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L-menthol: An emerging athletic performance enhancing aid

Maitham Khanafer

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Abstract

Background: L-menthol is an emerging athletic performance-enhancing aid. This review serves as an analytic report of existing controlled trials in the scientific literature that have investigated this compound as a sports aid when administered via the novel method of mouth rinsing followed by expectoration. Methods: Data on menthol mouth rinsing was sourced from EBSCO PowerSearch, Medline, and NCBI. In addition to a systematic review of existing literature, the current review outlines a detailed protocol to evaluate menthol mouth rinsing on a treadmill time-to-exhaustion test. This protocol adds an additional feature of conducting blood work examining any changes in energy substrate availability subsequent to menthol mouth swirling. It will also provide further investigation on whether a pre-exercise mouth rinse administration is sufficient to elicit an ergogenic response, which could then be compared to protocols that implemented more frequent mouth rinsing intervals. Results: Nine controlled trials involving menthol mouth rinsing were found. Data showed that menthol mouth rinsing could provide ergogenic effects when used in heated environments by non-heat acclimated individuals. What exactly causes these ergogenic effects is still not fully known, but it is predicted that menthol activates and then desensitizes ion channels in the mouth known as Transient Receptor Potential Cation Channel Subfamily M Member 8 (TRPM8) that subsequently result in a feeling of coolness, while also reducing thermal discomfort and improving breathing comfort. It is still inconclusive whether administration is best prior to exercise, at different intervals throughout an activity, in the latter stages of exercise, or a combination of all. Conclusion: L-menthol is a promising ergogenic aid and swirling it might provide a practical aid for athletes that train and compete in heated environments

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First Advisor

John Carbone, PhD, RD

Second Advisor

Colleen Croxall, PhD

Third Advisor

Ann R. Eisenberg, PhD

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L-MENTHOL: AN EMERGING ATHLETIC PERFORMANCE ENHANCING AID

By

Maitham Khanafer

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Supervising Instructor:	John Carbone, PhD, RD
Honors Advisor:	John Carbone, PhD, RD
Department Head:	Colleen Croxall, PhD
Honors Director:	Ann R. Eisenberg, PhD

SECTION 1: Abstract

Background: L-menthol is an emerging athletic performance-enhancing aid. This review serves as an analytic report of existing controlled trials in the scientific literature that have investigated this compound as a sports aid when administered via the novel method of mouth-rinsing followed by expectoration. **Methods:** Data on menthol mouth rinsing was sourced from EBSCO PowerSearch, Medline, and NCBI. In addition to a systematic review of existing literature, the current review outlines a detailed protocol to evaluate menthol mouth rinsing on a treadmill time-to-exhaustion test. This protocol adds an additional feature of conducting blood work examining any changes in energy substrate availability subsequent to menthol mouth swirling. It will also provide further investigation on whether a pre-exercise mouth rinse administration is sufficient to elicit an ergogenic response, which could then be compared to protocols that implemented more frequent mouth rinsing intervals. **Results:** Nine controlled trials involving menthol mouth rinsing were found. Data showed that menthol mouth rinsing could provide ergogenic effects when used in heated environments by non-heat acclimated individuals. What exactly causes these ergogenic effects is still not fully known, but it is predicted that menthol activates and then desensitizes ion channels in the mouth known as Transient Receptor Potential Cation Channel Subfamily M Member 8 (TRPM8) that subsequently result in a feeling of coolness, while also reducing thermal discomfort and improving breathing comfort. It is still inconclusive whether administration is best prior to exercise, at different intervals throughout an activity, in the latter stages of exercise, or a combination of all. **Conclusion:** L-menthol is a promising ergogenic aid and swirling it might provide a practical aid for athletes that train and compete in heated environments.

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SECTION 1: Introduction

L-menthol or Levomenthol is an organic compound found in peppermint, and other mint oils.[1] In today's world, it is also produced synthetically, and is found in many products such as pharmaceutical drugs, toothpastes, and mouthwash. When this compound is applied across the body, it initiates and desensitizes nociceptors, which are nerve cell endings responsible for initiating pain. This mechanism produces a counter irritant effect accompanied with the activation of pain-relieving pathways.

Menthol is speculated to activate ion channels known as Transient Receptor Potential Cation Channel Subfamily M Member 8 (TRPM8), that promote a cooling effect.[2] This ion channel is also known as cold and menthol receptor 1 (CMR1). The activation of this channel via menthol could be beneficial for athletes as it helps alleviate the feeling of thermal discomfort that accompanies exercise activity. TRPM8 is also activated with oral application of L-menthol, through thermoreceptors present on the oral mucosa. When menthol acts on those receptors, sensory information is sent to the brain, which then promotes a cooling sensation. L-menthol oral application could also inhibit the Transient Receptor Potential Cation Channel, Subfamily A, Member 1 (TRPA1), the channel responsible for the sensation of pain. This inhibition results in mediating pain responses potentially providing some benefit for an athlete.[3]

These effects have prompted scientists in the past 10-15 years to test this compound as a potential athletic performance enhancing aid. Applying menthol on garments, ingestion, and topical application are all methods of administration that have been explored through controlled trials, with promising results emerging demonstrating improved athletic performance outcomes.[4]

L-menthol has also been theorized to be involved in other mechanisms that offer a potential competitive edge for athletes. Labored breathing is a common symptom of endurance activity; menthol helps by increasing breathing depth and/or rate.[5] Menthol is also associated with increased alertness. It is however still to be determined if this effect is significant in the context of performance improvement.[4] Nevertheless, the activation of TRPM8 ion channels, and its subsequent physiologic effect is what reigns as the main theory for the reason behind any potential ergogenic effects.[2] This is further supported by the trials reviewed in the results section of this report.

Performance enhancement is the focus of much research, largely because of the lucrative benefits. Whether for financial gain, popularity, or legacy, athletes are seeking all avenues that may provide them an advantage over their competitors. This has led scientists to amplify their investigations on sports supplementation; a tool used to improve athletic performance. In a report by Fortune Business Insights, the dietary supplement industry was worth $61.20 billion in 2020. The global dietary supplements market is projected to reach $128.64 billion in 2028.[6]

With this exponential growth in the supplement industry, scientists have also proposed creative methods of supplement administration. In 2004, Carter and colleagues demonstrated that mouth rinsing a carbohydrate solution followed by expectoration resulted in similar performance-enhancing effects to ingestion.[7] This novel method of administration has also been tested using caffeinated solutions, leading us to our main discussion of this review which is examining the effect of L-menthol mouth rinsing on athletic performance.[8] The current review looks at previous study protocols that involve menthol mouth rinsing, and proposes a study protocol that incorporates a blood testing component.

SECTION 2: METHODS

Previously published literature on the topic of menthol mouth rinsing was sourced from EBSCO PowerSearch, Medline, and NCBI using the keywords and phrases: L-menthol, mouth rinse or mouthwash, performance, time trial, sprint, time to exhaustion, running, exercise, heat. Nine different study designs involving L-menthol mouth swirling were identified. Our findings also include additional means of menthol administration such as topical gel application, combining menthol with a carbohydrate solution, and ingesting menthol.

Studies were screened and selected based on confirmation of controlled study design, identification of study population, sample size, nature of exercise activity, means of menthol administration, setting in which the study was conducted, the outcomes measured, and repetitiveness and/or novelty in study protocols.

SECTION 3: RESULTS

The data in Table 1 highlight previously implemented protocols involving menthol mouth rinsing, along with their respective findings.

Table 1: The studies are presented in descending order of their publication year, covering study year, means of menthol administration, frequency of mouth rinse, and outcomes tested.

Study	L-menthol Application	Mouth Rinse Interval	Performance Type	Exercise Outcome	Thermal Sensation Outcome	Other Outcomes Tested
Jerram et al., 2023[9]	M-MR vs PLA	Two swills before two exercise blocks, and a third swill after exercise	Two 3-minute 15-a-side rugby-specific conditioning blocks	M-MR had no effect on physical performance metrics relative to PLA ($P > 0.05$)	Position-dependent M-MR attenuation of TS from baseline to Swill 1 ($P = .003$,) and Swill 2 ($P = .002$), compared with PLA	Subjective palatability of M-MR: Liked oral sensation at baseline, but per athlete's report, "it gets worse the more I have."

Tsutsumi et al., 2022[10]	W-IG, M-MR, and M-IG	Every 5 min while running	TTE	Running times improved with M-IG vs. W-IG (P = 0.01) Running times were similar between M-MR and W-IG (P = 0.15)	N/A	Breathing comfort: improved with M-IG compared to W-IG (P < 0.001). No difference between M-MR and W-IG (P = 0.09) or M-IG (P = 0.05)
de Camargo et al., 2022[11]	Combined M-MR and topical menthol gel vs PLA and CON	Every 2 km of the exercise	Three 10 km runs	Performance time improved in M-MR (P = 0.03) and PLA (P = 0.003) compared to CON; no difference between M-MR and PLA	TS was lower (P = 0.01) with menthol vs CON	Heart Rate, RPE, and Thermal Comfort: Increased over time under all conditions (P < .0001)
Parton et al., 2020[12]	M-MR or a control mouthwash	30-s prior to the main fixed RPE trial, and at 10 min intervals	Fixed-RPE of 16 cycling time trial	No differences in exercise duration were observed compared to control	In males, TS was lowered with M-MR except at the 18-min time point (Start P = 0.007), 6-min (P = 0.025), 12-min (P = 0.04), 18-min −0.45 (P = 0.136, End (P = 0.05) In females, TS was lower only across the first 12-min of exercise with M-MR (Start (P = 0.050; d), 6-min (P = 0.034;), 12-min (P = 0.048), 18-min (P = 0.739;), End (P = 0.330)	Core body temperature: increased with time (P < 0.001) but not between M-MR vs CON

Best et al., 2021[13]	Mouth swills of CHO, M-MR or a combination	Every 10 km starting at 5 km	40 km cycling time trial (TT)	Small differences between M-MR and CHO (d = 0.225) and M-MR and combination (d = 0.275)	TS affected by distance cycled, but no effect from mouth swills	Cooling sensation significant interaction between solution and location of oral cooling intensity (P < 0.001
Jefferies et al., 2018[14]	M-MR, PLA, ice ingestion	Administered at 85% of baseline TTE	TTE on a cycle ergometer at 70% Wmax –	6% increase in TTE	No significant difference between M-MR and PLA (P = 0.080)	Core temperature: no difference between conditions (P = 0.852) Thermal comfort: increased comfortability with time (P < 0.001) RPE: increased with time (P < 0.001); no difference between conditions (P = 0.674)
Flood et al., 2017 [5]	Oral M-MR vs PLA	1.5 minutes before trial, then 10 min intervals	Fixed-RPE of 16 cycling time trial	8% increase in TTE	Lower TS in M-MR vs PLA (P = 0.036)	Core and skin temperature: No changes in M-MR (P > 0.05) Heart rate: No significant change in M-MR (P > 0.05)

Stevens et al., 2015[16]	Precooling by ICE, mid-cooling by M-MR, or CON	M-MR at 0.2 km mark of every 1 km during 5km run	Three self-paced 5-km running time trials on a non-motorized treadmill	Performance time improved in M-MR compared to CON ($P = 0.01$) and ICE ($P < 0.01$)	Lower TS in M-MR compared to CON ($P < 0.05$)	Rectal temperature: significant decrease with ICE compared to CON and M-MR ($P < 0.01$) Skin temperature, Heart Rate, and Oxygen uptake: no significant changes across all conditions throughout the trial
Mündel & Jones, 2009[5]	M-MR vs PLA	Every 10 minutes	65% Wmax until volitional fatigue	Improved exercise time in M-MR vs PLA ($P = 0.043$)	N/A	Heart rate: effect of time ($P < 0.001$) in both M-MR and PLA Core temperature: increased ($P < 0.001$) in both M-MR and PLA Plasma lactate levels: increased ($P = 0.001$) in both trials with no differences observed between M-MR and PLA ($P = 0.817$) Plasma glucose levels: remained constant ($P = 0.797$) in both M-MR and PLA

Keywords: Carbohydrate (CHO), Control (CON), Ice Slurry (ICE), Maximal Work Capacity (Wmax), Menthol Ingestion (M-IG), Menthol Mouth Rinse (M-MR), Placebo (PLA), Rating of Perceived Exertion (RPE), Time-to-Exhaustion (TTE), Time Trial (TT), Thermal Sensation (TS), Water Ingestion (W-IG).

Given that mouth rinsing is a newer method of Levomenthol administration, the variability in protocols strengthens the body of available research. L-menthol application across the listed protocols was compared to placebo solutions, and/or menthol, water, and ice slurry ingestions. Combined methods of administration were also investigated, along with a variability in the initiation point, and the frequency of the mouth swirling. Diversity in the performance types is also demonstrated with some studies replicating real life exercise scenarios, and others being in a laboratory setting. We review the protocols from Table 1, in reverse-chronological order by publication date.

Contrary to the other selected studies, Jerram and colleagues tested effectiveness of menthol mouth rinsing on the elite athlete population.[9] Rugby players performed two 3-minute 15-a-side rugby-specific conditioning blocks that were both preceded by a menthol or placebo mouth rinse. A third swirl was done after exercise. Their findings show that menthol mouth swirling had no effect on any of the physical performance outcomes tested ($P > 0.05$). However, there was an improved thermal sensation in comparison to placebo; this effect was only seen in players playing in the Forwards position.

A unique feature of this protocol is the exercise type, and the sample population. The study also tested results between players playing in the Forward position vs Backs. The temperature of the surrounding environment was at around 23° C which leads to the question of whether any of the outcome results would be amplified if the protocol was done in a heated setting.[9] With no improvement in performance, and the protocol testing a short bout of an endurance activity, it begs to ask if any benefits will arise from menthol mouth rinsing (M-MR) if the exercise duration was longer. In addition, thermal sensations were attenuated for the Forwards only, but there was no subsequent performance improvement. This may highlight that a perceptual improvement in thermal sensation does not necessarily enhance athletic performance.

A couple of reasons could have led to differences between both player positions. Forwards usually have a higher body fat percentage in comparison to Backs which makes them more susceptible to heat stress. Additionally, Forwards had a higher acceptability of the solution even after the third swirl. In regard to performance differences, Backs cover greater running distances with higher intensity runs.

Tsutsumi and colleagues had 13 male regular-trained runners participate in a treadmill time to exhaustion (TTE) trial, with either an M-MR, menthol ingestion (M-IG), or water ingestion (W-IG) intervention every 5 minutes after the initiation of the activity.[10] Participants returned for three different TTE tests at an interval of 7-10 days, each time having a different intervention. The running times were longer with M-IG, with a significant difference when compared with W-IG ($P = 0.01$), though the running times for M-MR were not significantly different from those of W-IG ($P = 0.15$). This may be linked to the higher prevalence of the TRPM8 ion channels in pharynx, and on the surface of the epiglottis.[17] With the activation of those receptors through menthol ingestion, a greater cooling effect is achieved, which could explain why time-to-exhaustion was longer with M-IG. The authors also tested Breathing Comfort (BC) post-intervention with increased comfort demonstrated in M-MR and M-IG compared to W-IG ($P < 0.001$).

This protocol was also done at thermoneutral conditions, and it is unknown if the activation of the TRPM8 ion channels will differ under heated environmental conditions following this study design. If so, utilizing M-MR in a heated environment might trigger a greater effect on athletic performance. With breathing comfort improving with menthol administration, it is also possible to have a better outcome on performance in a hotter environment.

In a unique approach, de Camargo et al. combined topical application of a menthol gel, with a menthol mouth rinsing solution (TOP-MMR).[11] The participants took part in two different 10 km running trials, having either a placebo (a mouth rinse and a topical gel with no performance-enhancing properties), or TOP-MMR. Both placebo and TOP-MMR interventions were implemented at every 2 km running interval. Participants also took part in an initial control trial with no interventions, to set a baseline time for comparison.

Running time was lower with TOP-MMR when compared to the control trial, but there was no statistical significance when compared with placebo ($P = 1.00$).[11] Improved thermal sensation (TS) was the only marker evaluated that showed statistical significance in TOP-MMR versus placebo ($P = 0.001$). The findings of this study propose that performance improvements due to menthol could be caused by a placebo effect, since there was no statistical difference in running times between TOP-MMR and the placebo intervention. With these statistical results, TS once again did not seem to contribute to any performance enhancement.

Parton and colleagues' protocol represents the only study that investigated sex differences in the outcomes of M-MR on exercise performance.[12] In this study of 11 men and 11 women, the activity consisted of a fixed intensity cycling exercise under heated conditions (~35 °C). This consisted of exercising at an RPE of 16 on the Borg Scale which represents “hard” exercise, as subjectively perceived by the participant. The participants’ highest power output maintained for 30 seconds in the first 3-minutes of the exercise was recorded, with the trial being stopped after the cycling power dropped to 70% of this value, for 30 consecutive seconds. A placebo or a menthol mouth rinse were administered prior to start and at 10-minute intervals.

Similar to the aforementioned studies, thermal sensation was lower in both sexes with menthol compared to control ($P < 0.05$).[12] However, this changed towards the latter stages of the exercise for the females as thermal sensation increased and there was no longer a statistical difference for menthol compared to control (P = 0.739). The authors highlight that after the administration of menthol, males opted for a higher risk strategy of increasing power output, while females were more conservative in adjusting their power. This is connected to why females became more thermally sensitive in the latter stages of the exercise, as they reduced their exercise workload which extended the duration of their trial and thus subjected them to a longer period of heat stress.

Best and colleagues also designed a distinctive protocol by combining L-menthol with a carbohydrate solution, and comparing its effect against isolated solutions of both compounds.[13] The protocol consisted of a 40 km cycling time trial while testing various physiological and perceptual measures. A baseline trial was done with no interventions for comparison. The uniqueness of this study was examining the intensity of oral cooling and the perceived intensity of cooling on the different regions of the participant's forehead, face, throat, and towards the upper chest region. As expected, the results showed that the greatest cooling sensation was with the menthol containing solution. This was depicted through a scale derived by the authors (0, No cooling; 1, Weak; 2, Moderate; 3, Strong; 4, Very Strong); with menthol solutions resulting in a cooling effect of above 2 across the trial, in comparison to values below 1 in the stand-alone carbohydrate solution. The greatest cooling effect was reported in the upper chest region in comparison to other regions of the forehead, mouth and throat.

In regard to performance measures, there were no significant differences (P = 0.07) between all three solutions on the 40 km time trial.[13] There were also no significant differences between either of the experimental trials in comparison with the baseline time trial (P= 0.165).

Analyzing differences in mean power output, none of the solutions provided any influence relative to baseline (P = 0.903).

A review of these results indicates that combining both menthol and carbohydrate did not provide any additive performance-enhancing effect. Post-hoc testing did, however, indicate a moderate difference in the 40 km cycling time between the baseline and trials that included an administration of the mouth swirling solutions (P = 0.297-1.000).[13] Further statistical analysis identified that the combined menthol and CHO solution was statistically equivalent to stand-alone CHO (P = 0.031) in terms of 40 km cycling time. This indicates that the moderate differences between baseline 40-km trial were more influenced by the carbohydrate in the solution than by the menthol. The data identifies that carbohydrate mouth swirling might have a greater effect on performance, and that improved perpetual effects such as TS may not play a huge role in performance enhancement.

The next set of studies reviewed indicate some effectiveness of M-MR in performance enhancement. Jefferies and colleagues' protocol consisted of a TTE on a cycle ergometer at 70% of max power.[14] Critical components of this study are that it was conducted at heated 35 °C environment, participants were not heat-acclimated, and they indicated that they had not visited a hot-weather country in 3 months prior to the study.

The participants went through a familiarization session with no interventions to record their baseline TTE. Interventions included a placebo mouth rinse, M-MR, and an ice slurry ingestion (ICE). A distinctive factor of this protocol was that the interventions were given when the participants were at 85% of their baseline TTE, which signifies a later stage of the exercise activity.

Both the M-MR and ICE had an improvement in TTE of 6% and 7%, respectively, in comparison to baseline TTE.[14] The results indicated a significant performance enhancement for both the M-MR and the ice slurry ingestion in comparison to placebo (menthol: P = 0.036, ICE: P = 0.040). Thermal sensation was not significantly different between all three interventions, but the authors indicate that there was a large effect size that might signify that thermal sensation was reduced in both M-MR and ICE. This data demonstrates that there might be a benefit from the perceptual effects of M-MR on exercise performance for those who are not heat acclimated.

This protocol demonstrated that adding the M-MR towards the end of the activity might have played a bigger role in attenuating any performance-altering discomforts. However, other studies reviewed demonstrated an improvement in performance when M-MR was administered prior to start or in earlier stages of exercise. This warrants follow-up in future controlled trials strictly comparing administration of M-MR at different time points to identify which method has the most significant effect on athletic performance enhancement

Flood et. al also demonstrated that M-MR produces an athletic enhancing effect on non-heat acclimated individuals in a heated environment (~35 °C). [15] The protocol consisted of participants cycling at a fixed RPE of 16 on the Borg scale. An RPE of 16 is what the participants perceived as "hard" or "very hard". In a similar fashion to the aforementioned study by Parton et al, participants cycled until their power output dropped below 70% of their baseline power for a consistent 2 minutes.[12] Menthol and placebo solutions were gargled 1.5 minutes before initiation of activity, and then at 10-minute intervals. The study was a randomized crossover design, and participants returned for multiple trials using a different intervention solution each time.

The results were notable, as the M-MR significantly increased exercise duration in comparison to placebo ($P < 0.05$).[15] Once more, thermal sensation was lower with the M-MR intervention ($P = 0.036$), a parameter that has been consistent across many of the studies reviewed in this report. Those results once again indicate that M-MR possibly produces the best outcomes in a heated environment, as opposed to those studies showing equivocal results at more moderate temperatures.

The experimental protocol by Stevens et. al was also conducted under heated conditions (33°C).[16] The trial consisted of a three different 5-km timed run, where participants had one of three interventions, M-MR, ICE, or control (CON). For the ICE intervention, participants were given two different boluses spaced 15 minutes apart prior to the start of the activity. M-MR was given mid exercise at the 0.2 km mark, and then every 1 km. Interestingly, the running times for the M-MR were significantly faster than those of the ICE and CON intervention ($P = 0.01$ and $P = 0.03$, respectively). The authors attributed those notable results with M-MR to an improvement in the thermal sensation. This study further confirms the added value of thermal sensation to elevating athletic performance in a hot environment.

The final study reviewed by Mundel and Jones had nine participants do a time-to-exhaustion aerobic cycling 65% of their peak power output.[3] The interventions include a M-MR or an orange flavored placebo every 10 minutes of the exercise. The protocol was done in heated conditions (~34 °C) in a chamber. Participants came on four different visits that followed the following progression: visit 1 consisted of setting baseline measures of highest oxygen consumption at the peak of exercise (VO_{2max}) and maximal work capacity (W_{max}), visit 2 was familiarizing with equipment and protocol, visits 2-4 also included exercising until volitional exhaustion with visits 3 and 4 involving either the M-MR or placebo intervention.

Cycling times were longer for 8 out of 9 participants with the menthol intervention, with a significant improvement (P = 0.043) in contrast to placebo.[3] The authors did not highlight thermal sensation as a possible contributing factor towards improved performance. Regardless, they linked this performance enhancement of M-MR to reductions of breathing effort and an enhanced capacity to adhere to heated conditions. Additionally, the authors added a blood work component to test for changes in plasma lactate and glucose following the administration of interventions. Plasma lactate increased over time in both M-MR and PLA, with no significant difference between both (P = 0.817). Plasma glucose remained constant across the trial for both M-MR and PLA (P = 0.797). These results indicate that M-MR administration, relative to placebo, did not provide any significant alteration in energy substrate availability that could have affected the course of exercise activity.

This section has reviewed the methodologies of study protocols that tested Levomenthol mouth rinsing as a potential athletic performance enhancer. With a high variability between protocol designs, we were able to connect some dots that provide a greater understanding of the actual effects of M-MR. To summarize, M-MR seems to provide the greatest effect when administered in heated conditions. This could be due to multiple reasons such as providing a feeling of freshness by improving sensitivity to thermal conditions, by improving breathing comfort, and optimizing ventilation to fit exercise needs.

It is, however, still inconclusive as to the best initiation point and frequency of M-MR administration. The studies showing performance-enhancing effects administered M-MR at different timepoints of exercise, and at different frequencies, with beneficial effects shown when administered in latter stages of exercise in Jefferies protocol, and when administered prior to exercise and at subsequent intervals throughout the trial.[14] However, the common denominator between studies that exhibited an athletic performance enhancing effect of menthol was when the trials were executed in a heated environment, suggesting that cooling sensations underlie menthol-related physical activity improvements (33 °C<).[9,10,11,12,13]

SECTION 4: DISCUSSION

L-menthol mouth rinsing is a novel method of application for athletic performance enhancement. The literature presents that the main theory leading to subsequent ergogenic effects of MMR is related to the activation of ion channel Transient Receptor Potential Cation Channel Subfamily M Member 8 (TRPM8), which promotes a cooling sensation that provides thermal comfort and reduces pain sensations.[2,3] In comparison to placebo, L-menthol mouth rinsing has shown an effect in increasing time to exhaustion, improving breathing comfort, and reducing thermal discomfort during exercise activity when exercising in heated conditions.

In this report, nine studies were reviewed with four studies showing an athletic performance enhancing effect when M-MR was administered.[5,14,15,16] All four of those studies were conducted under heated conditions, which indicates that effects such as improved breathing comfort only contribute to improving performance when the environmental conditions are heated.

There is also high variability in initiation point and frequency of M-MR administration in the four studies that exhibited athletic performance enhancement. Jefferies et .al[14] administered M-MR at 85% of baseline TTE, Flood et al.[15] initiated 1.5 minutes before trial and then at 10 minute intervals, Stevens and colleagues[16] initiated at the 0.2 km and then at every 1 km of a 5 km run, and Mundel & Jones[5] administered the mouth swirls every 10 minutes of the protocol. With all those protocols showing performance enhancement, and no studies showing statistical significance as to the best initiation/frequency of administration; the preference of the user could play a big role in determining the best practical recommendation. It is important to note that even though performance was not enhanced in the protocol by Jerram and colleagues, there was a report by an athlete stating, "it gets worse the more I have."[9] This indicates that a lower frequency of M-MR might be ideal, but this could vary based on individual tolerance and palatability.

Both Jefferies et al[14] and Flood et al[15] conducted TTE protocols with M-MR administration increasing time to exhaustion in both. Again, both studies were done in heated conditions. In Jefferies and colleagues' protocol, TS was not significantly lowered, noting that in this protocol M-MR was administered in the latter stages of the exercise. With TTE improving in this protocol, it indicates that lowering TS does not necessarily contribute to enhancing performance. This statement is supported knowing that as TS was lowered in the studies done by Jerram et al[9], Parton et al[12], and de Camargo et al[11], where performance was not improved.

When looking at the data reviewed it seems that breathing comfort and improved respiratory responses due to M-MR administration can be one of the main contributors in improving exercise performance. In the study by Tsutsumi and colleagues, BC was improved in both M-MR and M-IG compared W-IG ($P < 0.001$).[10] In the protocol done by Stevens et al, M-MR increased expired air volume when compared to CON in the first 2km of the trial ($P < 0.05$).[16] It is still inconclusive whether respiratory changes due to M-MR produce a sizable effect since not many of the studies reviewed breathing-related test outcomes.

Out of the nine protocols, 5 studies showed no improvement in exercise performance.[9,10,11,12,13] These studies share the attribute of being conducted in thermoneutral conditions (~25 °C). Nonetheless, these protocols showed very high variability in design with some of them being running or cycling time trials, TTE test, and a protocol focused on rugby-based exercises. The other protocols that showed a performance enhancement did share similarity in protocol designs, thus it seems that the detrimental factor is the heated environment.

In terms of practicality, an L-menthol solution is an easy-to-carry sports aid. It can accompany long distance sport athletes as they tackle symptoms of endurance activity that include worsened ventilation and irritation due to heated conditions. Swirling and expectorating menthol for a few seconds is also relatively practical, and should not alter the flow of exercise activity. Ultimately based on preference and practicality to the athlete, rinsing L-menthol will most likely provide a beneficial supplemental aid for those looking for an extra athletic advantage when exercising in the heat. According to the literature reviewed, this advantage might work best for individuals that are not acclimated to a hot environment. An important factor to consider is palatability and acceptability, as it could get less palatable the more times a user administers menthol.

SECTION 5: PROPOSED PROTOCOL DESIGN

As part of this review, we designed an experimental protocol that investigates the potential effects of menthol as an athletic performance-enhancing aid. This study design could be implemented with a multidisciplinary approach combining students from different academic programs (i.e., dietetics, exercise science, clinical laboratory sciences). This protocol also adds an additional feature of conducting blood work examining any metabolic changes subsequent to menthol mouth swirling. It would allow for measurement of concentrations of energy substrates, such as glucose, lactate, and free fatty acids. This would allow for determination of any effects of menthol (vs. placebo) on energy availability.

Since there is some discrepancy in knowing what the most optimal time of M-MR initiation is, we propose testing it as an immediate pre-exercise intervention, consistent with three of the reviewed studies. A limitation of our protocol is not controlling the surrounding temperature. In practical terms, this is not feasible without a thermal chamber. The proposed design does, however, further explore if changes occur in energy substrates through the blood work test; a parameter not included in the majority of the available literature related to menthol mouth rinsing as an ergogenic aid. This would allow us to further investigate if athletic performance is enhanced from perceptual effects (i.e., lowering thermal sensation) versus physiological (i.e., changes in energy substrate availability).

Study Design:

Participants will perform a treadmill time-to-exhaustion (TTE) protocol to test the effect of rinsing Levomenthol 5 seconds prior to start. This will commence during two different visits that are preceded by a familiarization session. A minimum of a 72-hour interval will separate all three visits, and the study will follow a randomized, blind, crossover design.

Familiarization Session:

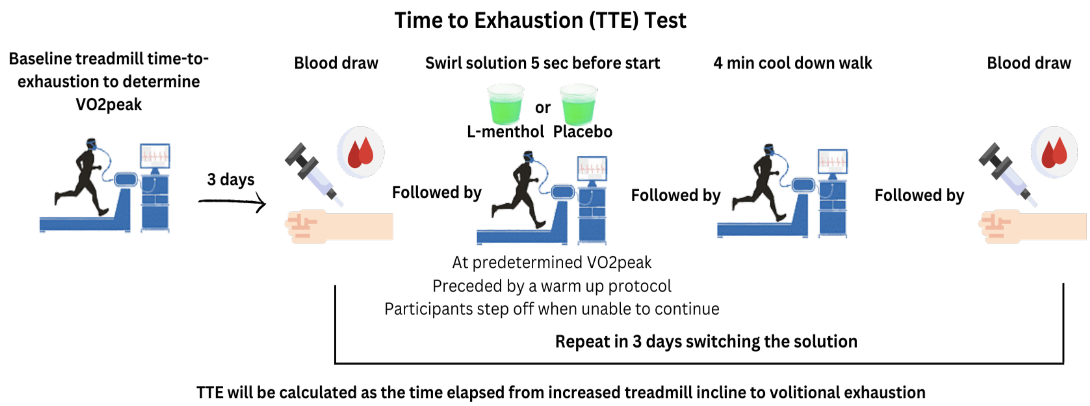
During the first visit, the participants will complete a baseline graded treadmill exercise test to determine peak oxygen consumption (VO_{2peak}) via open-circuit spirometry with a metabolic cart. The participants will also be informed on the flow of the protocol, to promote familiarization before beginning the main experimental phase of our protocol.

Experimental Procedure:

During visits 2 and 3, participants will complete two separate TTE performance tests, with randomized order of menthol or placebo (i.e., sucralose-sweetened dH_2O) mouth rinses swirled and expectorated immediately prior to the beginning of the test. The TTE will follow continuous progression of: 1) 4mL blood draw from the right median cubital vein (MCV); 2) a 4 min walking (3mph) warm-up at 0% incline; 3) 4 min run at baseline testing speed at 0% incline;

4) increase of treadmill incline to that achieved at baseline VO_{2peak} test cessation; 5) participants step off the treadmill when unable to continue running; 6) 4 min walking (3mph) cool-down at 0% incline; 7) 4mL blood draw from the left MCV. TTE will be calculated as the time elapsed from increased treadmill incline to volitional exhaustion.

An illustration of the study design is presented here:



Outcome Measurements:

Table 2 features the different outcomes that can be tested using this protocol.

Table 2:

Performance Measures	Physiological Measures	Perceptual Measure
Time to Exhaustion (seconds/minute)	Blood levels of: Glucose (mg/dL), lactate (mmol/L), free fatty acids (mmol/L)	Thermal sensation (ASHRAE 9-point analogue sensation scale where −4 = "very cold", 0 = "neutral", and 4 = "very hot")[18]

In conclusion, L-menthol mouth swirling is a novel method of administration that is emerging as an up-and-coming ergogenic aid. It is indicative through the data reviewed that it could be most effective when used in a heated environment. Our study design protocol will provide further investigation on whether a pre-exercise M-MR administration is sufficient, which could then be compared to protocols that implemented more frequent mouth rinsing intervals. This protocol further investigates whether L-menthol mouth swirling produces any changes in energy substrate availability through conducting blood tests to measure parameters such as blood glucose, lactate, and free fatty acids. With promising data on the ergogenic effects of L-menthol, we look forward to identifying what means of administration are the most optimal for athletic-performance enhancement.

SECTION 6: REFERENCES

1. National Center for Biotechnology Information. PubChem Compound Summary for CID 16666, l-Menthol. https://pubchem.ncbi.nlm.nih.gov/compound/l-Menthol. Accessed Mar. 27, 2023.

2. Andersen HH, Olsen RV, Møller HG, Eskelund PW, Gazerani P, Arendt-Nielsen L. A review of topical high-concentration L-menthol as a translational model of cold allodynia and hyperalgesia. *Eur J Pain*. 2014;18(3):315-325. doi:10.1002/j.1532-2149.2013.00380.x

3. Stevens, C.J., Mauger, A.R., Hassmèn, P. *et al.* Endurance Performance is Influenced by Perceptions of Pain and Temperature: Theory, Applications and Safety Considerations. *Sports Med* 48, 525–537 (2018). https://doi.org/10.1007/s40279-017-0852-6

4. Stevens, C.J., Best, R. Menthol: A Fresh Ergogenic Aid for Athletic Performance. *Sports Med* 47, 1035–1042 (2017). https://doi.org/10.1007/s40279-016-0652-4

5. Mündel T, Jones DA. The effects of swilling an L(-)-menthol solution during exercise in the heat. *Eur J Appl Physiol*. 2010;109(1):59-65. doi:10.1007/s00421-009-1180-9

4. Stevens, C.J., Best, R. Menthol: A Fresh Ergogenic Aid for Athletic Performance. *Sports Med* 47, 1035–1042 (2017). https://doi.org/10.1007/s40279-016-0652-4

6. Dietary supplements market size, share & covid-19 impact analysis, by type (vitamins, minerals, enzymes, fatty acids, proteins, and others), form (tablets, capsules, liquids, and powders), and regional forecasts, 2021-2028. *Dietary Supplements Market Size & Trends* | Growth, 2021-2028. https://www.fortunebusinessinsights.com/dietary-supplements-market-102082. Accessed March 27, 2023.

7. Carter JM, Jeukendrup AE, Jones DA. The effect of carbohydrate mouth rinse on 1-h cycle time trial performance. *Med Sci Sports Exerc*. 2004;36(12):2107-2111. doi:10.1249/01.mss.0000147585.65709.6f

8. Wickham KA, Spriet LL. Administration of Caffeine in Alternate Forms. *Sports Med*. 2018;48(Suppl 1):79-91. doi:10.1007/s40279-017-0848-2

9. Jerram ML, Baker D, Smith TB, Healey P, Taylor L, Black K. A 0.1% l-menthol mouth swill in elite male rugby players has different effects in forwards and backs. *International Journal of Sports Physiology and Performance*. 2023:1-9. doi:10.1123/ijspp.2022-0191

10. Tsutsumi Y, Momma H, Ebihara S, Nagatomi R. L-menthol administration facilitates breathing comfort during exhaustive endurance running and improves running capacity in well-trained runners: A randomized crossover study [published online ahead of print, 2022 Aug 31]. *Eur J Sport Sci*. 2022;1-9. doi:10.1080/17461391.2022.2115404

11. de Camargo R, Vilaça A, Mota GR, Elsangedy HM, Haile L, Sasaki JE. The Influence of L-menthol on Time Trial Running Performance in Recreational Runners [published online ahead of print, 2022 Apr 12]. *Res Q Exerc Sport*. 2022;1-9. doi:10.1080/02701367.2021.2014049

12. Parton AJ, Waldron M, Clifford T, Jeffries O. Thermo-behavioural responses to orally applied l-menthol exhibit sex-specific differences during exercise in a hot environment. *Physiol Behav*. 2021;229:113250. doi:10.1016/j.physbeh.2020.113250

13. Best R, Crosby S, Berger N, McDonald K. The Effect of Isolated and Combined Application of Menthol and Carbohydrate Mouth Rinses on 40 km Time Trial Performance, Physiological and Perceptual Measures in the Heat. *Nutrients*. 2021; 13(12):4309. https://doi.org/10.3390/nu13124309

14. Jeffries O, Goldsmith M, Waldron M. L-Menthol mouth rinse or ice slurry ingestion during the latter stages of exercise in the heat provide a novel stimulus to enhance performance despite elevation in mean body temperature. *Eur J Appl Physiol*. 2018;118(11):2435-2442. doi:10.1007/s00421-018-3970-4

15. Flood TR, Waldron M, Jeffries O. Oral L-menthol reduces thermal sensation, increases work-rate and extends time to exhaustion, in the heat at a fixed rating of perceived exertion. *Eur J Appl Physiol*. 2017;117(7):1501-1512. doi:10.1007/s00421-017-3645-6

16. Stevens CJ, Thoseby B, Sculley DV, Callister R, Taylor L, Dascombe BJ. Running performance and thermal sensation in the heat are improved with menthol mouth rinse but not ice slurry ingestion. *Scand J Med Sci Sports*. 2016;26(10):1209-1216. doi:10.1111/sms.12555

17. Alvarez-Berdugo D, Rofes L, Casamitjana JF, et al. TRPM8, ASIC1, and ASIC3 localization and expression in the human oropharynx. *Neurogastroenterol Motil*. 2018;30(11):e13398. doi:10.1111/nmo.13398

18. Zhang, H., Huizenga, C., Arens, E. *et al.* Thermal sensation and comfort in transient non-uniform thermal environments. *Eur J Appl Physiol* 92, 728–733 (2004). https://doi.org/10.1007/s00421-004-1137-y