraction force and each colored line indicates an individual MU FR. The red lines indicate median MU FR at 20%, 30%, and 40% of MVC. These medians were calculated from the instantaneous FRs during 17.5%–22.5%, 27.5%–32.5%, and 37.5%–42.5% of MVC, respectively. The change in MU FR (ΔMU FR) across three 10% MVC intervals.