INTERNATIONAL JOURNAL OF -EXERCISE SCIENCE-

Original Research

The Effects of Combined Fasting and Exercise on Inflammatory Cytokine Concentrations in Healthy Adults: A Randomized Crossover Study

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Abstract

International Journal of Exercise Science 18(6): 1252-1268, 2025. https://doi.org/10.70252/APHT9483 The object was to assess the effects of adding exercise to a 36-hour fast on the inflammatory cytokines IL-6, TNF-α, and MCP-1. The study was a randomized crossover design with counterbalanced conditions in community members living near a single academic institution. Participants included twenty healthy adults (11 male). The intervention consisted of two 36-h water-only fasts, one of which was initiated with a bout of treadmill exercise. Venous blood was taken at baseline, 12-h, 24-h, and 36-h of fasting. Area under the curve and timepoint analyses were computed. The area under the curve for MCP-1 was 210.4 ± 61.4 pg/ml higher in the fasting combined with exercise condition compared to fasting alone ($F = 4.69$, $p = 0.04$). No difference between conditions was observed in areas under the curve for IL-6 ($F = 0.02$, $p = 0.88$) or TNF-α ($F = 3.74$, $p = 0.06$). MCP-1 concentrations decreased over the course of both conditions ($F=19.77$, $p < 0.01$) with much of the reduction taking place between hours 12 and 24 ($F=19.77$, $p < 0.01$). Concentrations of IL-6 remained unchanged ($F = 0.85$, $p = 0.48$) while TNF-α increased ($F=8.60$, $p < 0.05$) in both conditions. A single fast has a mixed impact on the cytokines MCP-1, TNF-α and IL-6. MCP-1 decreases, while TNF-α fluctuates in a diurnal pattern, and IL-6 experiences no change during a water-only fast. Adding exercise to the beginning of a fast diminishes the decline in MCP-1 but has no impact on TNF-α or IL-6.

Keywords: Cytokines, intermittent fasting, behavioral intervention, physical activity

Introduction

With caloric intake, the body experiences metabolic strain that induces endocrine and immunological responses to process food and restore homeostasis.[1] Due to modern eating patterns and food availability, most people spend more than 16 hours per day in a fed state which can lead to chronic postprandial-inflammation.[2] While inflammation is an essential response of our body's defense mechanisms and recovery, a chronic state of systemic low grade-

inflammation has been linked to obesity, type 2 diabetes, cardiovascular disease, cancer, and other debilitating ailments.[3,4]

The inflammatory processes in the body are mediated, in part, by cytokines. Cytokines are released from nearly every cell in the body and encompass a broad array of proteins involved in cell-signaling pathways that reduce or induce inflammation through enhancing immune function. Proinflammatory cytokines include Interleukin 6 (IL-6), Tumor Necrosis Factor alpha (TNF-α), Monocyte Chemoattractant Protein-1 (MCP-1), and a host of others.[5] Behavioral strategies that reduce proinflammatory cytokines may help minimize inflammation and reduce the incidence of chronic illnesses. Among such strategies, both fasting and exercise have been independently proposed as effective means of lowering inflammation.[6]

The opposite is true of many foods found in the modern Western diet, some of which appear to specifically induce inflammation.[7] It follows that fasting provides respite from these changes and reduces the associated inflammatory cytokines.[8] A reduction of inflammatory cytokines has been observed when subjects are maintained on a fasting regimen for several weeks.[9] Additionally, acute fasting (defined here as a single bout of complete caloric restriction) has demonstrated a reduction in circulating monocyte levels, including CD14+ and CD16+, after 22 hours of water-only fasting[10], a beneficial response as pro-inflammatory cells are linked to chronic inflammation and increase risk of cardiometabolic disease.[11]

In addition to fasting, exercise interventions have been effective in lowering inflammation. Regular long-term patterns of physical activity have been linked to a systemic reduction of oxidative stress and inflammation including reduced levels of IL-6, TNF-α, and MCP-1.[9,12] Immediately following an acute bout of exercise, IL-6 concentrations increase which inhibits an endotoxin-induced increase in plasma TNF-α.[13] While exercise may cause damage to contracting skeletal muscles and increase oxidative stress, Dimitrov, et al. suggests that increased catecholamine levels due to sympathetic nervous system activation in exercise may also inhibit the production of TNF-α.[14] Similar suppressive effects of exercise have been noted in MCP-1 concentrations after 3 months of consistent exercise when performed 3 days per week[12], but acute bouts of exercise may not have similar effects. Although multi-week intermittent-fasting regimens yield mixed effects on inflammation, several studies indicate that acute fasting can rapidly influence inflammatory biology. A single 24-h fast reduces basal cytokine expression in adipose tissue and plasma (e.g., IL-1β, IL-6, TNF-α) in preclinical models.[15] In humans, short-term fasting decreases the number and inflammatory activation state of circulating monocytes, with refeeding inducing a transient rebound.[10,16] Observational and interventional work during Ramadan, an ecologically valid time-restricted fasting model, has reported reductions in IL-6, TNF-α, and CRP in many cohorts.[17] These data provide a mechanistic and translational basis to test whether an acute fasting bout of 36 hours alters circulating inflammatory markers in our target population and if an initial bout of exercise alters these outcomes.

Multiple studies have evaluated the effects of fasting and exercise on inflammation independently, often using fasting protocols such as time restricted eating, intermittent fasting, or caloric restriction.[18,19] These fasting protocols still involve some degree of caloric intake, which may explain the variability in outcomes. However, there is a paucity of work specifically

evaluating the combined effects of acute water-only fasting and exercise on inflammatory responses. The purpose of this secondary analysis was to evaluate the effects of combining exercise and fasting on the inflammatory cytokines TNF-α, IL-6, and MCP-1. These cytokines were measured in a 36-h water-only fast and compared with those of a 36-h fast initiated with a bout of treadmill exercise. Water-only fasting was selected to eliminate the confounding metabolic effects of nutrient intake and to isolate the physiological consequences of complete energy deprivation on immune signaling. We hypothesized that an acute fast would begin to dampen proinflammatory markers and that adding a component of exercise would amplify these changes.

Methods

Because this is a secondary analysis, the methods for this study have been published previously.[20] A summary of the methods is provided for context. A randomized crossover design with counterbalanced treatment conditions was used to compare the influence of fasting alone to fasting combined with vigorous exercise on the production of pro-inflammatory cytokines. These two conditions included a 36-h water-only fast beginning at 8:00 PM and ending at 8:00 AM, 36-h later.[21] Approval from the university's institutional review board was obtained before initiating any aspect of this study (IRB2019-319). This research was carried out fully in accordance with the ethical standards of the *International Journal of Exercise Science*.[22]

Participants completed two treatment conditions on identical days of the week, with a minimum 6-d washout between each session. Condition order was randomly assigned to participant numbers by the primary investigator before the study began.[23] Before each laboratory session, participants were screened for contraindications to participation as outlined hereinafter. The outcome variables measured were body mass index (BMI), percent body fat, fat mass, and plasma IL-6, TNF-α, and MCP-1 concentrations.

Participants

Thirty-one potential participants were screened for the study. Eleven were disqualified for reasons outlined in Figure 1. The remaining 20 individuals (11 male and 9 female) were randomly allocated to condition order. The demographic characteristics of the participants are outlined in Table 1.

All participants were capable of participating in vigorous physical activity without restrictions as measured by a Physical Activity Readiness Questionnaire (PAR-Q).[24] Any "yes" response on the PAR-Q excluded the participants.

Potential participants were excluded from the study if they:

- were diagnosed with a metabolic disease, inflammatory disease, orthopedic impairment, eating disorder or food allergy;
- were taking medications that altered metabolism;
- were taking anti-inflammatory medications;
- were consuming 60 mg or more of caffeine daily;
- were pregnant, lactating, or postmenopausal;

- did not have a BMI between 18.5 and <30 kg·m^{-2};
- were practicing ketogenic, carbohydrate, or calorie-restricted diets and/or
- were not over the age of 18 years old.

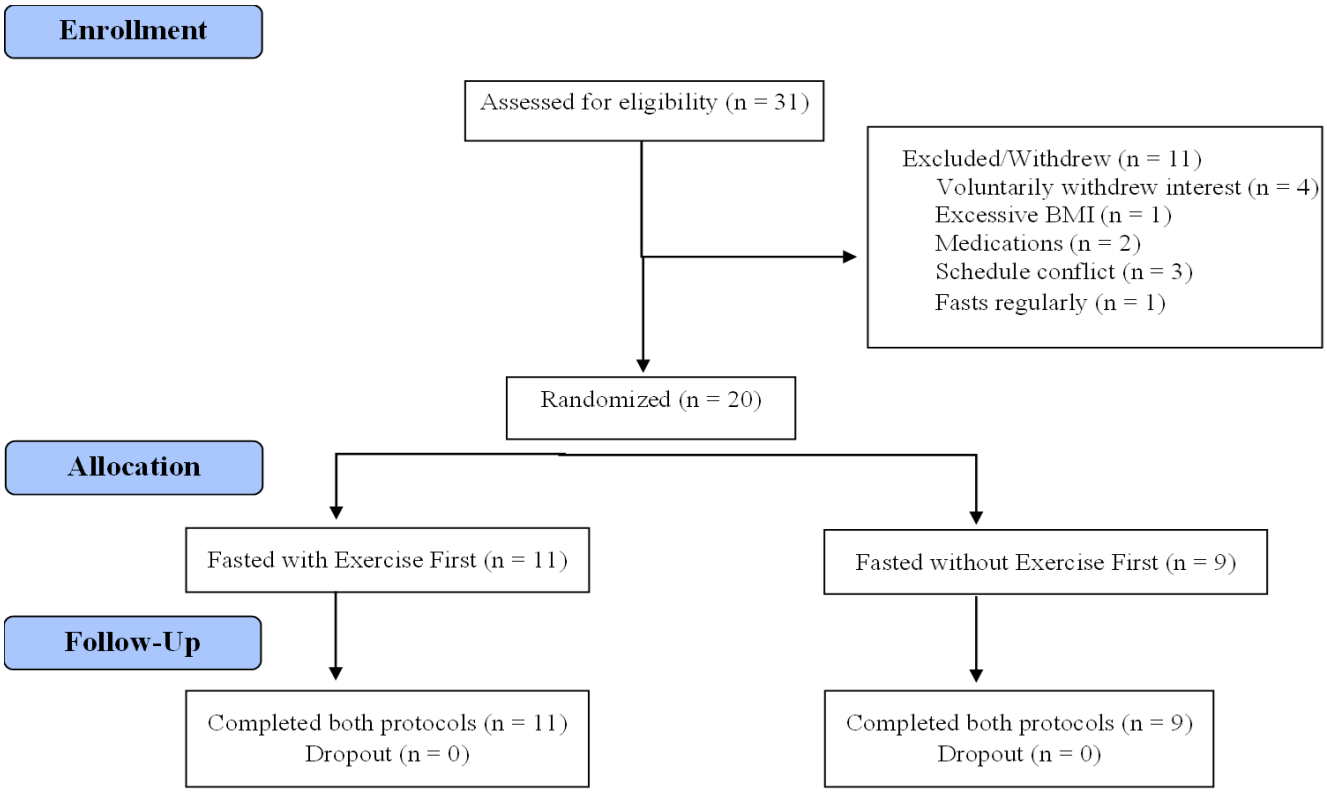

Figure 1. Participant flow diagram

Table 1. Demographic characteristics of participants

	Male (n=11)		**Female (n=9)**		**Cumulative (n=20)**	
	Mean	***SD***	***Mean***	***SD***	***Mean***	***SD***
Age (years)	26.5	7.1	25.8	4.3	26.2	5.8
BMI (kg/m^2)	24.7	3.1	22.7	3.6	23.8	3.4
Body Fat %	18.8	6.3	27.8	4.5	22.9	7.1
Visceral Adipose (g)	443.6	275.5	133.5	116.6	304	265.9
Exercise time (min)	49.3	5.9	54.7	7.8	51.7	7.2
Ethnicity	***n***	**%**	***n***	**%**	***n***	**%**
Asian	1	9	2	22	3	15
Caucasian	8	73	7	78	15	75
Hawaiian/Pacific Islander	2	18	0	0	2	10

Protocol

Measurements.

At the beginning of each session, body weight and height were assessed. Weight was obtained using a digital scale (Seca, Hamburg, Germany) with a precision of ±0.1 kg , while participants

wore athletic shorts and a T-shirt, standing barefoot. Height was measured by a stadiometer accurate to ±0.1 cm (Seca). Body composition, including fat mass, body fat percentage, and visceral adipose tissue, was evaluated with a GE iDXA scanner (GE, Fairfield, CT).[25] Each testing day began with calibration of the iDXA device using a calibration block supplied by the manufacturer. All scans were processed using Encore software version 17.

Inflammatory Cytokines.

Venipuncture occurred every 12-h (baseline, 12, 24, and 36-h) to collect two 4-mL EDTA tubes of blood. Each 4-mL tube was inverted to allow for mixture and both samples were centrifuged for 15 min at 1500*g* at 3°C within 10 min of collection. The plasma was then aliquoted and placed in separate vials and stored in a −80°F freezer until ready for analysis. IL-6, MCP-1, and TNF-*a* levels were quantified using standard 96-well microplate multiplex kits (MilliporeSigma, Catalog # HMHEMAG-34K). The manufacturer lists the percent coefficients variation (%CV) as follows: IL-1 intra-assay %CV <10% and inter-assay %CV <15%; MCP-1 intra-assay %CV <10% and inter-assay %CV <15%; and TNF-*a* intra-assay %CV <10% and inter-assay %CV <15%.

Orientation.

Prior to engaging in any part of the study, all participants provided informed consent and were thoroughly briefed on the study's overall objective and the procedures involved. They were instructed to maintain their usual daily routines and sleep habits throughout the testing period to minimize variability. Additionally, participants were asked to abstain from consuming caffeine or other stimulants on the day of testing and to avoid strenuous physical activity during the 24 hours leading up to their session. At the start of each visit, compliance with these guidelines was confirmed verbally by reiterating these instructions and asking the participant if they were compliant. To further verify consistency across testing days, baseline levels of insulin and glucagon were measured and showed no significant differences, indicating that participants began each session in a comparable metabolic condition.[20] Any deviations from the prescribed pretest protocols would have resulted in the session being rescheduled. No deviations were recorded during these pre-screenings.

Treatment sessions.

Participants were instructed to follow their typical eating habits in the days leading up to the fasting period but were required to refrain from consuming any food for four hours prior to the standardized meal. This approach was designed to prevent intentional overconsumption before fasting and to ensure consistency in baseline blood measurements.[26] At the beginning of the initial session, consent was obtained, and participants underwent orientation and received instructions. Also, within this initial visit, anthropometrics, demographic data, and a baseline blood sample were obtained. After completing these preliminary procedures, participants were provided with a standardized meal. The completion of this meal marked the beginning of a 36-hour water-only fast, during which no food or beverages—caloric or non-caloric—were permitted (with the exeption of water), including gum or mints. Follow-up blood samples were taken at 12, 24, and 36 hours into the fasting period. Each participant was randomly assigned to

begin with either an exercise or nonexercise condition. In the nonexercise condition, participants resumed their typical daily routines after consuming the standardized meal and completing measurements. In contrast, during the exercise condition, participants engaged in a prescribed workout session beginning 30 minutes post-meal, following the same sequence of measurements.[27]

Standardized meals.

Participants were given the same standardized meal to initiate each fast. The energy needs for each participant were estimated using height, weight, age, and sex to predict basal metabolic rates[28], and an activity factor of 1.55 was used to estimate total daily energy requirements.[29] An activity factor of 1.55 was used to represent a "moderate/low-active" energy requirement representing individuals who are sedentary at work or school but who undertake moderate physical activity 1-3 times per week. This level was verified for adults ages 18-64 by Black et al. as a general representation of healthy adults in affluent societies such as the sample tested in this study.[30] Meals were standardized based on macronutrient content consisting of 60% CHO, 25% fat, 15% protein and consisted of combinations of raw almonds, string cheese, crackers, apple slices, beef jerky, and commercially packaged peanut butter and jelly sandwiches. Meals were assembled using both commercially available prepackaged foods and commercially available boxed foods that were weighed to match caloric and macronutrient requirements of the participant. Participants were given 25% (basal metabolic rate 1.55 0.25) of their daily caloric requirements in the standardized meal. The same foods were given on both test days, and participants were instructed to consume all the food provided for each meal with meal adherence being assessed in each session by direct observation. The standardized meal fed to participants before each fast was 614.8 ± 85.2 kcal.

Exercise protocol.

Participants exercised on a treadmill at a grade and speed that brought their estimated heart rate reserve (HRR) to 70%.[31] The grade and speed combinations were made based on the preference of the participant to increase heart rate (HR). Participants exercised in this manner until an equivalent number of calories was expended as given in the standardized meal in an attempt to create a calorie neutral start to the fast. The formula used to calculate the participant's target heart rate was as follows:[32]

$$\text{Target HR} = [\%\text{target intensity} \times (HR_{max} - HR_{rest})] + HR_{rest} \quad [1]$$

Maximal HR estimation was calculated using the following formula:[33]

$$HR_{max} = 208 - (0.7 \times \text{age}) \quad [2]$$

Participants were fitted with a strap-on heart rate monitor (Garmin, Olathe, KS) and instructed to be seated for 5 min to establish resting HR (HR_{rest}). Once HR calculations were complete, subjects began the exercise. The speed and grade were adjusted to meet the target HR within the first 5 min of exercise. Once this speed and grade were set, they were not adjusted for the

remainder of the exercise intervention. Two participants were unable to maintain the exercise at this intensity, they were allowed to take a 60-s break, after which the exercise was resumed.

The length of exercise was individualized and calculated to expend a similar number of calories to that consumed from the standardized meal. This calculation was based on the standard American College of Sports Medicine–established metabolic calculation converting oxygen to calories by multiplying liters of oxygen by 5. The equations that were used include:

American College of Sports Medicine metabolic equation:

$$V \cdot O_2 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} = 0.2S + 0.9SG + 3.5 \quad [3]$$

Equations to estimate time on treadmill:

$$\text{Minutes of exercise} = (E/5 \times 1000)/(\text{kg} \times (0.2S + 0.9SG + 3.5) \quad [4]$$

where

E = calculated energy based on standardized meal in kilocalories

kg = weight of the participant in kilograms

S = speed of the treadmill in meters per minute

G = grade of the treadmill min = time on treadmill

All calculations were performed in a preset, protected spreadsheet to ensure accuracy. Indirect calorimetry was used continuously throughout the exercise to verify energy expenditure (COSMED, Rome, Italy). Measured energy expenditure during the exercise bout on the fast and exercise day averaged 587.6 ± 120.1 kcal. The average METs during the prescribed exercise were 9.14 ± 1.37. The average respiratory quotient (R) (measured by indirect calorimetry) throughout the prescribed exercise was 0.95 ± 0.14.

Statistical Analysis

Data were analyzed using SAS software version 9.4 for Windows. The sample size for this study was calculated a priori using an alpha of 0.05, and conservatively estimating a 10% difference in TNF-α concentrations between conditions. This provided a moderate effect size of 0.66. A sample of 20 participants was needed to yield 80% power.

Evaluation of the impact of fasting over time was performed using a general linear mixed model and follow up tests were conducted using differences of least square means. Analysis was performed between individual time segments of fasting intervention at which venipuncture was completed (0 vs 12-h, 12 vs 24-h, 24 vs 36-h).

Area under the curve (AUC) analysis was completed to compare cytokine concentrations between conditions. AUC estimations were calculated using trapezoidal sums with one

observation per subject by treatment with area under the curve as the dependent variable. The area under the curve was computed to represent a total response with a single number as a measure of intensity of the response and the magnitude of the change between conditions. Plasma cytokine concentrations were examined using repeated measures mixed model ANOVA. Where there was a significant interaction between time and a cytokine concentration between conditions, a post hoc least significant difference pairwise comparison was used to examine significant differences. Baseline values were used as a covariate. All results are presented as means ± SD. The data used to support the results of this study are available in the Open Science Framework repository.[34]

Results

Sex differences

There were no significant differences between sexes in their response to the intervention. Specifically, there was no significant sex by condition interaction for any measured cytokines (MCP-1; 208.96 ± 65.93 pg/ml, F=2.99, p = 0.1029; TNF-α; 15.76 ± 9.56 pg/ml, F=.15, p = 0.7031). Because no differences in sex were observed, men and women were then analyzed together.

Area under the curve between conditions

There was a significant difference between conditions for MCP-1 over time course of the fast. The difference in area under the curve for MCP-1 was 210.4 ± 61.4 pg/ml (F = 4.69, p = 0.0369) with MCP-1 having a greater area under the curve during the fasting intervention preceded by a bout of treadmill exercise (See Table 2). No significant difference was observed between areas under the curve for IL-6 (28.0 ± 182.4 pg/ml, F= 0.02, p = 0.8807) or TNF-α (16.1 ± 9.3 pg/ml, F= 3.02 , p = 0.1003).

Table 2. Area under the curve for inflammatory cytokines over a 36-hour fast started with or without exercise.

	Condition	AUC (pg/ml × hours)	F-value	P-value
MCP-1	No Exercise	2501.5 ± 141.0*	4.69	0.0369
	Exercise	2711.9 ± 140.9		
TNF-α	No Exercise	195.0 ± 5.01	3.74	0.0608
	Exercise	211.2 ± 5.02		
IL-6	No Exercise	8738.6 ± 116.8	0.02	0.8807
	Exercise	8766.7 ± 116.9		

MCP-1 = Monocyte chemoattractant protein-1, TNF- **α** = Tumor necrosis factor alpha, IL-6 = Interleukin 6. *Statistical difference between conditions for area under the curve. Results are reported as mean ± the standard deviation

Plasma MCP-1 concentrations saw a significant overall reduction over the course of the fast in both conditions (F=19.77, p < 0.0001; See Table 3). This reduction in MCP-1 took place primarily between hours 12 and 24 and then remained low between hours 24 and 36 (Figure 2). Plasma concentrations of IL-6 (Figure 4) remained similar over the course of the fast (F = 0.85, p = 0.4768). There was no difference between conditions observed at any time point (See Table 3). TNF-α increased slightly over the course of the non-exercise fast (F=8.60, p = 0.0463) but the change was not linear. Concentrations increased over the first 12 hours of the fast (F=8.60, p < 0.0001),

then decreased from hours 12 to 24 and then went up again from hours 24 to 36 (Figure 3). When comparing the values of TNF-α between conditions at the same time of the day, there were no differences with the exception that hour 36 was lower than hour 12 in the exercise condition (See Table 3).

Table 3. Time course changes in plasma concentration of inflammatory cytokines over a 36 hour fast started with or without exercise.

	Condition	0 hrs	12 hrs	24 hrs	36 hrs
MCP-1 (pg/ml)	No Exercise	77.24 ± 30.73[a]	76.50 ± 32.91[a]	63.37 ± 24.44[b]	57.39 ± 23.60[b]
	Exercise	75.64 ± 25.12[a]	88.19 ± 31.06[b]	68.85 ± 24.34[c]	67.85 ± 26.10[c]
TNF-α (pg/ml)	No Exercise	5.32 ± 8.75[ac]	6.07 ± 10.04[bc]	4.98 ± 7.08[ac]	5.74 ± 9.03[c]
	Exercise	5.24 ± 8.76[a]	6.39 ± 10.32[b]	5.57 ± 9.89[ac]	5.68 ± 9.37[c]
IL-6 (pg/ml)	No Exercise	243.67 ± 200.38[a]	246.25 ± 205.69[a]	240.14 ± 200.22[a]	241.83 ± 198.65[a]
	Exercise	239.54 ± 193.85[a]	240.39 ± 202.18[a]	238.76 ± 201.38[a]	244.56 ± 207.01[a]

MCP-1 = Monocyte chemoattractant protein-1, TNF- α = Tumor necrosis factor alpha, IL-6 = Interleukin 6. Within each row superscript letters that are different represent means that are statistically different from baseline in either condition ($p \leq 0.05$). Results are reported as mean ± the standard deviation

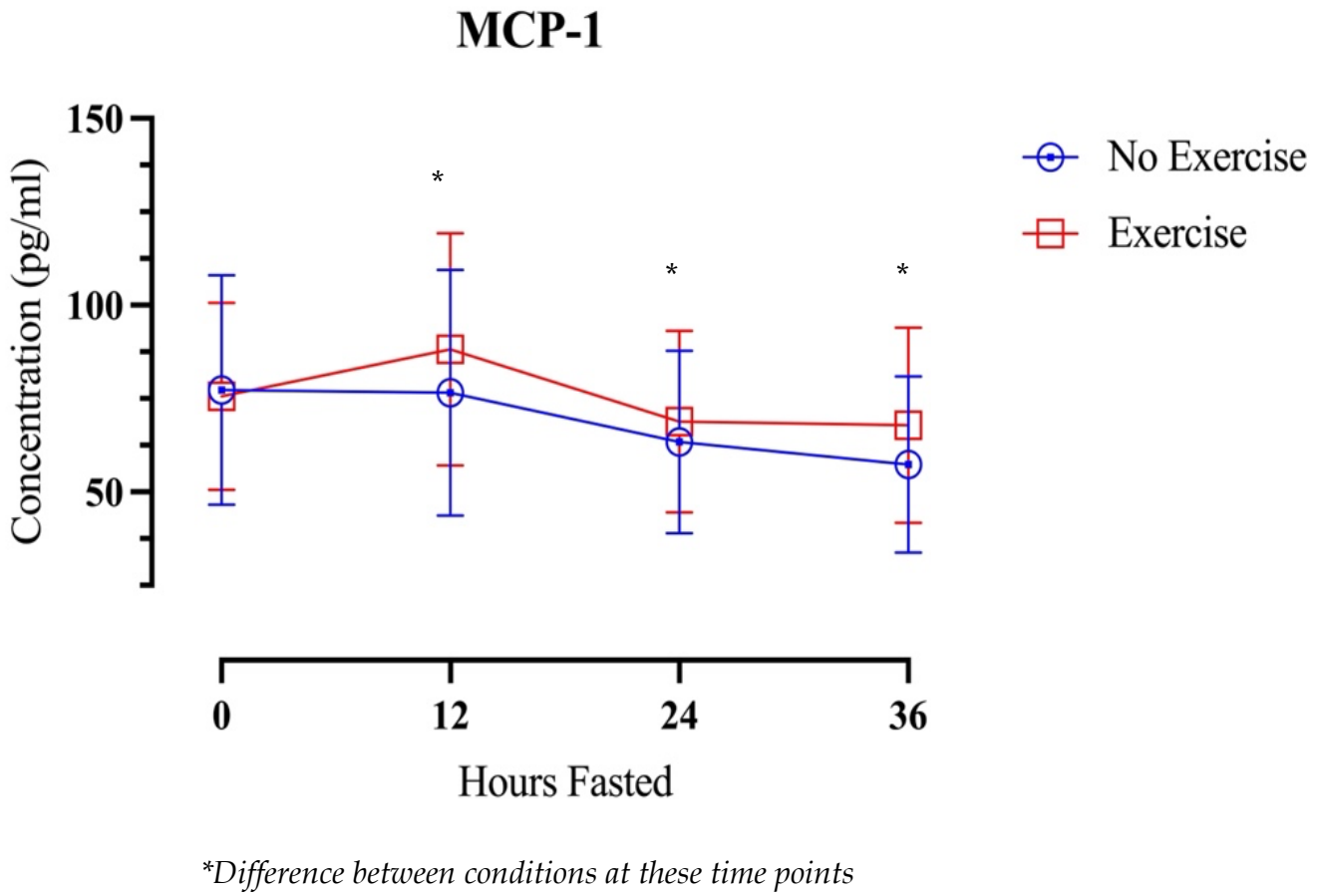

Figure 2. Changes in plasma concentration of MCP-1 over a 36 hour fast started with or without exercise.

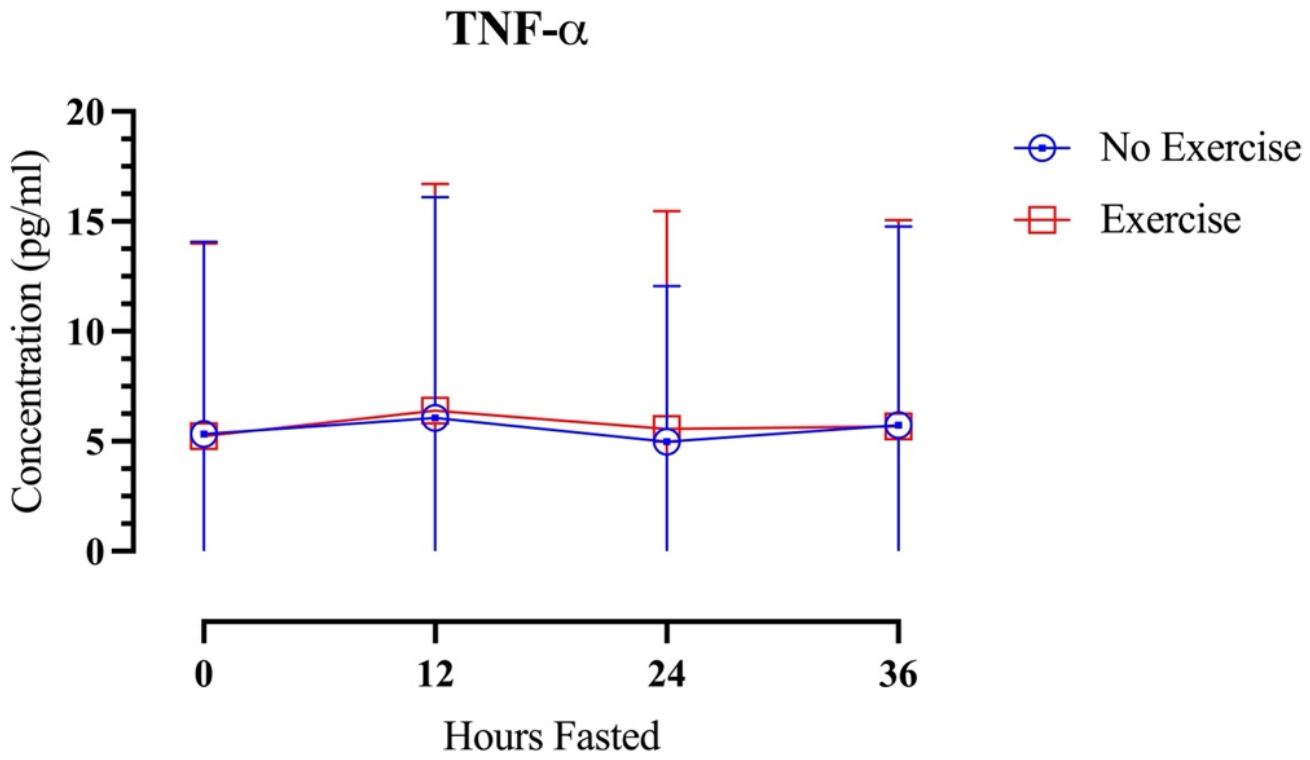

Figure 3. Changes in plasma concentration of TNF-α over a 36 hour fast started with or without exercise.

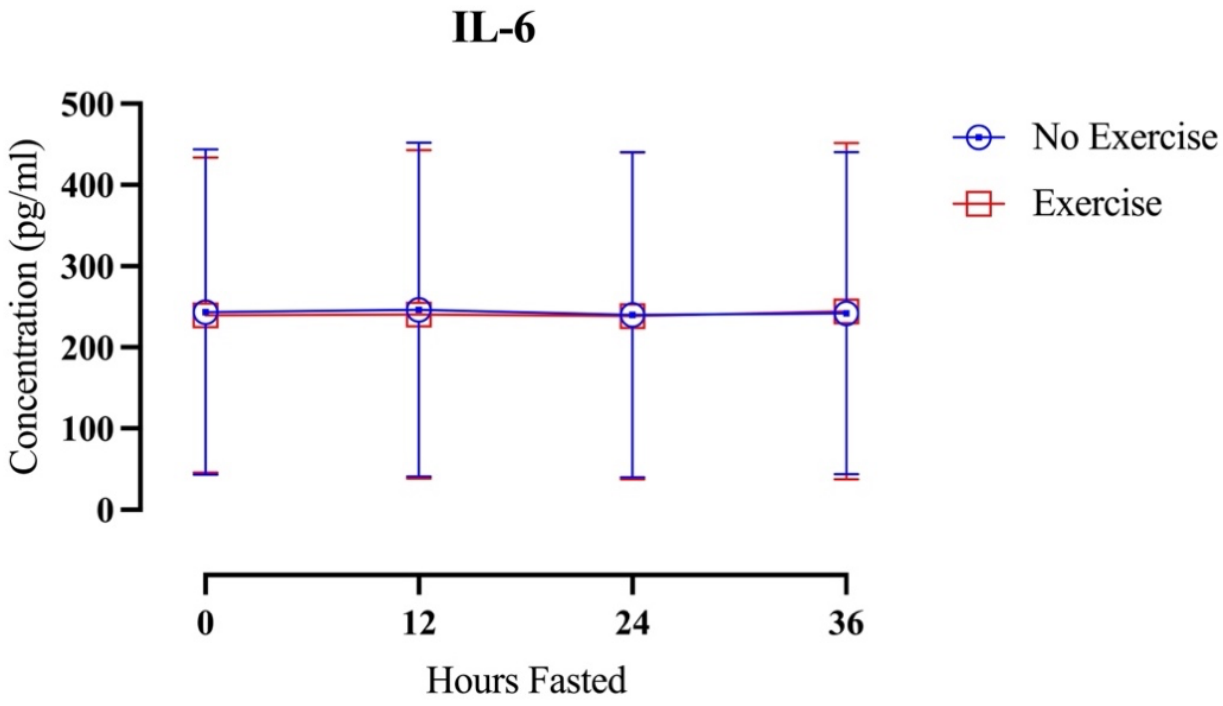

Figure 4. Changes in plasma concentration of IL-6 over a 36 hour fast started with or without exercise.

Discussion

This study aimed to assess the combined anti-inflammatory effects of exercise and acute fasting. Our hypothesis was that an acute fast would reduce proinflammatory cytokine concentrations, and the addition of a single bout of exercise at the beginning of the fast would enhance these effects. Contrary to our hypothesis, we found no significant differences in the overall effect (AUC) for IL-6 or TNF-α between conditions. While MCP-1 concentrations decreased in both conditions over the course of the fast, the magnitude of decline was reduced in the exercise condition. These results suggest that initiating an acute fast with exercise does not enhance the anti-inflammatory benefits of that fast, and may actually blunt any anti-inflammatory effects within this short window. It is also possible that long-term fasting and exercise interventions may be required to fully appreciate any synergistic effects.

Our results align with previous studies examining the impact of exercise on MCP-1. Based on a prior study, it appears that after a brief episode of exercise, MCP-1 temporarily increases.[35] This increase is thought to be a response to tissue injury, resulting in the recruitment of leukocytes to aid in an immune response.[35] Mooren et al. also observed this after an exercise session following a 36-hour fast (30-60 minutes of biking at 60% of VO2 max). In this study they observed an immediate increase in MCP-1 following the exercise, which persisted one hour after exercise compared to pre-exercise fasting concentrations.[36] Our study supports these findings by demonstrating an initial elevation of MCP-1 which persisted to 12 hours following the bout of exercise. Furthermore, our results revealed that the combined effect of exercise and fasting resulted in a diminished decline of MCP-1 values compared to fasting alone. This suggests that the anti-inflammatory effects of an acute fast were inhibited by an initial spike of MCP-1 following a bout of exercise. Other research has investigated long-term exercise routines, such as 12-week training programs, which have shown a decrease in MCP-1 levels in participants.[12] This suggests that while acute exercise increases MCP-1 concentrations, long-term interventions may ultimately lead to reduced MCP-1 and, consequently, decreased inflammation over time.

Unlike MCP-1, there was no difference between conditions for TNF-α or IL-6. These findings are consistent with a systematic review and meta-analysis examining the impact of intermittent fasting and energy-restricted diets on concentrations of TNF- α and IL-6 which found that neither dietary mode changed the concentrations of these markers.[8] Likewise, acute exercise bouts have shown little impact on concentrations of TNF-α and IL-6.[37] Specifically, one study demonstrated that TNF-α peaks around 2 hours after an exercise challenge and had a half-life of about 18 minutes.[38] Therefore, it is possible that any changes in TNF-α concentration resulting from exercise were transient and not observable by 12 hours. Additionally, TNF-α has been found to increase immediately after longer bouts of exercise, such as marathon running, but shorter bouts of exercise did not affect these concentrations.[39] Thus it is possible that the amount of exercise in our study was insufficient to alter TNF-α concentrations.

While there was no significant difference in TNF-α levels between the first and last blood draws, levels increased during the initial 12 hours and decreased by 24 hours in both conditions, though these fluctuations were not significantly different from baseline. The 12-h blood draw, conducted in the early morning, may explain the increased concentration due to the diurnal

effects of this pro-inflammatory cytokine. TNF-α plays a role in sleep regulation, and previous research suggests that pro-inflammatory markers are associated with sleep patterns, explaining the observed overnight increase.[40] Although these findings do not support our hypothesis, they align with prior research regarding diurnal effects of TNF-α.

Serum levels of IL-6 also did not differ between conditions. Similar to TNF-α, IL-6 levels have not been shown to decrease during acute fasting.[41] Although the literature lacks a precise definition of fasting, there is evidence suggesting that sustained adherence to fasting over time, as observed in practices like Ramadan fasting, may reduce concentrations of IL-6 and other proinflammatory cytokines in healthy adults.[42]

In contrast, one study observed that exercise acutely raised IL-6 concentrations by up to 100-fold during the exercise session itself.[43] This increase is attributed to the production of IL-6 within the muscles and reaches peak concentrations at the end of exercise or shortly after, subsequently returning to pre-exercise levels.[44] Due to this rapid rise and fall, any immediate changes in IL-6 concentrations may have been missed, given the intervals at which blood samples were collected in our study. While adherence to a long-term exercise regimen (12 or more months) has been shown to reduce IL-6 levels[45], acute fasting or a single bout of exercise seems to have a limited impact.

The participants in this study were generally healthy, young, active, and non-obese, with a mean BMI of 23.8 ± 3.4 kg/m². Research suggests that older, less healthy, and overweight populations are more susceptible to increased levels of inflammatory cytokines, including MCP-1, TNF-α, and IL-6.[42,43] Given that the participants in this study likely had lower baseline inflammation levels, it is possible that the reduced baseline expression of these markers contributed to the non-significant findings. While we did not observe significant differences between conditions in this group, it is noteworthy that even healthy individuals with relatively low inflammation levels can experience reductions in cytokines through behavioral changes.[46] Likewise, there was possibly insufficient statistical power to detect smaller effects in the inflammatory marker TNF-α in response to exercise where the p-value was slightly above the conventional threshold for significance. As a result, a conclusion that exercise has no impact on TNF-a should be interpreted with caution. Further studies with larger sample sizes may be needed to confirm this finding.

Additionally, the results of this study are also constrained by the interval at which blood samples were collected. While this interval was chosen a priori to observe how values changes over the course of a 36 hour fast, it limited our ability to see more acute changes that take place immediately after exercise. In addition, a significant portion of the trial was conducted under free-living conditions, introducing potential variability based on any differences in day-to-day schedule. However, it was deemed impractical to keep the participants in the laboratory for 36 hours. Other cofounders that may have influenced the results but were not recorded include general nutritional habits (especially those within 24 hours of the intervention), habitual sleep patterns, and habitual physical activity patterns including non-exercise physical activity. Nevertheless, participants reported to the laboratory every 12 hours, where they were reminded of the study protocols, specifically not engaging in strenuous activity during fasting periods.

Another factor for consideration is that we used a single activity factor of 1.55 to calculate the standardized experimental meal. Choosing a single, population-typical activity factor increases internal consistency in the meal stimulus across experimental conditions; however, it does assume that participants' habitual physical activity levels approximate the moderate/"low-active" reference. An activity factor error of ±0.35 (e.g., 1.2 vs 1.55 or 1.55 vs 1.9) corresponds to an absolute total energy expenditure change of ~400–600 kcal/day and thus could change the meal by ~100-150 kcal. We acknowledge this limitation and suggest that future work incorporate measured resting metabolic rate and individualized physical activity estimates to further reduce uncertainty in per-participant caloric dosing.

Furthermore, differences between menstrual cycle phases were not accounted for by this study. Some studies have reported differences in cytokine levels between the follicular and luteal phases[47] both at rest and after exercise while others indicate no differences.[48] However, our study found no difference in cytokine levels between men and women, potentially indicating no change in cytokine levels between menstrual phases, though the number of women included may have been underpowered to observe any differences. Despite conflicting results between studies, future research should account for menstrual cycle phase when examining the impact of fasting and/or exercise on inflammatory markers in women.

This study makes a valuable contribution to the literature by exploring the effects of fasting alone and fasting combined with an initial bout of strenuous exercise on inflammatory markers over a 36-hour period. This allows for a more controlled look at the impact of exercise on inflammation and aids in establishing the time course of the changes brought about by exercise. The results suggests that any pro- or anti-inflammatory effects resulting from the intervention are likely short-lived apart from MCP-1, which lasted at least 12 hours. Additionally, participants refrained from strenuous physical activity 24 hours before and during the intervention, except during the controlled exercise condition, which minimized potential variability between conditions and participants. Finally, while various studies have separately assessed the impact of acute fasting and exercise on inflammation, this study stands out as one of the first attempts to consider both interventions simultaneously.

Although inflammation plays a crucial role in the body's immune defense and tissue repair, persistent low-grade inflammation has been associated with obesity, type 2 diabetes, cardiovascular disease, cancer, and other health disorders.[3,4] Reduced chronic low-grade inflammation has been independently associated with long-term adherence to exercise and fasting protocols. MCP-1, a pro-inflammatory cytokine, was significantly reduced over time in an acute 36-hour water-only fasting condition. This pattern was maintained, albeit to a lesser extent, when a vigorous bout of exercise was added to the beginning of a similar fast. Compared to fasting alone, adding vigorous exercise resulted in elevated MCP-1 for at least 12 hours after the exercise bout, while IL-6 and TNF-α levels remained unchanged between conditions.

Further research could explore how both fasting and exercise influence inflammation in individuals with higher basal levels of inflammation, since changes in these cytokines may be more pronounced in these individuals. Additionally, future research may benefit from exploring different inflammatory cytokines that were not included in this study, such as C-reactive protein

and other inflammatory markers, such as innate immune cells.[48] Addressing chronic low-grade inflammation has the potential to alleviate a variety of noncommunicable chronic diseases.

Acknowledgements

We would like to thank the research assistants who helped collect data and analyze plasma samples for this study.

Research reported in this publication was supported by a mentoring environment grant from Brigham Young University.

Research reported in this publication was supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under the Award Number TL1TR002368. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

The data used to support the findings of this study have been deposited in the Open Science Framework repository. DOI 10.17605/OSF.IO/VM7BA

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