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The Effects of a Single Bout of High- or Moderate-Intensity Yoga Exercise on Circulating Inflammatory Mediators: A Pilot Feasibility Study

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Abstract

Background: There is a knowledge gap in the physiological effects of short-term yoga exercise interventions.
Objective: To evaluate the feasibility of a randomized controlled trial (RCT) assessing the acute effects of a yoga exercise protocol practiced at 2 intensities (high or moderate) on temporal responses of a battery of systemic circulatory cytokines in healthy yoga-naïve adults.
Methods: This study was a three-arm, pre-post pilot-RCT employing a single bout of yoga exercise intervention. Groups were high-intensity yoga (HY, n = 10), moderate-intensity yoga (MY, n = 10), and a sedentary, no-intervention control group (CON, n = 10). Blood samples were collected at baseline and post-intervention at 6 timepoints (0-, 30-, 60-, 120-, 180-minutes, and 24-hours post-intervention) and were processed with a pre-defined inflammatory panel of 13 cytokines. Heart rate (HR) was assessed with a Polar H10® device. The PROMIS Pain intensity Questionnaire was used to assess body soreness.
Results: We demonstrate feasibility of recruitment, randomization, and retention of participants based upon predetermined metrics, including: proportion of eligible to enrolled participants (55%); recruitment period (11-months); participant retention (97%); completion rate for questionnaires (99%); completion of physiological measures (98%); and adherence to the yoga exercise protocol (88%). Cytokine levels over time were heterogeneous within and between groups. Responses of a subset of cytokines were positively correlated with 1 another in high- and moderate-intensity yoga exercise groups but not in the control group. Median values for HR were 91 (IQR: 71–95) in the HY, 95 (IQR: 88–100) in the MY, and 73 (IQR: 72–75) in the CON. Pre-post changes in body soreness after the yoga exercise intervention were most evident in the HY group.
Conclusion: Along with observed trends in select cytokines, findings encourage a more definitive trial aimed at understanding the short-term effects of yoga exercise on inflammatory immune markers and pain in sedentary healthy adults. Clinicaltrials.gov ID# NCT04444102.

Keywords

Short-term yoga exercise, healthy adults, inflammation, cytokines

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Introduction

In the past few decades, the practice of yoga has increased in the United States.[1] This has been catalyzed in part by a growing body of literature demonstrating benefits for a wide range of health conditions, including management of chronic pain, cardiovascular, and biopsychosocial disorders.[2] However, the physiological pathways underlying these observed therapeutic effects remain poorly understood.[3,4] Even fewer studies have explored the physiological effects of short-term interventions or acute bouts of yoga exercise.[5-7] A better understanding of the impact of yoga on fundamental physiological dynamics associated with allostasis is critical to understanding both its initial and long-term impacts on disease prevention and rehabilitation.[8]

One set of key physiological events believed to underlie the therapeutic effects of conventional exercises, yoga, and other related mind-body movement therapies (MBMTs), such as Tai Chi and Qigong, are local immune-mediated processes, including inflammation, tissue remodeling, and regeneration. These changes are believed to be triggered by bottom-up processes occurring when local exertion is applied to the myofascial system.[9] These events also have systemic effects when different immune mediators are secreted into the bloodstream during and after physical activity, and have sometimes collectively been referred to as myokines and exerkines.[10-12] Simultaneously to these bottom-up changes, top-down influences may also be importantly activated by MBMTs practice, including modulation of the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) through the secretion of adrenocorticotropic hormone (ACTH) and catecholamines (adrenaline and noradrenaline).[3,13] Both bottom-up and top-down processes are known to modulate the expression of inflammatory cytokines on immune cells locally and systemically, which activates or inhibits the inflammatory transcriptome, after physical activity.[3]

A prior systematic review of in vivo animal stretching studies also supports the importance of bottom-up myofascial processes, beginning at the myofascial system.[14] For example, local and systemic mechanical forces that occur during the physical practice of yoga (i.e., static and dynamic postures) likely trigger myofascial activation and remodeling. These processes take place through crosstalk between local inflammatory and resident cells, including modulation of cytokine and chemokine expression, and local and systemic secretion.[10-12] A growing body of research, including animal and human studies, supports the essential role of the myofascial system in regulating inflammatory pathways.[15-18] Preclinical studies of physical interventions targeting the myofascial system suggest a range of inflammatory effects, from therapeutic to injurious, depending on the intensity, duration, and frequency of interventions.[15,16,19-23] Studies of active stretching in rats with experimentally induced lower back inflammation, for example, reported that active physical stretching could reduce lesion size and neutrophil count.[16] Another study of lower back inflammation in pigs also reported that the average lesion area was significantly reduced by stretching.[20] Finally, a study using a mouse model of breast cancer found a reduction in tumor size along with increased markers of inflammation resolution following a daily regimen of active stretching.[21] In contrast, experimental models of local mechanical tissue injury have shown that higher intensities and frequencies of stretching can lead to deleterious inflammatory effects.[24,25]

Clinical sports medicine studies provide additional evidence for the impact of tissue-level forces on cytokine systemic levels. Studies conducted in healthy adults have shown a proinflammatory response after an acute intervention (e.g., running, cycling, and walking) with an increase in systemic cytokines (e.g., IL-6, TNF-α, IL-1).[26] These acute changes have typically been attributed to myofascial stress. In contrast, long-term exercise interventions have been associated with an overall reduction of systemic proinflammatory cytokine basal levels.[27] Briefly, after each bout of exercise, a peak of inflammatory mediators due to myofascial stress will occur. With training, these post-exercise peaks will become less pronounced. At the same time, basal levels of inflammatory mediators (e.g., produced by other body tissues, such as liver or adipose tissue) have been observed to decrease with exercise.[28] However, the mechanisms underlying decreases in basal systemic levels by training have yet to be fully elucidated.[29] Collectively, these findings support the need for a closer look at the physiological role of systemic inflammatory mediators in response to acute bouts of yoga exercise.[30]

Based on current knowledge gaps of short- and long-term interventions of yoga exercise in healthy populations, we designed a pilot study exploring systemic cytokine dynamics after an acute bout of yoga exercise in yoga-naïve healthy participants. The study had 2 main aims: (1) To evaluate the feasibility of conducting a randomized controlled trial (RCT) to study the acute effects of high- and moderate-intensity yoga exercise protocols compared to a sedentary control group on inflammatory outcomes, and (2) To preliminarily characterize the temporal responses of a battery of systemic circulatory cytokines in response to high- and moderate-intensity yoga exercise. Specifically, exploratory sub-aims of aim #2 were designed to evaluate if: (a) an acute bout of yoga exercise impacts circulating levels of a battery of inflammatory cytokines in a measurable way; (b) cytokines associated with the myofascial system show responses to yoga exercise similar to other exercise interventions; (c) cytokine responses to yoga are intensity-dependent; and (d) responses of specific cytokine are positively and/or negatively associated with others in response to a bout of yoga exercise. Findings are discussed in the context of designing a fully powered clinical trial to evaluate the acute effects of yoga in healthy populations.

Material and Methods

This trial's supporting CONSORT checklist for pilot studies is available as supplementary information (Checklist S1).[31]

Ethics Statement

The study was approved by the Mass General Brigham/Partners Institutional Review Board (Protocol #: 2019P001716). Written informed consent was obtained from all subjects during the first visit before any intervention. The study was registered at clinicaltrials.gov ID# NCT04444102.

Study Design

This study was a three-arm, pre-post pilot randomized clinical trial of a single bout of yoga exercise intervention. The 3 groups were: high-intensity yoga exercise (HY), moderate-intensity yoga exercise (MY), and a sedentary, no intervention control (CON). In-person outcome assessments were conducted at baseline and post-intervention time points (0-, 30-, 60-, 120-, 180-minutes, and 24-hours post-intervention).

Yoga exercise groups. Both interventions were administered as an individual one-on-one yoga exercise session with a certified Hatha yoga instructor (DM-V). The yoga posture protocol for both exercise groups consisted of the same sequence of postures. Both interventions were delivered over 60 minutes, in the same room, around the same time of the day (8:30 to 9:30 AM) and utilizing the same props (blocks and mats). Briefly, the protocol started with 5 minutes of instruction about finding a body posture representing approximately 100% (i.e., high-intensity group) or 50% (i.e., moderate-intensity group) of an individual's range of motion that was pain-free. The formal routine began with 5 minutes of warm-up, followed by a set of yoga postures targeting ten anatomical groups (Supplementary Table S1).[32] Participants were reminded throughout the intervention to target the appropriate level of intensity (100% or 50%) and given feedback, specific cues, and potential modifications, meaning a more accessible version of the original posture, were offered depending on the feedback received by the participant during the intervention. No explicit instructions in other yoga components (e.g., breathing, imagery) were provided.

Control group. Participants randomized to the control group remained in a quiet room in the same clinical facility by themselves for 1 hour and were asked not to engage in any physical, mental, or emotional activity that could evoke distress.

Eligibility Criteria

Healthy participants, relatively sedentary (<30-minutes twice a week of high-intensity exercise, e.g., running), yoga- and MBMT-naïve, ≥40 to ≤60 years old, non-smoking, and with BMI ≥19 and ≤29, were recruited using Partners Rally, an online platform that supports collaboration between the public and research community to help advertise and recruit participants for different clinical trials, and flyers publicly posted in the Boston area. Exclusion criteria included: any history of chronic inflammatory disease or recent acute illness (<1 month) (e.g., rheumatoid arthritis, cancer, acute or chronic musculoskeletal pain), use of any medication in the preceding week (both prescribed or over-the-counter, except vitamins, supplements, herbal medicine, and birth control pills), and practice of structured exercise at least twice a week for more than 30 minutes.

Randomization

An allocation sequence (i.e., random permuted blocks of sizes 3 and 6) was generated and uploaded to the Research Electronic Data Capture (REDCap) online system. After obtaining informed consent, baseline data, and the first blood samples, the study coordinator randomized subjects within the REDCap platform.

Study Visits

Visit 1. Visit 1 started with participants arriving in a fasting state. After informed consent was obtained, baseline blood draws were taken by venipuncture within the antecubital vein. Participants were then offered a light snack and asked to complete baseline questionnaires, followed by randomization. Participants were equipped with a wearable device placed on their chest (Polar H10®) to record heart rate (HR) during the assigned intervention. At the end of the intervention, participants were moved to a procedure room, wherein nurses performed serial blood sampling (i.e., subsequent 5 samples). At the end of visit 1, participants randomized to yoga exercise groups were invited to a brief qualitative interview.

Visit 2. At the end of visit 1, participants were asked to return the next day in a fasting state between 8:00 and 8:30 AM for the last blood sample (#7) and the second administration of the PROMIS Pain intensity questionnaire.

Blinding

Participants and the yoga instructor were not blinded to group allocation. However, nurses and laboratory technicians were blinded during sample collection and laboratory analyses. The data analyst was unblinded to the allocation after all laboratory analyses were completed.

Outcomes

The primary goal of this study was to assess a priori-defined metrics of feasibility. Feasibility was evaluated

based on: (1) percentage of eligible to enrolled participants (>60%); (2) the number of months required to complete the recruitment process (within 9 months); (3) retention rate (i.e., proportion of participants completing 2 study visits >70%); (4) completion rate of questionnaires (>75% participants with full completion); (5) completeness of physiological measures (i.e., >80% of HR and blood measures collected and processed); and (6) the adherence rate to the yoga exercise protocol, defined as the percentage of the total number of poses each participant was capable of performing (>75%).

Adverse events (AEs) were systematically queried by the study coordinator immediately after the intervention and during the qualitative interview. Participants were also instructed to contact the study coordinator if they experienced physical symptoms that concerned them after the intervention and testing session ended.

Secondary outcomes included serum levels of a battery of cytokines measured at multiple time points to characterize the impact of a single bout of yoga exercise and to inform potential markers to be used in a future, fully powered trial. At baseline and 24 hours after intervention, patients completed online (REDCap) the PROMIS Pain intensity questionnaire, a 3-item measure assessing pain intensity using a 5 point Likert-type scale with anchors, no pain to very severe pain (Q1: worse pain intensity in the past 7 days, Q2: average pain intensity in the past 7 days, and Q3: current pain intensity).[33] The PROMIS Pain intensity instrument was used to characterize pre-post changes in body soreness after the yoga exercise class. HR was assessed to characterize the metabolic intensity of the yoga exercise interventions. A baseline questionnaire collected information on age, gender, ethnicity, education, alcohol consumption, and BMI.

Laboratory Data Collection

Blood sample collection. After baseline collection, at the end of the yoga exercise intervention and the control group's resting period, an indwelling peripheral venous catheter was placed in the antecubital vein, and consecutive blood samples were drawn into 2 tubes (10 mL each with Vacutainer® serum and CPT™ tubes) within a 15-minute window of placing the catheter (post-intervention time 0) and then at 30-, 60-, 120-, and 180-minutes post-intervention. Patients returned the following morning for venipuncture collection of 24-hour blood samples.

Blood sample handling and storage. Serum was collected following standard operating procedures described elsewhere.[34]

Cytokine detection. Participants' serum samples per each time point were quantified for each of the cytokines within each sample run in duplicate. Following a standardized manufacturer manual, the samples were analyzed using premixed beads coated with different human inflammation panel detection antibodies (LEGENDplex™ Human Inflammation Panel 1—BioLegend, San Diego CA) for the following cytokines: IL-1β, IFN-α2, IFN-γ, TNF-α, MCP-1 (CCL2), IL-6, IL-8 (CXCL8), IL-10, IL-12p70, IL-17A, IL-18, IL-23, and IL-33 selected on the basis of previous MBMT and Sports Medicine studies.[5,35] A high throughput CytoFLEX Platform (Beckman Coulter, Life Sciences) was used to read whole plates, and data analysis was performed using the LEGENDPlex™ data analysis online software (BioLegend® and GOGNIT— informatics).

Statistical Methods

Demographic and HR data were analyzed with descriptive statistics. Continuous raw data were tested for normality using the Shapiro-Wilk Test. Means and standard error of the mean (SEM) were reported for normally distributed data, and medians and interquartile ranges were reported for non-normally distributed data. Nominal data were reported as absolute numbers and percentages.

Exploratory analysis of cytokine levels utilized all available data per participant. Undetectable cytokine levels at a given time point were not included in analyses and treated as truncated data.[36] Levels of each cytokine at each timepoint were summarized with means and SEM per group. Exploratory longitudinal inferential analyses were calculated with SAS package (version 9.4, SAS Institute Inc, Cary, NC, USA) for each cytokine to explore time-by-group interactions using unadjusted analysis of response profile commonly used when dealing with an incomplete data set. To further characterize cytokine responses, raw data from the first 6 time points were used to calculate net Area Under the Curve (netAUC) adjusted for each participant's baseline values. This analysis compares increases and decreases of each cytokine over time using the trapezoid rule to summarize the change in each cytokine level with a single value.[37,38] Comparisons of netAUCs between groups were assessed using an unadjusted general linear regression model. Finally, to characterize correlations between the magnitude of initial changes (baseline to first timepoint after the intervention) between 7 cytokines commonly evaluated in myofascial exercise studies,[10,11,39,40] Pearson correlations were used to generate matrices of association between these 7 cytokines and plotted using heatmaps. Positive or negative relationships between changes among the 7 cytokines were then represented with heptagon strength diagrams.[41]

The Prism 9 software (GraphPad Software, Inc, San Diego, CA, USA) was used to calculate descriptive statistics for HR, PROMIS Pain Intensity score, and exploratory cytokine analyses. Given the pilot nature of this study, these analyses were intended to be exploratory to inform future hypotheses and study design features.

Based on a prior pilot in our laboratory, the sample size for this study was expected to be sufficient for evaluating a priori-defined metrics of feasibility.[42,43] Data from all subjects were reported and analyzed according to the intention-to-treat principle.

Table 1. Baseline Characteristics of the Study Participants by Randomized Treatment Assignment.

	Randomized groups		
	Mean ± SD, n (%)		
Variable	High-intensity yoga exercise group (HY)	Moderate-intensity yoga exercise group (MY)	Sedentary control group (CON)
Gender female, n (%)	4 (40%)	8 (80%)	4 (40%)
Age	50.6 ± 6.1	52.6 ± 6.1	52 ± 5.9
Race/Ethnicity, n (%)			
Caucasian	5 (50%)	4 (40%)	8 (56.7%)
Hispanic	1 (10%)	3 (30%)	0 (0%)
Black	2 (20%)	1 (10%)	1 (10%)
Asian	3 (30%)	2 (20%)	1 (10%)
Pacific islander	1 (10%)	0 (0%)	0 (0%)
Other	1 (10%)	1 (10%)	0 (0%)
Education some college, n (%)	9 (90%)	8 (80%)	10 (100%)
Alcohol (#drinks/week)	1.6 ± 1.8	.8 ± 1.9	.7 ± 1.6
BMI	23.4 ± 3.5	27.1 ± 2.5	27.1 ± 3.1

Results

Despite randomization, there were modest imbalances in gender and BMI between groups (Table 1). The HY was slightly younger and had a lower BMI compared to MY and CON. MY had a greater proportion of females than HY and CON, and was older and had a higher BMI than HY.

Feasibility Measures. Figure 1 summarizes participants' flow in the study, which occurred between January 2020 and February 2021. This period coincided with the beginning of the COVID-19 pandemic social distancing mandates. Regarding our a priori-defined metrics of feasibility: (1) 31 of 56 eligible subjects were enrolled and consented (55%, CI: 57; 54), falling short of our 60% target; (2) overall recruitment took 11 months, longer than our 9-month target; (3) 29 of 30 participants attended both study visits (retention rate: 97% CI: 98; 96), exceeding our 70% target; (4) 89 out of 90 (99%) baseline and follow-up questionaries were fully completed, also exceeding our 75% target; (5) 412 of 420 blood samples (98%) were successfully collected and processed. For HR, 29 out of 30 measurements during the intervention were achieved (97%), exceeding our goal of 80%; (6) the yoga exercise protocol adherence, defined as participating in either the original or modified versions of each pose, was 88% (original posture 66% and modified posture 22% of the time). Participants with BMI >25 were more likely to opt for a modified version of poses (31%) compared to those with a BMI <25 (9.3%). No differences in adherence or modification rate were seen according to age and gender.

No serious adverse events were reported during or after each yoga exercise intervention. Post-intervention soreness was assessed with the PROMIS pain scale. Four participants in the HY group and 2 in the MY group reported a modest increase (i.e., 1- or 2-point change) in pain 24 hours after the intervention, while none in the CON reported an increase in pain.

Cytokine Levels Before and After Yoga Exercise. Levels of 12 inflammatory cytokines were successfully measured; IL-1β was excluded from further analysis due to validation issues. Levels varied across the seven timepoints and 12 cytokines in complex ways (Supplementary Table S2).

Given the complexity of temporal dynamics, our main synthesis of effects of exposure to yoga exercise on cytokine levels relied on netAUC analyses (Figure 2). Comparisons of the mean netAUC between groups revealed 3 main patterns: (1) in all cases, the average netAUC within each group was negative (i.e., relative to baseline values, subsequent values were lower); (2) for 7 out 12 cytokines (IFN-γ, TNF-α, IL-8, IL-10, IL-12p70, IL-17A, and IL-33) the magnitude of netAUC change for the control group was greater than for the 2 yoga groups. In other words, cytokines level decreased more, relative to baseline, in the control group compared with the two yoga exercise groups; (3) the magnitude of changes between the 2 yoga groups did not show any consistent response (Supplementary Table S3).

Figure 3 shows the correlation matrix heatmaps and correlation strength diagrams, which characterize the interrelationships of initial post-exposure changes in 7 cytokines (i.e., IFN-γ, TNF-α, IL-6, IL-8, IL-10, IL-17A, and IL-33) known to be relevant to myofascial physiology during exercise.[10,11,39,40] The change in a given cytokine level immediately following intervention was compared to changes in the other 6, with blue representing positive correlation (same direction of change between the 2 cytokines being compared) and red representing negative correlation (opposite direction of change), with the width of the lines indicating strength of that interrelationship. Noteworthy patterns include: (1) Generally, across all 3 groups,

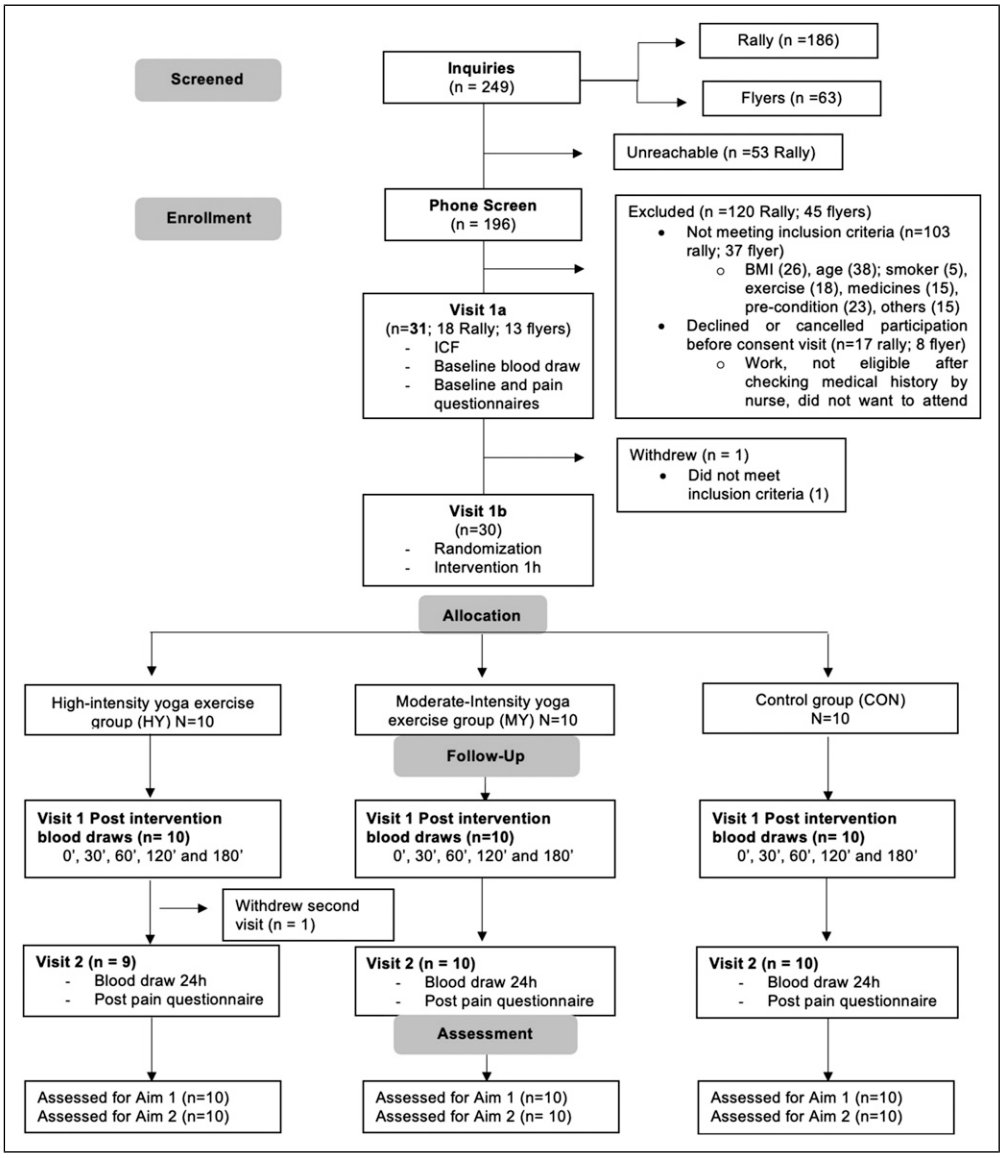

Figure 1. Study flow diagram.

changes in cytokines were highly interdependent; (2) in both yoga exercise interventions, these correlations tended to be more positive, whereas in the control group there was more of a balance between positive and negative correlations, and; (3) IL-33 positively correlated (intercorrelation coefficients of at least .6) with all other 6 cytokines, but only in the HY. Similarly, in the MY, these intercorrelations were all positive but weaker. In contrast, in the CON, IL-33 had mixed positive and negative intercorrelation coefficients. Supplementary Table S4 provides the confident intervals (CIs) for these intercorrelation coefficients.

Heart Rate. Median values for HR were 91 (IQR: 71, 95; range 31) in the HY, 95 (IQR: 88, 100; range 32) in the MY, and 73 (IQR: 72, 75; range 14) in the CON. HR trajectories in both yoga exercise groups increased during the intervention, more so in the MY compared to the HY (30% and 24.6% compared to CON, respectively) (Figure 4).

Discussion

To our knowledge, this is the first randomized study to evaluate the effects of a single bout of yoga exercise on immune-mediated markers of inflammation in yoga-naïve and healthy adults. Our findings support feasibility for the majority of evaluated criteria, which is noteworthy given that the study was conducted during the COVID-19 pandemic. We successfully collected and analyzed serum samples of 12 cytokines and identified candidate cytokines to be included in future studies. Below we discuss how results from this pilot study inform the

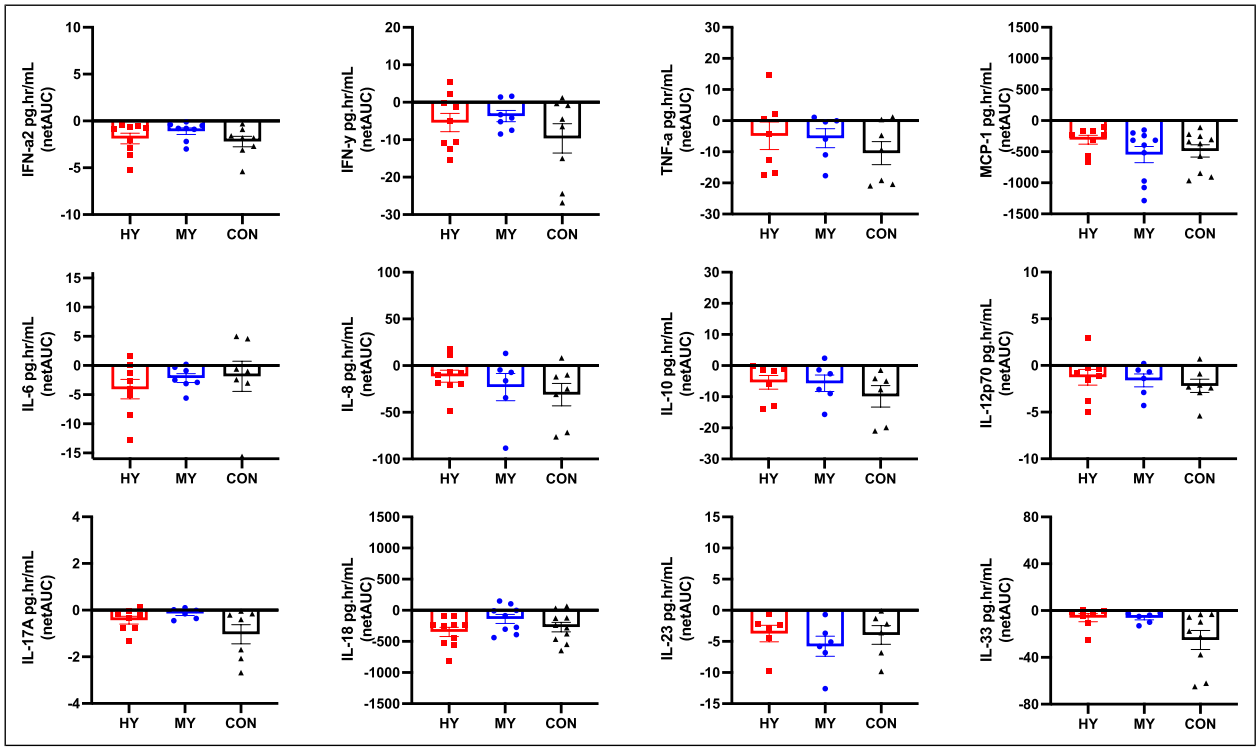

Figure 2. Each bar graphs denote each group net area under the curve (netAUC) mean and standard error of the mean (SEM) per each cytokine. Timepoints: baseline, 0-, 30-, 60-, 120-, and 180-minutes were included in the netAUC calculation. High-Intensity yoga exercise group individual netAUC in squares (HY); moderate-intensity yoga exercise group individual netAUC in circles (MY); control group individual netAUC in triangles (CON).

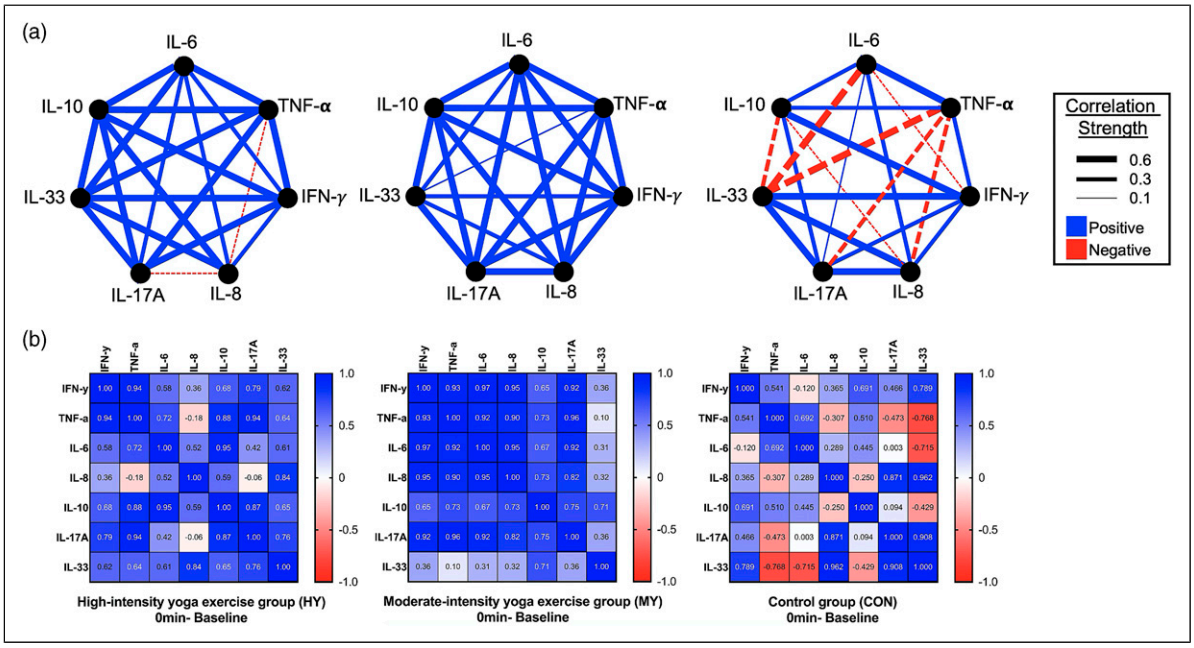

Figure 3. Delta correlations between the first time point after the intervention (0-minutes) and baseline. (A) Positive (continuous line) and negative (dashed line) correlations in which the width of the line represents 3 correlation strengths: weak, mild, and moderate correlation strength. (B) Heatmaps depict delta difference correlations between 7 cytokines in the high-Intensity yoga exercise Group (HY); moderate-intensity yoga exercise group (MY); and control group (CON).

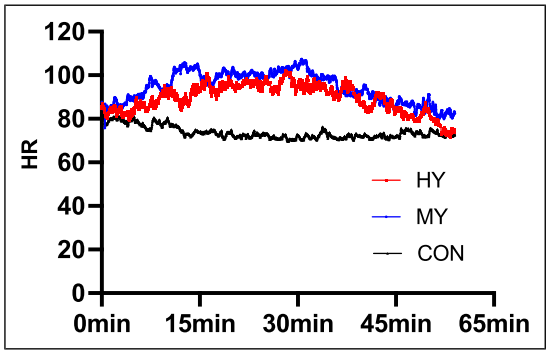

Figure 4. Measures of heart rate to characterize changes in each group. One-hour longitudinal trajectories of heart rate (HR) for each group. High-intensity yoga exercise group (HY), moderate-intensity yoga exercise group (MY), and control group (CON).

design of a future study and, more broadly, outline insights from our preliminary findings regarding the impact of a single session of yoga exercise on systemic inflammatory processes.[44]

Feasibility (Aim 1)

We fulfilled 4 of 6 a-priori defined feasibility metrics (retention rate, completion rates of questionnaires, completion rate of physiological measures, and protocol adherence). The remaining 2 feasibility metrics were close to target; the eligible to enrollment rate was 55% vs. 60%, and the number of months to complete the recruitment process was 11 vs. 9 months. Despite concerns about engaging in any social activity during COVID-19 social distancing measures, especially activities requiring visits to hospital environments, we were still able to recruit 30 yoga-naïve, eligible individuals who were willing to be randomized and provide biological samples at multiple time points. Once enrolled, all but 1 participant returned for visit 2, and the majority were highly compliant with all protocols. Of note, 14 (47%) participants of our sample were other than Caucasian, supporting our ability to recruit a diverse population. This success was due to active recruitment in highly diverse neighborhoods in the Boston area using flyers posted in public spaces. Collectively, these metrics suggest that recruitment and retention in future larger, powered trials with a similar design should be feasible.

The adherence rate to the yoga exercise protocol also indicates feasibility of using the same posture sequence and pacing in future trials. The inclusion of posture modifications (i.e., less physically demanding versions of the original postures) allowed participants and/or the yoga instructor to opt for them when the original version was difficult to achieve. We also noticed that adherence was impacted by differences in BMI at baseline (Table 1). A BMI >25 at baseline in the MY may have caused this group to opt for more posture modifications. Higher BMI may also explain the counterintuitive slightly higher HR in the MY compared to the HY (30% and 24.6% compared to CON, respectively).[45] We consider this finding relevant because previous studies among yoga naïve individuals with chronic health conditions and physical limitations have highlighted the importance of the protocol's design for adherence.[46,47] For example, a study among women with abdominal obesity allowed for the adapting of each posture to the needs, capabilities, and limitations of this population and gradually increased the difficulty of the postures over the course of the intervention.[48] In addition to BMI, gender was also skewed in the MY towards females; future larger randomized studies should stratify by BMI and gender to minimize confounding effects of the participants' characteristics. Future studies might also consider assessing postural range of motion (ROM), heart rate variability (HRV) metrics, and more detailed metabolic activity (e.g., mean VO2, peak VO2, % VO2max, and kcal expenditure) to better characterize the yoga interventions.[5,49] Finally, with respect to delayed onset of muscle soreness (DOMS), our post-intervention results for body soreness were not surprising. Future studies should include and compare subjective and objective tools to evaluate body soreness after short- and long-term yoga exercise interventions. These may include the Likert scale of muscle soreness and quantitative sensory testing (QST), as well as metrics that account for participants' level of sedentarism and intervention intensity, 2 relevant variables for the evaluation of DOMS.[50,51]

Exploratory Characterization of the Temporal Response of Circulating Inflammatory Cytokines to a Short-Term Yoga Exercise Intervention (Aim 2)

We demonstrated that collecting and analyzing blood samples taken at multiple time points (7 in total per participant) for quantitative time-course descriptive analysis is feasible. However, in this pilot study, we observed a great deal of heterogeneity within and between groups, and over time. We speculate that a number of factors may have contributed to this variability. First, the lack of an adequate physical activity washout period between participants' variably active travel mode (e.g., time amount and intensity of walking required) to the study site and the first baseline blood sample likely contributed to variability across individuals at baseline. This factor also may explain why netAUC was more negative in the control group, as they experienced a longer period of sedentary activity. This bias may have masked other influences of the yoga intervention cytokine dynamics.[9,35] Second, because of our small sample size and chance, randomization with respect to biological factors known to impact systemic inflammation (e.g., BMI and age) was not balanced.[52] Of note, differences in BMI and age may have contributed to the lack of observed difference between the HY and MY groups. Third, it is possible that circadian variation also contributed to variability in cytokine levels. It is

well established that some cytokines have circadian cycles. Therefore, it is possible that baseline values varied between individuals due to their variability in their circadian cycles. Additionally, these circadian cycles (e.g., increases in the early morning) could have confounded the effect of exposure to yoga exercise on cytokines.[6,53]

Few yoga studies have evaluated the impact of short-term (e.g., single bout) interventions on systemic levels of cytokines for comparison with this study. Three studies measured changes in systemic levels of different inflammatory cytokines (e.g., IL-6, IL-10, and TNF-α). The first 1 compared Bikram (hot) yoga exercise vs. room temperature (RT) yoga exercise among yoga experts. Only the hot yoga group experienced a significant increase in IL-6 after the intervention.[5] The second study exposed healthy yoga female practitioners to 3 conditions during 3 separate visits (restorative yoga, movement control, and passive-video control) and measured changes in levels of IL-6 and TNF-α before and after each intervention. Findings indicated increases for both cytokines post-intervention.[6] Another yoga-related study measured, after a single bout of yogic breathing, levels of inflammatory cytokines in saliva at baseline and 5 timepoint post-intervention (0-, 5-, 10-, 15-, and 20-minutes). Their results show an overall reduction of IL-1β, IL-8, and MCP-1 in the yogic breathing group compared to the control group.7 2 main differences between our study and the first 2 yoga exercise trials are: (1) differences in study design (i.e., cross-over vs. parallel); and (2) previous exposure to yoga exercise (i.e., yoga-naïve vs. yoga experts or beginners). With respect to the third yoga breathing study: (1) they used saliva instead of serum to quantify cytokines levels;[54,55] (2) their intervention targeted the respiratory system, whereas our protocol used yoga's physical postures without any systematic breathing exercise technique; and (3) the intervention's duration was different (20- vs. 60-minutes). Future short-term yoga studies should more systematically explore biological interactions between yoga postures and breathing exercises on systemic levels of inflammatory mediators.

While there are few studies of the impact of a single bout of yoga on circulating inflammatory mediators, it is broadly known that a single bout of more conventional exercise (e.g., endurance, resistance, and sprint training) stimulates systemic changes in cytokine levels during physical activity and recovery among healthy individuals, and this immune response reflects the magnitude of physiological stress (e.g., intensity) experienced by the exerciser.[35,56-60] Other research on mechanical forces targeting the myofascial system has also observed changes in the dynamics of circulating cytokines (often referred to as myokines).[9]

In contrast to short-term studies, there are more robust studies on the effects of long-term yoga practice on inflammation. A systematic review summarized results from 15 studies assessing the long-term effects of yoga on inflammatory markers among healthy and/or disease individuals. Results showed no agreement on the effect of yoga on levels of C-reactive protein (CRP), TNF-α, and IL-6.[61]

Our pilot data reveal a number of trends/patterns worthy of further investigation in fully powered studies. Although serum values of individual cytokines varied considerably within and between groups over a 24-hour period, exploratory analyses revealed trends toward group-by-time effects for IFN-α2, IL-6, and IL-33, suggesting that an acute bout of yoga exercise may impact circulating levels of cytokines. These findings reflect differences in temporal patterns between MY and CON for IFN-α2 and IL-33, while for IL-6, this interaction effect reflects differences between the 2 yoga groups.

To determine whether different cytokines show unique responses to yoga exercise, the first 6 timepoints were used to compute netAUC. Although, in all cases, the average netAUC was negative, a closer look at the 12 graphs reveals a subset of cytokines showing unique trends of responsiveness to the yoga exercise intervention (IFN-γ, TNF-α, IL-8, IL-10, IL-12p70, IL-17A, and IL-33). Specifically, these cytokines exhibited a lower rate of decline in both yoga exercise groups compared to the control group. Of note, 6 of these cytokines have been reported to be important myokines involved in metabolic, inflammatory, and regenerative processes occurring in the muscle microenvironment through the co-dependent cross-talk between resident and migrant immune cells during and after exercise.[11,12,39,62] IL-12p70, a potent driver of Th1 immune responses, has relevant roles in fibrosis and tissue regeneration and warrants further investigation regarding its potential functions on myofascial and yoga physiology.[63] Our findings also suggest a difference between the yoga exercise and control groups for IL-33, a crucial cytokine released by fibro-adipogenic progenitor cells (FAP) to enhance the recruitment of Treg cells to help in the regeneration process while contributing to the extracellular matrix production needed to support myofascial repair.[64]

Changes in cytokines after a bout of yoga exercise were positively correlated, suggesting that a single bout of yoga exercise may cause coordinated and inter-dependent post-interventional dynamic changes across different cytokines.[10,59,65,66] IL-6 and IL-33, 2 important cytokines with medium to strong positive correlations between these 7 myofascial-associated cytokines, indicate that understanding their dynamic changes, both individually and in relation to other cytokines, will provide granular insights into the temporal, local, and systemic dynamics associated with physical adaptation of the myofascial system through inflammation, tissue remodeling and regeneration processes during and after yoga practice.[11,12,39,62]

Neither netAUC nor correlation matrices analyses reveal a difference in the response of systemic levels of cytokines to 2 different intensities of yoga exercise (high vs. moderate). This finding differs from studies in the sports medicine literature, where the intensity of single bouts of different exercise modalities led to differences in circulating levels of cytokines. As noted above, heterogeneity caused by multiple factors may have obscured detection of yoga intensity effects. This parameter of yoga should be systematically explored in future larger trials.[35,56-60]

The Relevance of Short-Term Yoga Exercise Studies for Understanding the Physiology of Yoga

How yoga promotes preventive or therapeutic effects during health and disease remains incompletely understood. Likely, the multimodal nature of yoga (i.e., breathing exercises, active body movements, and meditation),[67] and the multifactorial etiological nature of these disease syndromes challenge our understanding of the physiological networks triggered during yoga practice.[68-72] Our study design purposely emphasized the physical component of yoga (i.e., body postures) among healthy and yoga-naïve individuals in order to begin understanding the response of the myofascial system to an acute physical challenge expressed through changes in systemic levels of inflammatory mediators (i.e., bottom-up effects).[27,30,62,73,74] Overall, we postulate that these inflammatory mediators might reflect how yoga practice stresses the myofascial system, allowing the recruitment of circulating immune cells towards myofascial stressed locations, along with activation of resident cells to start mechanisms of tissue remodeling, regeneration, and pro-resolution.[75] All these beneficial effects are thought to be modulated by the same inflammatory pathways involved in acute/chronic inflammation and pain.[76] In other words, a controlled increase of inflammatory mediators after an acute bout of exercise would be an allostatic mechanism to allow the body to adapt to a subsequent physical challenge with potential beneficial effects in the longer term. The widespread belief that only reductions in inflammatory mediators expression represent healthy change ignores the nuance of the complex role that physiological inflammation plays in maintaining homeostasis and dynamically adapting to an evolving environment. The context in which these increases or decreases occur is crucial. Indeed, recent exercise studies suggest that measurements of systemic biomarkers during and after short-term interventions (i.e., single bout of exercise) are fundamental to understanding training adaptation to exercise among healthy individuals.[58] Currently, the best example to elaborate on these complex dynamics is given by the classical pleiotropic inflammatory mediator IL-6. Basal levels of IL-6 have multiple origins (e.g., adipose tissue, liver, brain, muscle, and blood leukocytes). When secreted by the myofascial system after a bout of physical activity, it is considered a myokine/exerkine with potent anti-inflammatory effects. In contrast, it is regarded as a pro-inflammatory cytokine when secreted by other tissues, such as adipose tissue.[28] Moreover dynamic and sometimes opposite changes may occur over time, as with myofascial training adaptation (i.e., IL-6 increases in short peaks after each bout of exercise) vs. co-occurring long-term decrease of basal IL-6 levels produced by non-myofascial tissues. Unfortunately, these processes are not yet fully understood and warrant future research.[29] Thus, a longitudinal future study exploring both effects (short- and long-term) might hypothesize that the time-effect on basal levels of IL-6 is not linear. We acknowledge that while yoga-based exercise and conventional exercise may have important differences, they likely evoke similar physiological responses. Although not observed in this pilot study, the magnitude of the myofascial effects likely depends on the modulation of 3 parameters: intensity, duration, and frequency of the intervention.[77,78]

Limitations

Our pilot study has multiple limitations. First, the randomization process did not prevent imbalances due to the small sample size, which likely influenced the yoga exercise protocol's adherence, the HR data, and potentially systemic levels of cytokines. Other baseline characteristics, such as age, BMI, diet, and even the level of cognitive stress, anxiety, and depression due to the COVID-19 crisis, might have influenced the systemic level of cytokines.[79,80] Additional sources of heterogeneity include the insufficient physical activity washout period prior to the start of the study, the lack of appreciation for potential circadian rhythms in specific cytokines, top-down neuro-muscular influences, and the potentially confounding added element of mental engagement (e.g., higher levels of focused attention) which was not isolated from the physical component.[81] Second, pilot studies are not powered to formally evaluate differences between groups.[52,82] Hence, inferential findings should not be over-interpreted, and future fully-powered studies should be considered for these purposes. Third, although feasible in this study, multiple timepoint sampling imposes logistic challenges for nurses and lab technicians, and it may be a cumbersome procedure for some participants. Currently, non-invasive and painless electrochemical biosensors for automatic, wireless, and real-time glucose detection levels are available. Future yoga exercise studies might benefit from this technology for cytokine detection at multiple body sites.[83]

Conclusions

A fully-powered RCT for understanding the short-term effects of yoga exercise on systemic inflammatory mediators is both feasible and warranted.

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Author Contributions

DM-V: Conceptualization (supporting), data curation (lead), statistical analysis (equal), funding acquisition (supporting), investigation (lead), methodology (equal), project administration (lead), software (equal), visualization (lead), writing (lead), and review & editing (equal). KS: Methodology (equal), supervision (supporting), and review & editing (equal). HL: Conceptualization (lead), supervision (supporting), and review & editing (equal). GY:

Supervision (supporting) and review & editing (equal). YZ: Visualization (supporting) and review & editing (equal). PR: Statistical analysis (equal) and software (equal). PW: Conceptualization (supporting), funding acquisition (lead), methodology (equal), resources (lead), supervision (lead), writing (supporting), and writing & editing (equal).

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Supplemental Material

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