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Original Article

Menthol-induced cutaneous stimulation combined with self-paced walking training improves knee extension performance in untrained older healthy females

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Abstract. [Purpose] The present study aimed to investigate whether self-paced walking training utilizing the facilitating effect of skin cooling with menthol gel application was effective in untrained older healthy females. [Participants and Methods] Forty-two untrained healthy older females (aged 60–69 years) were divided into the following three groups: (i) Walking training with menthol group: G_M, (ii) Walking training group: G_W, and (iii) Control group: G_C. The participants in G_M and G_W performed self-paced walking for 30 minutes a day, 2 times a week, for 6 weeks. Menthol gel was applied to the front of the thigh of the participants in G_M. Maximal voluntary contraction and rate of force development were measured pre- and post-training and walking speed was measured during the training. The number of steps taken and walking speed in daily activity were measured and the average of these parameters per day were calculated. [Results] The main findings were [1] knee extension muscle strength increased in G_M and G_W, and [2] rate of force development only improved in G_M. [Conclusion] These results suggest that walking training utilizing the facilitating effect of skin cooling enhances muscle function in untrained older healthy females and that the present skin cooling method with menthol gel application may be recommended as a training strategy.
Key words: Walking training, Rate of force development, Menthol

INTRODUCTION

Age-related muscle strength decline is associated with the reduction of fast muscle fiber[1, 2]. Atrophy of fast muscle fiber is an important hallmark of sarcopenia and may predispose to falls and hip fractures in older adults[3, 4]. Fall risk is associated with a decline of muscle function with aging and not only influences muscle strength obtained by maximal voluntary contraction (MVC), but also the rapid development of force in older adults[5, 6]. Therefore, training stimuli that improve rate of force development (RFD) may be of benefit.

The American College of Sports Medicine (ACSM) guidelines recommend 30 minutes of brisk walking per day to maintain cardiovascular health[7]. However, previous studies have shown that walking exercise alone does not increase muscle strength, an important factor in maintaining health with aging[8].

Previous studies have shown that low-intensity exercises with skin cooling (i.e. 35% maximal voluntary contraction) may

increase muscle strength[9]. Cutaneous stimulation with skin cooling has also been shown to modulate polysynaptic neural pathways, and a neural connection between skin cold receptors (transient receptor potential melastatin 8: TRPM8) and the motor neuron pool has been observed[10–12]. Skin cooling over the working muscle, without reducing the temperature of the muscle, facilitates preferential recruitment of large motor neurons[10] and reduces the recruitment threshold of fast muscle fibers[12]. Several previous studies have also demonstrated a change in motor unit (MU) recruitment pattern, and MU recruitment thresholds were lower during voluntary contractions through spinal afferent input with electrical stimulation[13]. It is believed that the recruitment order of MUs during voluntary contraction is not fixed and can be modulated via spinal afferent input. Spinal afferent input with skin cooling methods increases RFD[11], and neuromuscular modulation has been observed with the topical application of menthol gel, in the same manner as TRPM8-mediated skin cooling, at low intensity contraction[14]. This physiological phenomenon may be useful in walking training, which has the potential to be an effective form of training. However, no study to date has observed the relationship between self-paced walking training with skin cooling and muscle function in older adults. If muscular strength can be increased with walking training, training strategies for improving muscular function in older adults can be greatly improved.

The purpose of present study was to investigate whether self-paced walking training with menthol gel applied to the skin over the working muscle increases MVC and RFD of isometric knee extension in untrained older healthy females. It was hypothesized that self-paced walking training alone would not increase MVC or RFD but MVC and RFD would increase with spinal afferent input of skin cold receptors, in the form of topical menthol application.

PARTICIPANTS AND METHODS

Forty two healthy older females aged 60 to 69 years with no exercise habit, but motivation to exercise, were recruited for the present study. All participants were initially screened for BMI (18.5–24.9) and to check that they had no low back pain and/or knee conditions, and from the 42 participants recruited, 2 dropped out after the initial measurements and the remaining 40 were divided into three groups, Walking training with menthol group: G_M, Walking training group: G_W, and Control (No Walking) group: G_C, one by one in that order then in reverse order (e.g. G_M, G_W, G_C, G_C, G_W, G_M, G_M...) based on left knee extension MVC of the quadriceps muscle from highest MVC to lowest MVC (Fig. 1). The study term was from September 4 to November 8, 2014.

All participants were informed of the purpose and risks associated with the present study and gave their written informed consent before taking part. The present study was approved by the Toho University Ethics Committee (No. 260632405823025).

Body composition, knee extension MVC, RFD, and number of steps taken per day were measured before and after the training protocol. G_M and G_W participated in self-paced walking for 30 minutes a day, 2 times per a week, for 6 weeks, and 2 g
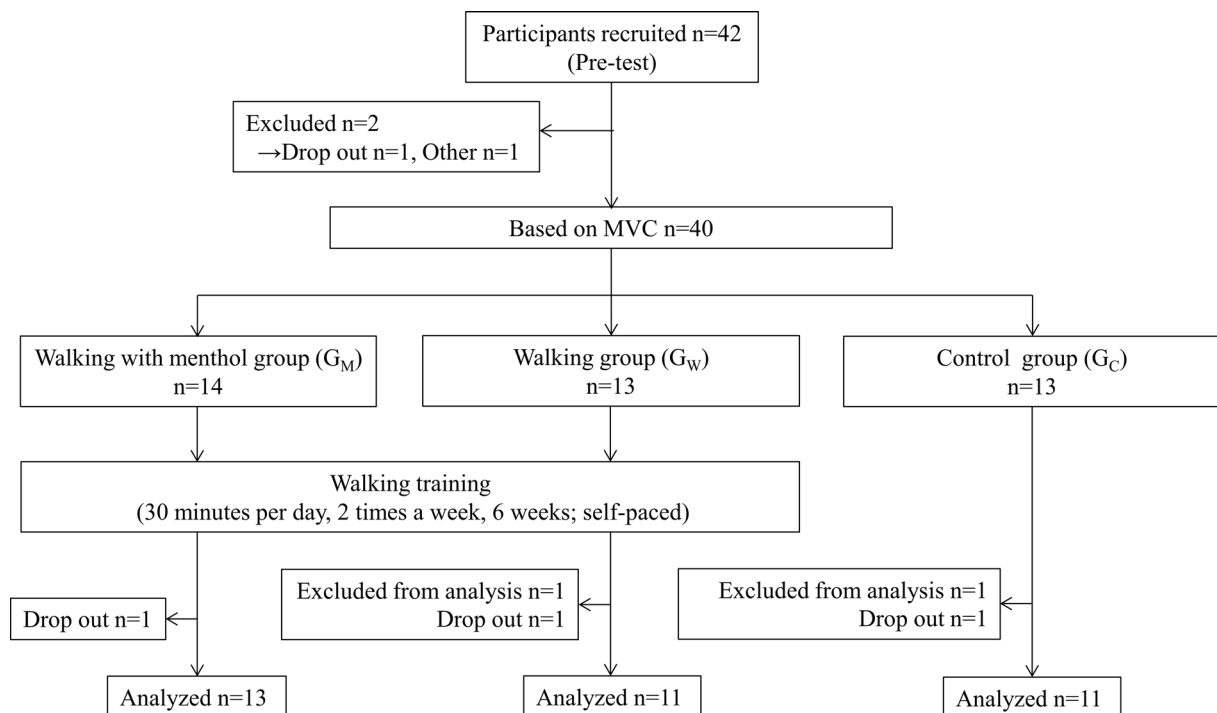

Fig. 1. Flow chart of participant recruitment and training and/or menthol application.
MVC: Maximal voluntary contraction.

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(each leg) of a specially prepared 5% menthol gel was applied to participants in G_M, to chemically stimulate cold receptors in the front of the thigh, just before each training session. After the training protocol, and accounting for drop out and excluded participants (N=5), data were measured and the changes were examined in the remaining participants (N=35).

G_M and G_W performed self-paced walking training around a 570 meter course under the supervision of a professional health and fitness trainer on assigned days (Tuesdays and Fridays) in the same predetermined park. Only participants who lived within 1 hour of the park by public transport were recruited for the study.

Lap times during walking training were measured each time to clarify exercise intensity. The walking speed was calculated by lap time. However, lap time was not measured on rainy days.

Participants wore a physical activity monitoring device (AM500N; ACOS Co. Ltd., Nagano, Japan) all day every day for a total of 9 weeks (3 weeks pre-training, first 3 weeks of training and last 3 weeks of training), except when sleeping and bathing. The device measured the number of steps taken and walking speed in daily activity, and the average of these parameters per day were calculated for the 3-week period before beginning the walking training protocol and for the first 3-week period of the walking training and last 3-week period of the walking training. The results of these three periods were then compared to determine the number of daily steps and daily walking speed.

Isometric knee extension torque MVC on the left was measured using a torque meter (type 9E05-B1–50; NEC Corporation, Tokyo, Japan), via a plate attached above the ankle and distal to the tibia, while the participant sat in an adjustable chair-like device with the hip at 90° flexion and the knee at 70° flexion. After warming up, a MVC of the quadriceps muscle (isometric knee extension), maintained for 2–3 s, was performed twice with a rest period of 90–120 s between each contraction. The larger of these two MVCs was determined as the MVC condition in the present study.

After a sufficient rest period, participants performed two maximal isometric knee extensions with the instruction: "as fast and forcefully as possible". Verbal encouragement was provided by investigators when participants contracted knee extensors. Trials where a 'torque drop' was observed (torque below zero) before the onset of contraction (counter movement) were disqualified and another trial was performed. RFD was derived from the slope of initial time–torque curve (torque/time) at 30, 50, 100, and 200 ms relative to the onset of contraction. Onset of contraction was defined as the moment when torque exceeded 7.5 Nm[15].

Torque output was digitized by an electronic converter (Power Lab; ADInstruments, Bella Vista, NSW, Australia) and was filtered at 100 Hz and stored at a sampling frequency of 1 kHz using computer software (LabChart7; ADInstruments).

Body composition was measured using electronic scales (InBody720; Biospace Corporation, Cerritos, CA, USA) to clarify factors of muscle hypertrophy and determine leg skeletal muscle mass.

Statistical analysis included A One-way analysis of variance (ANOVA) of baseline data. A two-way ANOVA was performed to compare groups (G_M, G_W and G_C) and the monitoring periods (3 weeks pre-training, first 3-week period of the walking training and the last 3-week period of the walking training), to compare groups during walking exercise (G_M and G_W) and the training times (week 1, 2, 3, 5 and 6), to compare groups before and after training (G_M, G_W and G_C), and to compare test periods before and after training (pre- and post-training).

A significant main effect was analyzed utilizing a post hoc analysis of simple main effects using the Bonferroni correction. The level of significance was defined as 5%. Data analysis was performed using computer software (Dr. SPSS II for Windows 11.0.1 J, Japan).

RESULTS

A One-way analysis of variance (ANOVA) of baseline data showed no significant differences among the groups. Baseline data are outlined in Table 1.

The number of daily steps and daily walking speed, excluding training days, for the three groups in pre-training, first 3

Table 1. Baseline data

Variable	Walking with menthol (G_M)	Walking (G_W)	Control (G_C)
	(n=13)	(n=11)	(n=11)
Female, n (%)	13 (100%)	11 (100%)	11 (100%)
Age (years)	64.2 ± 3.1	63.9 ± 2.4	65.7 ± 2.7
Height (cm)	153.7 ± 3.4	155.6 ± 5.0	155.8 ± 4.5
Weight (kg)	52.5 ± 4.9	51.7 ± 6.5	53.3 ± 6.6
BMI (kg/m^2)	22.2 ± 1.6	21.3 ± 1.9	21.9 ± 1.8
Body fat ratio (%)	31.0 ± 4.2	28.4 ± 5.2	31.2 ± 3.2
Skeletal muscle mass of leg (kg)	5.6 ± 0.6	5.7 ± 0.8	5.8 ± 1.0
Knee extension muscle strength (Nm)	101.7 ± 14.0	101.8 ± 20.6	110.0 ± 22.3

Data are mean ± SD. SD: Standard deviation.

weeks of training and last 3 weeks of training, are presented in Table 2.

A two-way ANOVA of mean number of daily steps showed no significant interaction between the groups and the monitoring periods (pre-training, first 3-weeks of training and last 3-weeks of training) (F=0.87, p=0.48) and a main effect was not observed in the groups and the monitoring periods (F=0.39, p=0.67; F=1.33, p=0.27, respectively).

A two-way ANOVA of mean daily walking speed showed no significant interaction between the groups and the monitoring periods (pre-training, first 3-weeks of training and last 3-weeks of training) (F=1.02, p=0.40) and a main effect was not observed in the groups and the monitoring periods (F=0.64, p=0.53; F=2.69, p=0.07, respectively).

Walking speed during walking exercise for G_M and G_W from week 1 to 6 is shown in Table 3. A two-way ANOVA of walking speed during walking exercise showed no significant interaction between the groups (G_M and G_W) and the training week (week 1, 2, 3, 5 and 6) (F1, 22=3.76, p=0.65), and a main effect was observed in the training week (week 1, 2, 3, 5 and 6) (F1, 22=105.62, p<0.01). A main effect was not observed in the groups (F1, 22=2.14, p=0.15).

The isometric knee extension MVC force for three groups pre- and post-training is presented in Table 4. A two-way ANOVA of MVC showed no significant interaction between the groups (G_M, G_W and G_C) and the test periods (pre-training and post-training) (F=0.79, p=0.46), and a main effect was observed in the test periods (pre-training and post-training) (F=18.09, p<0.05). Post hoc analysis of MVC showed significant increases with post-training when compared with pre-training in G_M and G_W (p<0.01 and p<0.05, respectively), and no significant increases in G_C (p=0.11). A main effect was not

Table 2. Mean number of daily steps and daily walking speed, excluding training days, in pre-training, first 3 weeks of training and last 3 weeks of training, for the three groups

	Walking with menthol (G_M)	Walking (G_W)	Control (G_C)
	(n=13)	(n=11)	(n=11)
Mean number of daily steps			
Pre-training (steps)	6,236 ± 1,873	5,581 ± 1,314	6,282 ± 2,481
First 3 weeks of training (steps)	5,457 ± 1,687	5,551 ± 1,966	6,123 ± 2,762
Last 3 weeks of training (steps)	5,946 ± 1,495	5,348 ± 1,047	6,252 ± 2,959
Daily walking speed			
Pre-training (m/min)	62.0 ± 4.4	60.2 ± 2.0	60.8 ± 3.4
First 3 weeks of training (m/min)	61.7 ± 4.8	59.7 ± 2.7	61.6 ± 3.6
Last 3 weeks of training (m/min)	62.1 ± 4.3	61.0 ± 2.6	61.8 ± 4.1

Data are mean ± SD. SD: Standard deviation.

Table 3. Change in walking speed during walking training in Walking (G_W) and Walking with menthol (G_M) from week 1 to 6

Training week	Walking with menthol (G_M)	Walking (G_W)
	(n=13)	(n=11)
Week 1 (m/min)	90.0 ± 6.6	92.6 ± 4.0
Week 2 (m/min)	92.5 ± 6.8	94.5 ± 3.3
Week 3 (m/min)	92.6 ± 6.6	95.3 ± 2.2
Week 5 (m/min)	94.7 ± 6.4	98.2 ± 4.2
Week 6 (m/min)	94.4 ± 7.0	98.9 ± 3.2

Data are mean ± SD. SD: Standard deviation.

Table 4. MVC of knee extension muscle strength pre- and post-training for the three groups

	Walking with menthol (G_M) (n=13)			Walking (G_W) (n=11)			Control (G_C) (n=11)		
	Pre-training (Nm)	Post-training (Nm)	Δ% (%)	Pre-training (Nm)	Post-training (Nm)	Δ% (%)	Pre-training (Nm)	Pre-training (Nm)	Δ% (%)
MVC	101.7 ± 14.0	112.9 ± 14.0**	11.0	101.8 ± 20.6	109.3 ± 19.6*	7.3	110.0 ± 22.3	115.5 ± 23.5	5.1

Data are mean ± SD. SD: Standard deviation; MVC: Maximal voluntary contraction.
Asterisks indicate significant differences between pre- and post-training for each group (*p<0.05, **p<0.01).

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observed in the groups (F=0.46, p=0.63).

The RFD in isometric knee extension torque for the three groups before and after training is presented in Table 5 and the raw data of one participant from G_M and G_W, respectively, is shown in Fig. 2. A two-way ANOVA of RFD showed no significant interaction between the groups (G_M, G_W and G_C) and the test periods (pre-training and post-training) in 0–30 ms, 0–50 ms, 0–100 ms, 0–200 ms (F=0.81, p=0.45; F=0.85, p=0.43; F=0.28, p=0.75; F=0.19, p=0.87), and a main effect was observed in the test periods (pre-training and post-training) in 0–30 ms, 0–50 ms, 0–100 ms (F=4.25, p<0.05; F=5.61, p<0.05; F=5.87, p<0.05, respectively). Post hoc analysis of MVC showed significant increases in post-training when compared with pre-training in G_M in 0–30 ms, 0–50 ms, 0–100 ms (p<0.05, p<0.05, p<0.05, respectively), and no significant increases in G_W and G_C. A main effect was not observed in the groups in 0–30 ms, 0–50 ms, 0–100 ms, 0–200 ms (F=3.03, p=0.06; F=2.27, p=0.11; F=0.79, p=0.46; F=0.13, p=0.87, respectively).

A two-way ANOVA of left leg skeletal muscle mass showed no significant interaction between the groups (G_M, G_W and G_C) and the test periods (pre-training and post-training) (F=2.14, p=0.13). A main effect was not observed in the groups and the test periods (F=2.08, p=0.15 and F=0.28, p=0.75, respectively).

Table 5. RFD in isometric knee extension force pre- and post-training for the three groups

	Walking with menthol (G_M) (n=13)			Walking (G_W) (n=11)			Control (G_C) (n=11)		
	Pre-training (Nm/s)	Post-training (Nm/s)	Δ% (%)	Pre-training (Nm/s)	Post-training (Nm/s)	Δ% (%)	Pre-training (Nm/s)	Post-training (Nm/s)	Δ% (%)
RFD									
0–30ms	453.0 ± 197.2	569.2 ± 179.2*	25.7	348.3 ± 120.8	385.4 ± 148.6	10.7	337.9 ± 212.6	372.8 ± 280.7	10.4
0–50ms	455.5 ± 187.0	578.8 ± 206.5*	27.1	373.6 ± 113.6	427.7 ± 154.7	14.5	355.9 ± 211.4	390.7 ± 275.3	9.8
0–100ms	389.3 ± 107.6	458.7 ± 161.1*	17.8	378.9 ± 105.2	416.7 ± 126.8	10.0	333.9 ± 181.7	372.6 ± 198.3	11.6
0–200ms	334.3 ± 59.8	371.1 ± 91.6	11.0	331.4 ± 90.6	353.7 ± 84.9	6.7	315.4 ± 121.9	353.4 ± 99.6	12.0

Data are mean ± SD. SD: Standard deviation; RFD: Rate of force development.
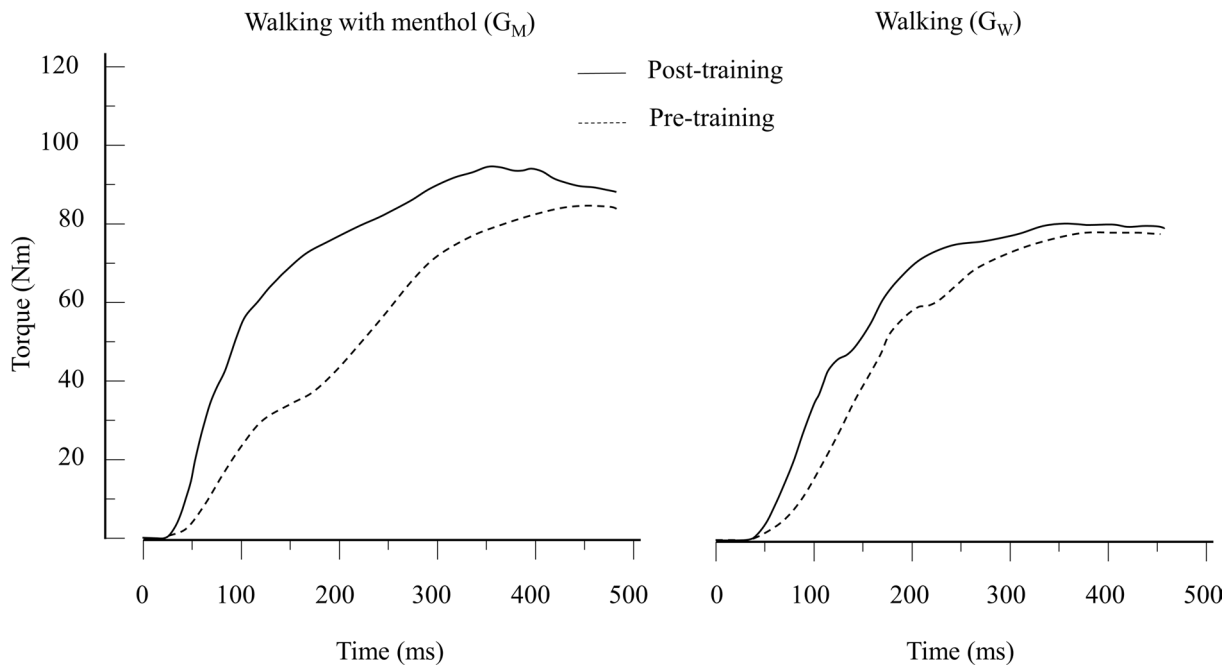Asterisks indicate significant differences between pre- and post-training in each group (*p<0.05).

Fig. 2. Pre- and post-training RFD raw data.
Left: Walking with menthol; Right: Walking (n=1, respectively). RFD: Rate of force development.

DISCUSSION

In older adults, the physiological benefits that can be derived from high intensity exercise are unnecessary and such exercise may even compromise safety. Therefore, the present study, exploring muscle function during self-paced walking training (moderate intensity exercise) with menthol gel applied to the skin over the working muscle, may be beneficial in the establishment of training methods and determining guidelines for maintaining health in older adults. Moderate intensity activities are defined as "those with a metabolic equivalent of the task (MET) value of 3 to 6"[16, 17]. This level of energy expenditure can be achieved by walking between 80.5–120.7 m/min (3–4.5 mph)[18]. Bohannon[19] defined a "comfortable" walking speed as about 78 m/min and a "maximum" walking speed as about 106 m/min, in females of mean age 65 years. In the present study, self-paced walking training was performed at a speed of about 90–100 m/min throughout the 6 week training period, within the comfortable walking speed defined as moderate intensity above.

The quadriceps femoris is activated at about 30% MVC during walking[20]. Muscle torque gains were 8% after knee extension training at 30% 1-repetition maximum (RM) load for 16 weeks (3 times/wk). In the present study, MVC of G_M increased more than in a previous report[21]. These results suggest that the physiological benefits that can be derived from high intensity training may be unnecessary in older adults. In slight contrast to the hypothesis, self-paced walking training increased MVC but did not increase RFD. In order to increase RFD with self-paced walking, spinal afferent input with the topical application of menthol is required.

In the present study, MVC in G_M and G_W increased with self-paced walking training for 30 minutes, two times per week, over a period of 6 weeks. Self-paced walking training was considered to be the equivalent of moderate intensity training[17]. In older adults with no exercise habit but motivation to exercise, MVC increased with walking training but RFD was unchanged. This result highlights the need for the topical application of menthol during walking training.

Previous literature on the effectiveness of moderate walking training shows conflicting evidence in relation to muscle strength[22, 23]. The reason for this is uncertain, however, age-related changes in neuromuscular activity may lead to higher activity of antagonist leg muscles (co-contraction)[24]. Therefore, inhibition of antagonist muscle activity should be a priority when employing neuromuscular training, suggesting that comfortable walking training may be effective in older people rather than fast walking which increases co-contraction[24]. Resistance training has been reported to reduce antagonist co-contraction and is considered to be a suitable mechanism for increasing muscle strength in older adults, even more so than in middle age[25], further suggesting that comfortable walking training is especially important for improvement of muscle strength in walking and other activities of daily living in older adults. The results of the present study suggest that the improvements in MVC observed may be due to the walking training which activated fast MUs in the motorneuron pool and/or inhibited antagonist muscle.

Rate of force development is influential in age-related neuromuscular activation, physical activity and fall risk[6, 26]. Therefore, in relation to age-related changes in muscle force characteristics, improvement of RFD is an important training factor in older adults. In the present study, RFD improved in G_M in response to stimulation of spinal afferent input with the topical application of menthol. However, no improvement in RFD was observed in G_W although an increase in MVC was observed.

Rate of force development is influenced by neural drive, muscle-tendon stiffness and muscle fiber type (particularly fast muscle fiber)[15, 27]. Unhjem et al.[26] found differences between older adults and young adults with training at 75–80% of 1RM intensity, and older adults exhibited substantial improvements in RFD (0–30 ms: 40.7%, 0–50 ms: 37.7%, 0–100 ms: 38.1%, 0–200 ms: 36.3%), and MVC (20.2%). In particular, RFD increased about two times more than MVC in all time periods. Therefore, they advocate the importance of neural factors in age-related decline of muscle strength. Other studies also reported high increases in RFD (more than 40%) with high intensity training[28, 29]. In the present study, MVC increased by 11.0% and RFD increased by 25.7% at 0–30 ms, 27.1% at 0–50 ms, and 17.8% at 0–100 ms in G_M. As the number of daily steps and daily walking speed were the same in G_M and G_W, it may be possible that recruitment of fast muscle fibers was activated by the topical application of menthol. Due to the physiological properties of the quadriceps femoris, innervation of fast muscle fibers is relatively high therefore a stronger contraction is possible. In order to perform a strong contraction, inhibition of the antagonist hamstrings is necessary. However, antagonist inhibition is weaker in older adults, especially in explosive exercise, making strong contractions more difficult to achieve. Therefore, the topical application of menthol could be a useful tool to assist with the inhibition of antagonist muscles during explosive exercise.

Limitations of the present study include: [1] Training intensity levels have not been determined relative to absolute intensity levels defined by parameters such as heart rate, cadence and oxygen uptake. Therefore, the changes in muscle function due to training intensity remain unclear and require further investigation. [2] Neuromuscular activity during walking training was not measured, therefore improvements in muscle function due to such factors, and their effect on walking performance, must also be investigated further.

In conclusion, the results of the present study reveal that self-paced walking alone is not enough to increase MVC and RFD in untrained older healthy females. However, with the topical application of menthol over the working muscle in self-paced walking training, increases in MVC and RFD can be achieved in untrained older healthy females. This information is vital for rehabilitation specialists and trainers because it shows that significant improvements in performance can potentially be achieved in older patients from a relatively simple and safe exercise protocol without having to employ potentially dangerous, high intensity exercise protocols which are generally associated with such gains.

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