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Toe-lift test: a novel, simple and noninvasive *in vivo* method for contractile evaluation of tibialis anterior muscle

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Abstract. Tibialis anterior (TA) muscle has frequently been used for scientific experiments, particularly for muscle contractile assays, because of its anatomical advantages. However, classical evaluation methods for the TA muscle, such as EMG and force transducer, require experimental skills to acquire reliable results. Furthermore, because sacrificing experimental animals is usually indispensable for both methods, sequential observations cannot be performed. Therefore, developing a simple, objective, and animal friendly evaluation system was warranted. In this article, we introduce a novel, simple, and noninvasive *in vivo* evaluation method for the TA muscle called the toe-lift test (TLT), which is not only easy to perform but also capable of detecting contractile strength precisely. Because the TLT does not require experimental animal sacrifice, performing assessments over time, such as in sequential observation, is possible. This novel method represents a solution to the need for a simple, noninvasive, and effective method for TA muscle contractile evaluation.

Key words: Tibialis anterior muscle — Contractile evaluation — Electromyography — Force transducer — Nociceptive withdrawal reflex

Introduction

The anterior crural muscles are frequently used for contractile muscle evaluation owing to their easy accessibility for manipulation due to their anatomical advantage. The anterior crural muscles are composed of the tibialis anterior (TA), extensor digitorum longus, and hallucis longus. Together, these muscles are responsible for foot dorsiflexion. Among these anterior crural muscles, the TA muscle is the most frequently used for functional assays because it is immediately beneath the skin, relatively large in size, and innervated with the peroneal nerve and its mixed fibre-type composition (Coombes et al. 2002). Many studies have described TA muscle characteristics, such as the biochemical characteristics of contralateral TA muscles (Kauvar et al. 2006; Zhou et al. 2006), twitch force in TA

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muscles under nerve repair (Shirley et al. 1996), and isometric tetanic force of normal TA muscles (Lovering et al. 2005) and for interspecimen comparison (Lopes-Martins et al. 2006). Among relevant studies, the method of evaluating TA muscle contractility is one of the most frequently discussed topics. The conventional evaluation method for TA muscle usually involves two traditional examinations, namely electromyography (EMG) (Alford et al. 1987; Courtine et al. 2009; McCaughey et al. 2010) for mechanical function evaluation and force transducer measurement (Coombes et al. 2002; Shin et al. 2008) for quantifying the contractibility of isolated muscle tissues. These evaluation strategies require high technical proficiency and, therefore, have technical limitations. Using EMG to evaluate muscle function objectively through accurate stimulation of motor units is difficult (Yue et al. 1995; Yao et al. 2000). Furthermore, although force transducer experiments can quantify the contraction force of the muscle tissue or even a single muscle fiber, they have some limitations, such as a short time window for operation, which might decrease the reliability of the results (Canaday and Fay 1976; Roche et al. 2015). In addition, every force transducer experiment requires experimental animal sacrifice, which makes this method unfavorable for sequential evaluation; furthermore, it is unmerciful from the animal protection point of view. Although novel functional evaluation methods for TA muscle or even the lower limbs have been reported recently (Shin et al. 2008; Mintz et al. 2016; Santos-Zas et al. 2017; Zotz et al. 2019), having well-trained personnel and needing expensive experimental apparatus are barriers. To overcome these hurdles and thus be able to assess muscle contractility easily, establishing a simple evaluation system that can also objectively and consecutively measure TA muscle strength is indispensable.

The nociceptive withdrawal reflex (NWR) is a rapid withdrawal response of the vertebrate when some part of its body exposed to a noxious stimulus (Sherrington 1910). These include stimuli sensed through the skin, joints, and muscles (Baxendale and Ferrell 1981; Schomburg 1990). The NWR has been observed with rabbits (Clarke and Harris 2004), mice (Thelin and Schouenborg 2008), and rats (Schouenborg and Kalliomäki 1990), which are often used as animal models for skeletal muscle contractility evaluation. An interesting phenomenon has been observed during our previous experiments on rats. When the plantar surface of the rat paw is stimulated, for example, by pinching it, the rat quickly withdraws its stimulated foot from the stimulus through simultaneous pulling up of its leg and a large dorsiflexion of the ankle joint. This observation is consistent with that of a study by Clarke and Harris (2004), although they also claimed the paw movement was affected by the stimulus location; the ankle joint movement transitioned from flexion to extension as the stimulus moved from the rostral to the caudal region of the paw. Because this withdrawal reflex of the paw could be constantly induced when appropriate regions were stimulated and this reflex of ankle dorsiflexion could be achieved through TA muscle contraction, we proposed that the NWR of the rat ankle could be used for evaluating TA muscle contractility.

Here, we report a novel TA muscle contractility evaluation method for rats called the toe-lift test (TLT). The foot of the experimental rat is tied to a weighted water bottle, and the maximum weight lifted through ankle dorsiflexion is measured after stimulating the rostral portion of paw plantar. Compared with the traditional methods of EMG and force transducer, the TLT is simpler to perform and does not requires special skills. The maximum weight the experimental rat lifted was measured, with the maximum weight defined as when the rat failed to lift the given task three times continuously. The TLT was first introduced in our previous study (Kou et al. 2019; Lin et al. 2020). In this method, experimental animal sacrifice is not required, and hence, sequential daily evaluation is possible. In this study, we examined contractility differences due to discrepancies in myofibre composition. According to previous studies, young rats possess higher contractility than senile rats do due to their predominant composition of fast myofibres (Bottinelli et al. 1991; Danieli-Betto et al. 1995). In addition, to confirm the consistency of this method with traditional muscle contraction evaluation methods, its sensitivity in distinctly detecting muscle contractility between young and senile rats was also assessed. Notably, the TLT is not only consistent with the two traditional evaluation methods but also more precise than these methods in detecting muscle contraction difference.

Material and Methods

Animals

All animal experiments complied with the Animal Research: Reporting of In Vivo Experiments guidelines were performed in accordance with the National Institutes of Health guide for the care and use of laboratory animals, and were approved by the Laboratory Animal Center of Taipei Medical University (approval No. LAC-2018-0127). Male Sprague-Dawley rats were purchased from BioLASCO (Taiwan). In total, 10 rats were used in this study: six rats were assigned to the 'young' group (8 weeks postnatal), and 4 rats were assigned to the 'elder' group (48 weeks postnatal).

EMG

The TA muscle was examined. The rats that underwent EMG were anesthetized with Zoletil (Virbac, Carros, France; intraperitoneally, 30 mg/kg) during the examination. Hair on the testing side of the hind limb was shaved, and the rats were placed in laying posture. An incision was made at the

lateral side of the thigh and extended toward the tibiofemoral joint gradually. The femoral biceps and gluteus muscle were separated to expose the sciatic nerve. The stimulation electrode (pen type) was attached to the exposed sciatic nerve, and it was hooked up without touching the muscle surface. Furthermore, signal receiver electrodes were inserted in the middle of the TA muscle. The stimulation was set at 100 μA/20 ms for 200 ms. A DS3 constant-current stimulator with a constant-current unit (CCU1; Upward Biosystems, Taipei, Taiwan) was used for performing EMG.

Force transducer measurement

The TA muscle was connected to the free arm of a force transducer (PH132-SS12LA Variable Force Transducer, Goleta, CA USA), immediately after their isolation, hoops were formed using polyglactin 910 suture 3-0 (Vicryl, Ethicon InC., NJ, USA) through stitches at the tendon of the TA muscle at both distal ends. The force transducer was connected to a MP36 data acquisition unit (MP36 BIOPAC Systems, Inc., CA, USA). The signal was recorded and stored in binary files on a laptop, and analysis software (Biopac Student Lab System Inc., CA, USA) was used in this study. Electrical stimulations generated by DG2A Train/Delay Generator (Digitimer Ltd., England) and a DS3 constant-current stimulator (Digitimer Ltd., England) with 100 μA/20 ms for 200 ms were given to rats through a pair of platinum electrodes that were placed on the surface of the central part of their TA muscle. A supramaximal stimulus (1 mA) with a pulse duration of 40 ms was used in all protocols.

TLT

The TLT was conducted to evaluate the maximum weight that the TA muscle of the rats could lift up. A brief introduction of the TLT was first presented in our previous work (Kou et al. 2019). Furthermore, an exhaustive annotation was provided. For the weight-bearing experiment, a water bottle filled with a baseline amount of water fastened with a plastic rope was tied transversely to the middle of the right hind feet of the rat with a rubber band (Fig. 1A). Then, the plantar side of the right paw, close to the middle of the third digit (rostral side of the foot), was pinched by hand as a pain stimulus during each trial of TLT (Fig. 1B). The strength of each pinch was measured using a pinch gauge (B&L Engineering, Santa Fe, Spring, CA, USA), which revealed 5.2 kg/pinch on average. A rat had ankle dorsiflexion (as TA muscle response) after pain stimuli. Furthermore, 10 g of water was added to the water bottle iteratively. A 15-min break was taken between each of the consecutive five trials. The maximum volume lifted by the test rats in each group was recorded. With the action of the gravity, after touched the surface of the TA muscle, the foot would be in extension position and the toes were in alignment with the water bottle. When the foot presented in extension after the dorsiflexion, the lifting motion was considered as completed (Fig. 1A). The lifting motion was considered incomplete if the test foot did not touch the surface of the TA muscle. The test was considered 'fail' when the rat failed to lift the bottle three times consecutively. Contrariwise, the test was considered 'pass' when the tested foot successfully touched the TA muscle.

Statistical analysis

The data of TLT, EMG, and force transducer method were normalized by the body mass (Edwen et al. 2014; Tamaki et al. 2019) in order to minimize the bias of body weight to contractile force. Further standardization yielded z scores. Because the variables were not normally distributed, the EMG amplitude, contraction force of the force transducer, and weight bearing (z scores) in the TLT in medians between the groups were evaluated using the Kruskal-Wallis H test. When the Kruskal-Wallis H test results were significant,

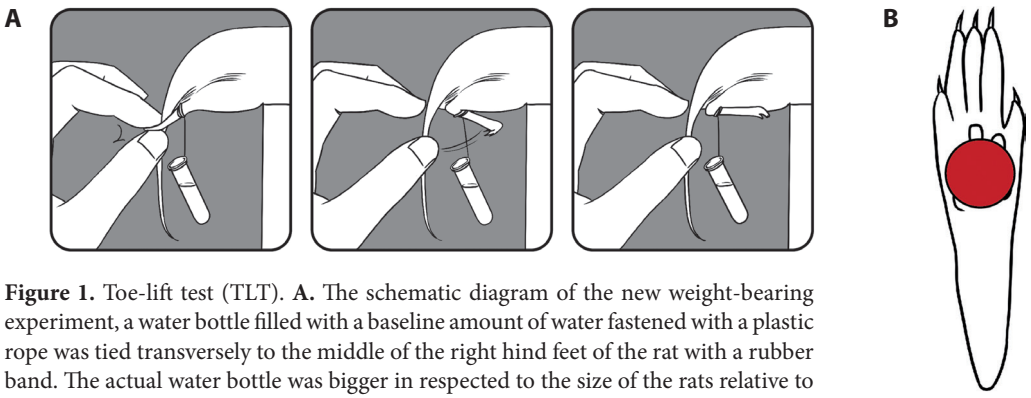

Figure 1. Toe-lift test (TLT). **A.** The schematic diagram of the new weight-bearing experiment, a water bottle filled with a baseline amount of water fastened with a plastic rope was tied transversely to the middle of the right hind feet of the rat with a rubber band. The actual water bottle was bigger in respected to the size of the rats relative to this schematic diagram. **B.** The stimulation point of the paw. The plantar side of the right paw, close to the middle of the third digit (rostral side of the foot), was pinched by hand as a pain stimulus during each trial of TLT.

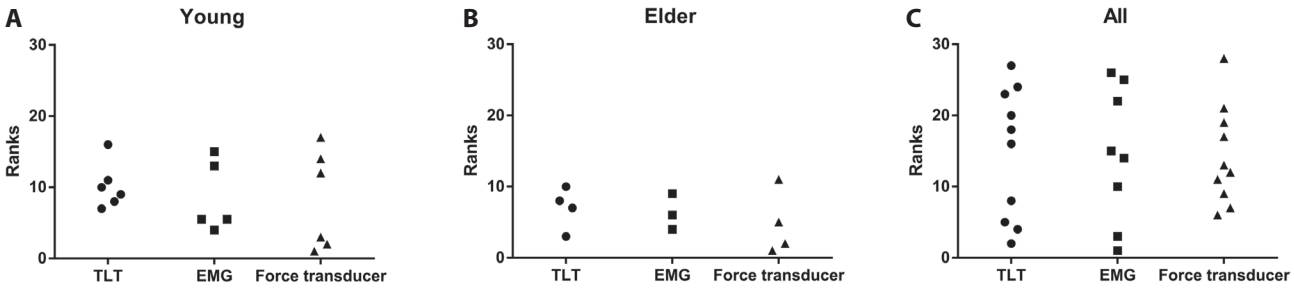

Figure 2. Mean rank of contractility evaluation of the tibialis anterior muscle by using the TLT, EMG, and force transducer in young (n = **6**), elder (n = 4), and all rats (n = 10). **A.** EMG data of rat No. 5 were unavailable. The corresponding z scores are shown in Table 2. **B.** EMG data of rat No. 4 were unavailable. The corresponding z scores are shown in Table 2. **C.** EMG data of young rat No. 5 and elder rat No. 4 were unavailable. The corresponding z scores are shown in Table 3. TLT, toe-lift test; EMG, electromyography.

Dunn's multiple comparisons were further performed to do the *post hoc* test between the groups. The results are presented with adjusted p values obtained from the *post hoc* test. The Wilcoxon rank-sum test was used to analyze the results of the three methods (TLT, EMG, and force transducer) between young and elder rats. Results were considered at a significance level of $p < 0.05$. The Kruskal-Wallis H test and Wilcoxon rank-sum test were performed using PRISM6 software (GraphPad Software, California, USA).

Results

Contractility evaluation of the TA muscle using TLT, EMG, and force transducer in rats

The body weights of young and elder rats were 330.5 ± 6.6 and 817.25 ± 62.0 g, respectively. The raw data and corrected data divided by the body weight in the TLT, EMG, and force transducer of young and elder rats are shown in Table 1. To avoid age bias, the difference between these three methods was evaluated in young, elder, and all rats (Tables 2 and 3; Fig. 2). No significant difference in contractility was observed between the three methods in young, elder, and all rats. The mean ranks of the TLT, EMG, and force transducer in young, elder, and all rats were 10.17, 8.60, and 8.17 (p = 0.7880; Table 2; Fig. 2); 7, 6.33, and 4.75 (p = 0.6509; Table 2; Fig. 2); and 14.7, 14.5, and 14.3 (p = 0.9941; Table 3; Fig. 2), respectively. These results indicated that the TLT was compatible with established evaluation methods (i.e., EMG and force transducer), and it was not affected by rat age.

Young rats showed higher contractibility of TA muscles than elder rats

The maximum weight-bearing capacities of young and elder rats in the TLT were 141.7 ± 16.0 and 237.5 ± 5.0 g, respectively, and their values after correction based on body weight were 0.4284 ± 0.0446 and 0.2916 ± 0.0172, respectively (Table 1). The corrected (divided by the body weight) amplitude of the TA muscles of young rats (0.0150 ± 0.0029) was significantly greater than that of elder rats (0.0068 ± 0.0025,

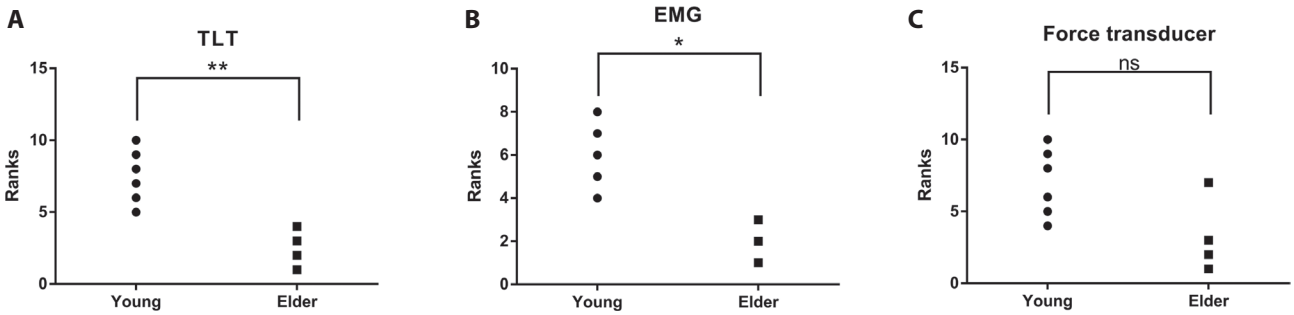

Figure 3. Differentiation of tibialis anterior muscle contractility between young and elder rats based on the TLT, EMG, and force transducer. **A.** Data consist of the rank of weight bearing in the TLT based on body weight. The corresponding z scores are shown in Table 4. **B.** Data consist of the rank of EMG amplitude based on body weight. The corresponding z scores are shown in Table 4. **C.** Data consist of the rank of the contraction force of the force transducer based on body weight. The corresponding z scores are shown in Table 4. * $p < 0.05$, ** $p < 0.01$, with young *versus* elder rats in the same condition based on the Kruskal-Wallis test. TLT, toe-lift test; EMG, electromyography; ns, non-significant.

Table 1. Body weight, measurement data by TLT, EMG and force transducer method in young and elder rats

		Young rats							Elder rats				
		1	2	3	4	5	6	Mean ± SD	1	2	3	4	Mean ± SD
BW (g)		328	336	337	330	319	333	330.5 ± 6.6	822	832	733	882	817.25 ± 62.0
TLT	Weight bearing (g)	140	170	130	130	130	150	141.7 ± 16.0	240	240	230	240	237.5 ± 5.0
	Weight bearing/BW (g/g)	0.4268	0.506	0.3858	0.3939	0.4075	0.4505	0.4284 ± 0.0446	0.292	0.2885	0.3138	0.2721	0.2916 ± 0.0172
EMG	Amplitude (mV)	4	4	6	5	NA	6	5.0 ± 1.0	5	8	3.5	NA	5.5 ± 2.3
	Amplitude/BW (mV/g)	0.0122	0.0119	0.0178	0.0152	NA	0.018	0.0150 ± 0.0029	0.0061	0.0096	0.0048	NA	0.0068 ± 0.0025
Force transducer	Contraction force (g)	8	7	14.5	7	31.5	16	15.2 ± 10.0	1	1.8	27	9	12.6 ± 13.0
	Contraction force/BW (g/g)	0.0244	0.0208	0.043	0.0212	0.0987	0.048	0.0427 ± 0.0122	0.0012	0.0022	0.0368	0.0102	0.0126 ± 0.0083

BW, body weight; TLT, toe-lift test; EMG, electromyography; NA, not available.

exact *p* value 0.0357; Table 4; Fig. 3). Furthermore, the corrected contraction force of the force transducer (0.0427 ± 0.0122) conducting the contractility of the TA muscle was greater in young rats than in elder rats (0.0126 ± 0.0083); however, the difference did not reach statistical significance (exact *p* value 0.0667; Table 4; Fig. 3).

The TLT was significantly better than EMG and force transducer in differentiating the contractility of TA muscles in young and elder rats

The aforementioned analyses confirmed that the contractility of TA muscles in young rats was greater than that in elder rats. Furthermore, we tested the capability of the TLT in differentiating the contractility of TA between young and elder rats. The corrected weight-bearing capacity of young rats was significantly more than that of elder rats, with the exact *p* value (0.0095) lower than that of EMG (0.0357) and force transducer (0.0667) (Table 4; Fig. 3). These results indicate that the TLT could distinguish between young and elder rats with greater sensitivity than could EMG and force transducer.

Discussion

Due to the advantage of its anatomical location, the TA muscle is popularly used for experiments. Although evaluation methods for TA muscle contractility are available, such as EMG and force transducer, a simpler method was needed. In this study, we introduced the TLT, a novel, simple, and noninvasive *in vivo* evaluation method for TA muscle contractility of rats. Compared with the classical methods of EMG and force transducer, the TLT has three advantages: simplicity, animal friendliness (ethically appropriate), and suitability for sequential observation. As mentioned, one of the purposes of this study was to establish a universal *in vivo* evaluation system for TA muscle contractility that is simple yet objective. EMG is one of the most commonly used evaluation methods for muscle contractility. However, a well-trained professional is required to dissect the dominant nerve (in this case, the peroneal nerve). Furthermore, skillful handling is essential to preserve not only the nerve integrity but also the vitality of both the nerve and muscle. Therefore, completing the entire evaluation within a limited period becomes another technical hurdle. The force transducer method has similar requirements. In fact, the technical burden of a force transducer is even higher than that of EMG because the muscle and its dominant nerve must be completely dissected and detached from the tibia. Contrariwise, in the TLT, neither dominant nerve dissection nor TA muscle detachment from the tibia bone is required. Using the biological characteristic of nociceptive withdrawal reflex, a rapid withdrawal response

Table 2. Evaluation of the contractility of TA muscle by TLT, EMG and force transducer method in young and elder rats

Rat No.	TLT				EMG				Force transducer			
	Weight bearing/ BW (g/g)	z score	Rank	Mean rank	Amplitude/BW (mV/g)	z score	Rank	Mean rank	Contraction force/BW (g/g)	z score	Rank	Mean rank
Young rats												
1	0.4268	−0.0359	10		0.0122	−0.9655	5.5		0.0244	−1.5000	3	
2	0.5060	1.7399	16		0.0119	−1.0690	4		0.0208	−1.7951	1	
3	0.3858	−0.9552	7	10.17	0.0178	−0.9655	5.5	8.60	0.0430	0.0246	12	8.17
4	0.3939	−0.7735	8		0.0152	0.0690	13		0.0212	−1.7623	2	
5	0.4075	−0.4686	9		NA	NA	NA		0.0987	4.5902	17	
6	0.4505	−0.0225	11		0.0180	1.0345	15		0.0480	0.4344	14	
Elder rats												
1	0.2920	0.0233	8		0.0061	−0.2800	6		0.0012	−1.3735	1	
2	0.2885	−0.1802	7	7.00	0.0096	1.1200	9	6.33	0.0022	−1.2530	2	4.75
3	0.3138	1.2907	10		0.0048	−0.8000	4		0.0368	2.9157	11	
4	0.2721	−1.1337	3		NA	NA	NA		0.0102	−0.2892	5	

Data of z score were analyzed *via* Kruskal-Wallis test. For abbreviations, see Table 1.

of the vertebrate upon a noxious stimulus, the TLT can be employed to precisely evaluate muscle contractility with minimal technical requirements. Because of the simplicity of the TLT, it can be easily performed even by someone new to handling experimental rats. Moreover, the consistency of results can be easily maintained with the TLT, which suggests that this novel evaluation method is highly reliable.

Furthermore, our data confirmed that this method was more sensitive than former methods. Fast and slow skeletal muscle types exist, which consist of different ratios of fast and slow myofibres; the contractility of the skeletal muscle is typically determined according to the ratio of the myofibre types (Pette and Staron 2000; Neunhäuserer et al. 2011). Skeletal muscles predominantly comprising fast myofibres possess strong contractility (Bottinelli et al. 1991; Gerdle et al. 2000). Conversely, resistance to fatigue was found to be high in skeletal muscles predominantly comprising slow myofibres (Herbison et al. 1982). A transition or shifting of myofibre proportions can occur with well-designed physical training or simply through natural processes, such as ageing. The proportion of fast myofibres decreases with age, leading to a decrease in contractile capability, and this phenomenon has been observed not only in humans but also in murines, such as rats (Danieli-Betto et al. 1995; Goodpaster et al. 2006).

Table 3. Evaluation of the contractility of TA muscle by TLT, EMG and force transducer method in all rats

Rat No.	TLT			EMG			Force transducer		
	Weight bearing/ BW (g/g)	z score	Rank	Amplitude/BW (mV/g)	z score	Rank	Contraction force/BW (g/g)	z score	Rank
Young rats									
1	0.4268	0.6747	23	0.0122	0.0400	15	0.0244	−0.2188	13
2	0.506	1.6811	27	0.0119	−0.0200	14	0.0208	−0.3438	11
3	0.3858	0.1537	16	0.0178	1.1600	25	0.043	0.4271	19
4	0.3939	0.2567	18	0.0152	0.6400	22	0.0212	−0.3299	12
5	0.4075	0.4295	20	NA	NA	NA	0.0987	2.3611	28
6	0.4505	0.9759	24	0.018	1.2000	26	0.048	0.6007	21
Elder rats									
1	0.292	−1.0381	5	0.0061	−1.1800	3	0.0012	−1.0243	6
2	0.2885	−1.0823	4	0.0096	−0.4800	10	0.0022	−0.9896	7
3	0.3138	−0.7611	8	0.0048	−1.4400	1	0.0368	0.2118	17
4	0.2721	−1.2910	2	NA	NA	NA	0.0102	−0.7118	9

Data of z score were analyzed *via* Kruskal-Wallis test. Mean rank evaluated for TLT: 17.70, for EMG: 14.50, for force transducer: 14.30. For abbreviations, see Table 1.

Table 4. Differentiation of TA muscle contractility between young and elder rats by TLT, EMG and force transducer method

Rat No.	TLT (g/g)		EMG (mV/g)		Force transducer (g/g)	
	Young	Elder	Young	Elder	Young	Elder
1	0.4268	0.292	0.0122	0.0061	0.0244	0.0012
2	0.5060	0.2885	0.0119	0.0096	0.0208	0.0022
3	0.3858	0.3138	0.0178	0.0048	0.0430	0.0368
4	0.3939	0.2721	0.0152	NA	0.0212	0.0102
5	0.4075		NA		0.0987	
6	0.4505		0.018		0.048	
Mean ± SD	0.4284 ± 0.0446	0.2916 ± 0.0172	0.0150 ± 0.0029	0.0068 ± 0.0025	0.0427 ± 0.0122	0.0126 ± 0.0083
Exact *p* value	0.0095**		0.0357*		0.0667	

Data of TLT were weight bearing (g) weighted by individual BW (g); data of EMG were amplitude (mV) weighted by individual BW; data of force transducer were contraction force (g) weighted by individual BW. Data were analyzed *via* Wilcoxon rank-sum test; * $p < 0.05$; ** $p < 0.01$. For abbreviations, see Table 1.

Compatible with previous reports, our data in this study also revealed stronger contractility in young rats compared with that in elder rats, and these distinctions could be more precisely detected with the TLT than with classic methods.

When choosing an evaluation method in an experiment, in addition to accuracy, cost is another crucial factor to be considered; in this respect, low cost is another advantage of the TLT. Unlike EMG and force transducer, the TLT requires no expensive precision apparatus, which makes the TLT affordable. Regarding the precise measurement of the maximum weight-bearing capacity, experimenters can define the interval of weight-bearing measurement according to the demand. For instance, 10 g as an interval was chosen in this study.

As mentioned, both EMG and force transducer require anatomical dissection of the TA muscle and peroneal nerve to complete the evaluation. This requires not only experimental skills but also experimental animal sacrifice. Although EMG can be performed by directly inserting the stimulating or sensor needle into the TA muscle without sacrifice, the evaluation accuracy may be affected and the animal often experiences intolerable pain. Therefore, anaesthesia is indispensable during the evaluation, which increases experiment complexity. With respect to animal ethics (McCaughey et al. 2010), the TLT is more merciful because it does not involve sacrificing the rat. Furthermore, because the whole procedure is accompanied by endurable pain induced by the operator through hand pinching, anaesthesia is unnecessary.

Another advantage of the TLT is that it can be used to perform sequential evaluation. Serial evaluation over time of the same rats is a crucial evaluation model, especially in clinical medicine. Generally, a treatment or an intervention is sequentially followed by application of a protocol or guidance. Therefore, treatment efficacy can appear or alter overtime. To objectively assess treatment without biases of individual animals, time, or space, an observation system employed over time is indispensable. EMG and force transducer can be performed sequentially; however, numerous experimental animals are needed because experimental animal sacrifice is unavoidable for each observation. By contrast, the TLT does not require experimental animal sacrifice and is capable of being applied in sequential evaluation, which fulfils the criteria of animal ethics and economical efficacy.

The TLT has some limitations. As the TLT involves the assessment of the lifting motion of the lower limb, a training effect might emerge, particularly during sequential evaluation. Another limitation of the TLT is possible operator bias, although this can be minimized easily with training. In addition, the limited number of animals is also the limitation in this study. Although current number of animals was sufficient for statistical analysis, indeed, increase the number of animals in both groups can increase the statistical power and thus better characterizing of the new method.

Conclusion

The TLT, a novel, simple and noninvasive *in vivo* evaluation method for the TA muscle, was introduced in this study. Unlike classical evaluation methods, this method is easy to perform and precise in determining contractile strength. Because the TLT does not require experimental animal sacrifice, conducting an assessment over time is possible, such as sequential observation. We hope our results represent a superior solution for TA muscle contractile evaluation.

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Authors contribution. HC and CMT designed the research, performed the research. HC, CMT, and YLH analyzed the data. HC and CMT wrote the manuscript. YCL, CYL and CYH analyzed the data and all authors approved the final version of the manuscript. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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