Complementary Therapies in Medicine 56 (2021) 102611

Contents lists available at ScienceDirect

Complementary Therapies in Medicine

journal homepage: www.elsevier.com/locate/ctim

Wet-cupping induces anti-inflammatory action in response to vigorous exercise among martial arts athletes: A pilot study

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ARTICLE INFO

Keywords:
Alternative medicine
Cupping
Incremental exercise
Interlukin-6
Tumor necrosis factor-α

ABSTRACT

Purpose: The aim of the present study was to investigate potential anti-inflammatory effects of wet-cupping prior to a moderate-to-vigorous exercise test among martial arts athletes.

Methods: Twenty-one male karate athletes voluntarily participated in this study and were randomly divided into 3 groups: vigorous exercise (VE, $n = 7$), cupping (CT, $n = 7$) and cupping plus vigorous exercise (VECT, $n = 7$). Participants in exercise groups performed an exercise test while participants in CT received cupping therapy, and participants in VECT received cupping therapy plus exercise. Inflammatory markers (i.e., interlukin-6, IL-6, and tumor necrosis factor-α, TNF-α) were assessed prior to, immediately, 30 min, and 24 h after cupping therapy, vigorous exercise test, and their combination.

Results: IL-6 values were significantly lower immediately after cupping intervention in CT as compared to baseline ($P < 0.025$). IL-6 significantly increased immediately and 30 min post-exercise in VE in comparison with baseline ($P < 0.025$). IL-6 was also significantly higher at 24 h post-exercise in CTVE as compared to baseline ($P < 0.025$). TNF-α values were significantly lower in CT as compared to VE and CTVE at immediately and 30 min post-exercise ($P < 0.01$). TNF-α significantly decreased immediately and 30 min after cupping intervention in CT as compared to baseline ($P < 0.01$). Conversely, TNF-α significantly increased immediately after exercise in VE as compared to baseline ($P < 0.025$). TNF-α also significantly increased at 30 min and 24 h post-exercise in CTVE in comparison with baseline ($P < 0.025$).

Conclusion: Our findings showed that exercise-induced augmentation in inflammatory markers were lower in athletes who received cupping therapy, suggesting such therapy may be an avenue to mitigate the inflammatory response to vigorous exercise among martial arts athletes. A large-scale clinical study is needed to confirm the findings of the present study.

1. Introduction

Among other cytokines, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are mainly considered as inflammatory responses of the body.[1] Pre-inflammatory cytokines such as TNF-α and IL-6 play a central role in regulating the body's immune response to inflammation.[2] TNF-α effectively causes local excitation in the inflammatory responses and helps the control of infections, whereas IL-6 causes the activation of lymphocytes as well as the production of antibodies,[2] indicating the vital role of these cytokines in our body's defense system.

Cytokine responses to exercise, especially with regards to IL-6 and TNF-α, are usually interpreted as a defensive immune mediator.[3] Previous studies have suggested that plasma IL-6 increases as a result of exercise, and these changes depend on exercise intensity, time of activity, proportion of activated muscles and an individual's endurance capacity.[4] For example, vigorous exercise has been reported to elevate blood inflammatory markers (i.e., IL6 and TNF-α). Interestingly, studies have shown that even without causing damage to the muscles, muscle contraction alone can result in a significant increase in IL-6 level.[5] Therefore, elucidating any strategies that could prevent and/or alleviate the inflammatory response to exercise training are warranted.

Various methods have been introduced to improve immune function, some of which have been proven to be effective strategies, such as nutrition-based intervention. [6] It has been suggested that in addition to

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https://doi.org/10.1016/j.ctim.2020.102611
Received 27 March 2020; Received in revised form 11 July 2020; Accepted 31 October 2020
Available online 5 November 2020

other interventions (to improve immune function) such as with herbal supplementation, holistic methods including wet-cupping might also be useful.[3] Wet-cupping is applying a sudden stress to the body's immune system in order to activate it against all internal and external factors.[7] Cupping prevents numerous illnesses using different mechanisms such as regulating the immune system, chemical-hormonal compounds, and the body's autonomic nervous system.[8] In addition, this method has also been suggested to be effective in curing some diseases such as nervous and migraine-related headaches, high blood lipidslipisa, sugar and urea levels, and kidney and gallbladder stones.[8]

From an immune function and anti-inflammatory action perspective, a recent review by Al-Bedah et al. (2019) suggested two theories by which cupping theory improves immune function and elicits anti-inflammatory action.[9] These theories include "Activation of Immune System Theory" and "Release of Nitric Oxide (NO) theory". The former has been reported to improve immune function through eliciting an artificial local inflammation, activating the complementary system, and augmenting immune system products like interferon and TNF, [9] whilst the NO theory has been suggested to elicit anti-inflammatory action through NO activation which is a signaling molecule that mediates vasodilatation and regulates blood flow and volume.[9] For example, a previous study has demonstrated a higher activity of myeloperoxidase, lower activity of superoxide dismutase, higher levels of malondialdehyde and nitric oxide in cupping blood in comparison to the venous blood. [10] Such immunomodulation and anti-inflammatory action of cupping renders it a potential intervention to prevent and/or modulate various diseases. However, whether cupping could alleviate the inflammatory responses to various conditions such as stress and vigorous exercise is not fully investigated, which warrants further investigations.

Taken together, considering the inflammatory responses to exercise, especially intense exercise, and beneficial effects of wet-cupping therapy on these responses, we investigated the potential modulatory effects of wet-cupping therapy on inflammatory responses (IL-6 and TNF-α) to vigorous exercise training. It was hypothesized that wet-cupping therapy prior to intense exercise training could effectively mitigate the vigorous exercise-induced augmentation in inflammatory markers among martial arts male athletes.

2. Methods

2.1. Study design

Following anthropometric measurements, participants randomly divided into 3 equal groups using a computer-generated randomization sequence. Participants were allocated to either vigorous exercise (VE, n =7), who performed a supervised vigorous exercise test, a cupping therapy (CT, $n = 7$), who only received cupping therapy, or a cupping therapy plus vigorous exercise group (CTVE, $n = 7$), who initially received cupping therapy and then after 24 h performed the exercise test, with the randomization sequence concealed in sequentially numbered, opaque sealed envelopes. To measure inflammatory makers, blood samples were collected at different phases at baseline and after incremental exercise. This study was conducted in accordance with the Declaration of Helsinki; with Ethics approval was obtained through the local Education Ethical Research Committee (Approval code: 32097-2016-09-20).

2.2. Participants

Twenty-one martial arts male athletes volunteered and participated in this study. Participants were eligible for inclusion if they were age 20–30 years, had at least 5 years tournament and karate training experience, had no addiction to drugs or alcohol, no history of renal, hepatic, cardiovascular disease, diabetes, and/or any physical injury preventing participation in an exercise test. Participants were advised that no new exercise should be commenced and not to use supplements during experimental trial. Before participating in the study, all procedures were explained to volunteers and after a full explanation of study procedures, written informed consent was obtained. No side effects were reported by participants after either cupping therapy, exercise testing, or their combination. Participants' demographic characteristics are shown in Table 1.

Table 1
Participants' demographic characteristics (mean ± standard deviation).

	VE	CT	CTVE	P value
Age (years)	24.6 ± 3.1	24.9 ± 3.6	25.3 ± 2.7	P > 0.05
Height (cm)	177.4 ± 5.9	174.9 ± 6.8	176.9 ± 4.7	P > 0.05
Weight (Kg)	80.5 ± 14.9	74.9 ± 7.2	80.9 ± 7.2	P > 0.05

VE, vigorous exercise group; *CT,* cupping therapy group; *CTVE*, cupping therapy + vigorous exercise group.

2.3. Exercise testing, blood collection, and inflammatory markers assessment

Participants in exercise groups (VE and CTVE) performed a moderate-to-vigorous exercise test.[11] Briefly, participants first performed a warmup session consisting of running at speed ranged from 4 to 6 km/hour for 2 min. Following this initial warm up, running speed then increased by 2 km.h^{-1} every two minutes up to 16 km.h^{-1}, with blood samples collected by venipuncture at different phases of exercise test, including prior to, immediately, 30 min, and 24 h after completion of the exercise test. Samples then were centrifuged at −20 °C, 3000 RPM for 10 min to extract the serum, and separated into aliquots then stored at −80 °C until analysis. Human serum IL-6 and TNF-alpha levels were measured by sandwich ELISA as per manufacturer's instructions (Bioscince, USA).

2.4. Cupping protocol

Participants in the cupping group were cupped by a trained phlebotomist as previously described.[10,12] Briefly, sterile disposable cups (5 cm in diameter) were used, with points selected for treatment including posterior neck, bilateral pre spinal areas of the neck, and thoracic spine. Once cleaned with antiseptic solutions, application areas were used to apply cups, followed with applying negative pressure through cupping pump. The cups were removed from the skin after 2–3 mins and then cupping sites were superficially incised (i.e., ≤1 mm oblique depth) using a 26-gauge disposable lancet. Next, 5–15 mL of blood was drained from each cupping site by pumping with vacuum for three times. Application sites were then covered using sterile pads. The entire treatment time totaled 20 min to treat both sides of the body.

2.5. Statistical analysis

All data (mean ± standard deviation) were analyzed using software MS Excel. Two-way repeated measures analysis of variance (ANOVA) was used to analyze the data, with one factor for time (baseline. immediately, 30 min, and 24 h after exercise) and one factor for groups (CT, VE, and CTVE). Post hoc was Bonferroni corrected. Statistical significance was set at $P < 0.05$.

3. Results

3.1. IL-6

A significant interaction effect for time and group was noted for IL 6 ($P < 0.05$, $\eta_p^2 = 0.251$). IL-6 values were significantly lower in CT as compared to VE at immediately and 30 min after exercise (both $P < 0.025$), with no significant differences found between CT and CTVE ($P > 0.05$). Further, IL-6 values were significantly lower at immediately after

Table 2
Mean and standard deviation for Interleukin-6 levels in three groups (Nanogram/deciliter) at different phases.

Time	Group		
	VE	CT	CTVE
Prior to exercise	1.251 ± 0.48	1.533 ± 0.46	1.392 ± 0.54
Immediately after exercise	1.737 ± 0.58	0.937 ± 0.44	1.388 ± 0.35
30 min after exercise	1.894 ± 0.69	1.289 ± 0.32	1.535± 0.3
24 h after exercise	1.704± 0.58	1.191 ± 0.49	1.626 ± 0.24

cupping intervention in CT, compared to baseline ($P < 0.025$). Conversely, IL-6 significantly increased at immediately and 30min after exercise in VE, both compared to baseline (both $P < 0.025$). However, IL-6 values were significantly higher only at 24 h after exercise in CTVE, compared to baseline ($P < 0.025$). It also should be noted that there was no significant difference between group for IL-6 at baseline ($P > 0.025$) (See Table 2 and Fig. 1).

3.2. TNF-α

A significant interaction effect for time and group was noted for TNF-α ($P < 0.001$, $\eta_p^2 = 0.400$). TNF-α values were significantly lower in CT as compared to VE and CTVE at immediately and 30 min after exercise (all $P < 0.01$). Further, TNF-α values significantly decreased immediately and 30min after cupping intervention in CT, compared to baseline (both $P < 0.01$). Conversely, TNF- α significantly increased immediately after exercise in VE, as compared to baseline (all $P < 0.025$). TNF-α also significantly increased at 30min and 24 h after exercise in CTVE, compared to baseline (both $P < 0.025$) It also should be noted that there was no significant difference between groups for TNF-α at baseline ($P > 0.025$) (See Table 3 and Fig. 2).

4. Discussion and conclusion

Cupping therapy has been of great interest among researchers for its effectiveness treating various chronic conditions such as low back pain, chronic arthralgia, radiculopathy, and respiratory -related disease.[8] However, whether such intervention is an effective avenue to improve exercise performance and alleviate its related deleterious conditions, such as increased inflammatory markers, has yet to be investigated. Therefore, this experiment was conducted to examine the potential beneficiary effects of wet-cupping therapy prior to vigorous exercise on selected inflammatory markers (i.e., IL-6 and TNF-α) among martial arts athletes.

Two novel findings of the present study were that cupping therapy significantly decreased resting inflammatory markers within martial arts athletes and that such therapy was able to preclude augmentation in inflammatory makers (i.e., IL6) post-exercise, manifesting with lower

Table 3
Mean and standard deviation of Tumor Necrosis Factor Alpha levels in three groups (Nanogram/deciliter) at different phases.

Time	Group		
	VE	CT	CTVE
Prior to exercise	4.887 ± 2.29	4.746 ± 1.02	5.0421 ± 0.27
Immediately after exercise	6.384 ± 1.8	2.744 ± 1.6	5.412 ± 0.63
30 min after exercise	6.833 ± 1. 82	2.648 ± 1.73	5.674 ± 1.13
24 h after exercise	5.41 ± 1.41	4.393 ± 1.27	6.202 ± 1.72

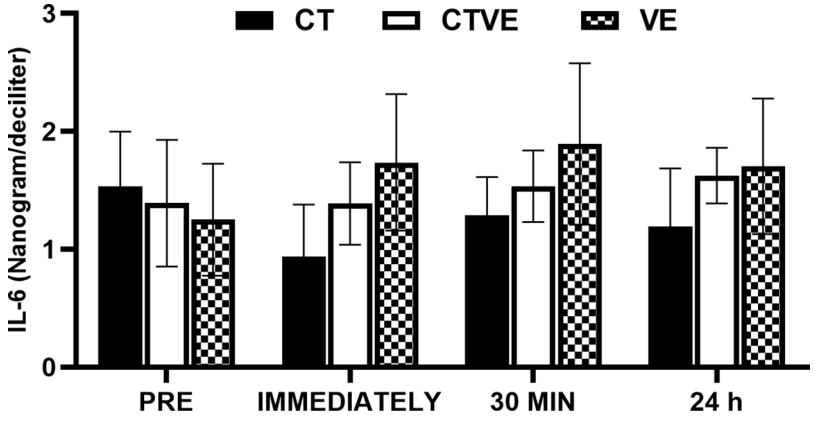

Fig. 1. Mean and standard deviation for Interleukin-6 (IL-6) in the three groups at different phases.

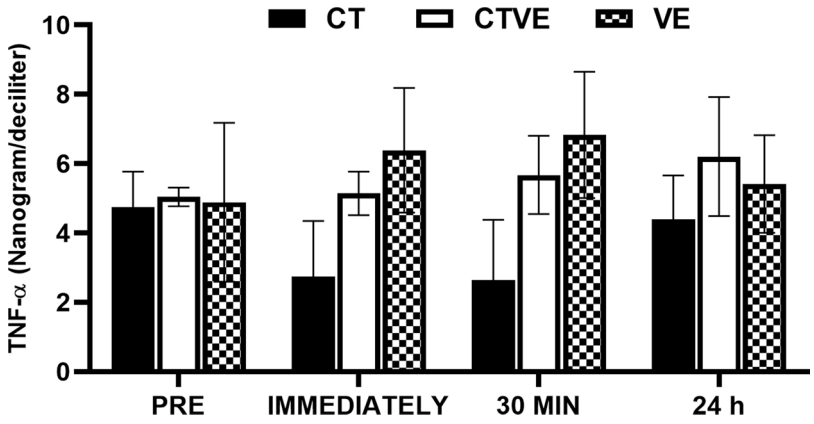

Fig. 2. Mean and standard deviation for Tumor Necrosis Factor Alpha (TNF-α) in the three groups at different phases.

inflammatory markers values at immediately and 30 min post-exercise in CTVE as compared to VE group. To our knowledge, we are the first to report such anti-inflammatory effects of cupping therapy before and after vigorous exercise. These findings build upon previous studies reporting various beneficial effects of cupping therapy on various systems in both health and disease, including lowering blood pressure and preventing cardiovascular disease progression among healthy individuals,[13,14] treating oral and genital ulceration in patient with Behçet's disease, [15] alleviating musculoskeletal pain, [16,17] reducing low density lipoprotein (LDL) and preventing the development of atherosclerosis, [18] lowering the number of lymphocytes, [19] increasing red blood cells[20] and reducing blood sugar level among diabetic patients.[21] With regard to cupping therapy-related immunomodulation and anti-inflammatory effects in agreement with our findings, Khalil and colleagues (2013) suggested that cupping appears to contribute to the immune system at cellular level and may provide a protective role by enhancing immunity, protecting the body from various disease development and progression.[22] Similarly, improved immune function has been reported following cupping in patients with chronic obstructive pulmonary disease.[23] Cupping has also been demonstrated to be effective in reducing rheumatoid arthritis-related inflammatory markers such as C-reactive protein (CRP)[24]. The exact mechanisms whereby cupping mediates such anti-inflammatory effects are not fully understood however, such beneficial effects appear to be orchestrated through "nitric oxide (NO) theory". Briefly, NO, a gaseous molecule resealed from endothelial cells, has been shown to contribute to blood pressure regulation, immune function, neurotransmission modulation, and several other physiological functions. [25,26] In the clinical setting, an upregulation of enzyme contributing to NO production (i.e., NO synthase) has been reported around skin acupuncture points in rodents. Another study conducted by Tagil and colleagues (2014) also reported an increased myeloperoxidase activity, decreased superoxide dismutase activity, and augmented malondialdehyde and NO levels in cupping blood in comparison to the venous blood. [10] Together, cupping therapy-induced augmentation in NO production elicits vasodilation that can bring about such anti-inflammatory effects. Another theory that may have contributed to observed anti-inflammatory action of cupping is "blood detoxification". In short, cupping therapy has been shown to augment blood circulation which in turn elicit removal of toxins and waste products from body. [27] This could presumably be accomplished by facilitating microcirculation, capillary endothelial cell repair, granulation, and angiogenesis in local tissues, resulting in a normalized functional state and muscle relaxation.[28,29] It should be noted that these mechanisms may have worked in tandem to produce such beneficial effects. Future studies focusing on these mechanisms, or their interaction, are needed to shed light on such anti-inflammatory effects of cupping.

Another main finding was that exercise caused significant augmentation in inflammatory markers (i.e., IL6, TNF-α). Our findings are in agreement with previous studies in which inflammatory markers found to be greater post-intense aerobic exercise.[30–32] Exercise has been reported to elicit secretion of IL-6 in several tissues such as skeletal muscle, brain, and peri-tendinous tissues.[33] Active (contracting) muscle appears to be a main source for IL-6 during exercise,[34–36] with circulating IL-6 increasingmore rapidly toward the end of exercise.[35,36] Exercise-induced augmentation in circulating IL-6 appears also to be associated with muscle glycogen availability, with lower availability leading to higher IL-6 levels during exercise.[37,38] An inverse correlation has also been found between pre-exercise muscle glycogen concentration and post-exercise muscle IL-6 messenger RNA.[39] Specifically, increases in exercise intensity occur concurrently with increases in carbohydrate metabolism through increased muscle glycogen turnover and uptake of circulating glucose by active muscle, [40,41] resulting in a greater release of IL-6. [42] However, mechanisms by which exercise increase TNF-α levels are not well-understood. However, it is suggested that this can be regulated by circulating lipopolysaccharides (LPS), known as TNF-α stimulus, that increases during exercise.[43–45] Interestingly, however, TNF-α levels have shown to be unchanged despite increased LPS.[45] It is also unlikely that this could be monocyte-mediated since monocyte activity has shown to be either unchanged[46] or reduced [47] with exercise. Upregulated post-exercise TNF-α may, therefore, be related to exercise-induced muscle damage that is associated with neutrophil infiltration and proinflammatory cytokine accumulation,[48] suggesting a potential role for increased TNF-α levels post-exercise.[49] Finally, preclinical experiments examining inflammatory responses to acute exercise also reported an upregulation of TNF-α in the lung [50] and adipose tissue, [51] suggesting these as other contributing sources of greater circulating TNF-α concentrations during exercise.

Although this study has uniquely shown the anti-inflammatory effects of wet cupping prior to exercise testing among martial arts male athletes, there are some limitations of the current study that should to be acknowledged. Firstly, a relatively small sample of martial arts male athletes was used in the present study that may limit the generalizability of the current results to all populations. Future studies utilizing a larger sample size from different populations (e.g., non-athletes, male, female) would elaborate upon these findings. Secondly, a sham cupping group was not included in the present study, which warrants further investigation.

In conclusion, our findings showed that exercise-induced augmentation in inflammatory markers was lower in athletes received cupping therapy, introducing cupping therapy as a potential to reduce the upregulation of these markers during stressful conditions such as exercise training, especially during vigorous exercise. Future studies utilizing a larger sample size are required to confirm the findings of the current study and to elucidate mechanism(s) underlying such beneficial effects of cupping therapy and its potential application in various health and disease conditions.

Author statement

NE and MN conceived and designed the study with NE and HS collecting the data. NE, MA, MN, and HS contributed in statistical analysis, data interpretation and manuscript drafting and revising. The final version of manuscript for submission was approved by all authors for submission.

Funding

The authors declare that no funding is involved in this study. They also would like to appreciate all the valuable assistances they received from Prof. M. Karimi and Prof. B. Tartibian for their knowledge and advice, Dr. S. M. Soroushzadeh for his experience in the cupping process, Mr. S. Ashtiani and Mr. O. Dehghani for their kind supports and helps.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ctim.2020.102611.

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