Avicenna Journal of Phytomedicine (AJP)

Original Research Article

A preliminary pilot study of the effect of oatmeal and oxygen–ozone (O_2-O_3) administrations on NRF2 pathway-related antioxidants after intense exercise

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Article history:
Received: Oct 14, 2024
Received in revised form: Jan 23, 2025
Accepted: May 24, 2025
Epub ahead of print

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Keywords:
Antioxidants
Intense exercise
Nuclear factor erythroid 2–related factor-2 (NRF2)
Oatmeal
Oxygen–ozone
White blood cells

Abstract

Objective: This preliminary pilot study aimed to evaluate how oatmeal and oxygen-ozone (O_2-O_3) administrations after intense exercise affect the NRF2 pathway, a key regulator of antioxidant responses.

Materials and Methods: A single-blind, repeated-measures design was conducted with ten participants (ages 20-36, body mass index<30 kg/m²) who completed high-intensity interval exercise (HIIE) protocols under four conditions: baseline, following O_2-O_3 sauna sessions, after oatmeal supplementation, and post-HIIE. Blood glucose, lactate, white blood cell count (WBC), and levels of NRF2 pathway-related antioxidants, body weight, and blood pressure were measured under four conditions.

Results: Significant fluctuations over time were observed in cardiovascular responses ($p<0.05$). Notably, cardiovascular responses following HIIE after supplementation differed compared to ozone sessions. Lactate, platelets, lymphocytes, and WBC increased significantly, while neutrophils decreased ($p<0.05$). glucose and most NRF2-related antioxidants remained stable; however, a gender-specific increase in superoxide dismutase (SOD) was found ($p>0.05$). Women showed a greater WBC reduction after oatmeal versus O_2-O_3 administration ($p>0.05$).

Conclusion: The study demonstrated that oatmeal and O_2-O_3 administrations may significantly affect cardiovascular responses and immune cell dynamics post-exercise, with gender-specific effects. The SOD increase among women and the greater reduction in WBC after oatmeal probably suggest its potential in enhancing antioxidant defenses and reducing inflammation. These findings probably underscore the role of dietary interventions in managing oxidative stress following intense exercise.

Please cite this paper as:
Abbasian S, Mortezae Z, Kargar Moghadam M, Moghavemi H, Ameri Afzadi H, Ghasemzadeh Rahbardar M, Sardar M.A. A preliminary pilot study of the effect of oatmeal and oxygen–ozone (O_2-O_3) administrations on NRF2 pathway-related antioxidants after intense exercise. Avicenna J Phytomed, 2025. Epub ahead of print.

Introduction

Free radicals, especially reactive oxygen species (ROS), are produced during cellular activities (Pizzino et al. 2017). Due to their high reactivity and unpaired electrons, ROS can damage proteins, deoxyribonucleic acid (DNA), lipids, and cell membranes (Juan et al. 2021). Exercise training significantly increases oxygen supply to muscles, potentially raising ROS production and harming essential biological components (Vickers 2017). However, the body has a strong antioxidant system to combat oxidative damage from ROS (Pizzino et al. 2017; Powers et al. 2022).

Studies show that nuclear factor erythroid 2–related factor-2 (NRF2), is crucial for alleviating oxidative stress in various tissues (Han et al. 2023; Rasmussen et al. 2023). Under oxidative stress, NRF2 is activated, translocates to the nucleus (Thiruvengadam et al. 2023) and suppresses inflammation and the nuclear factor kappa B (NF-κB) signaling pathway by activating heme oxygenase-1 (HO-1) (Payandeh et al. 2020).

Scientific research has highlighted the enhanced antioxidant activity of avenanthramide (AVA), a compound commonly found in oat (*Avena sativa*) (Ji et al. 2003; Koenig et al. 2014; Koenig et al. 2016; Zhang et al. 2020). The antioxidant capabilities of AVAs depend on α, β-unsaturated carbonyl and hydroxyl groups which act as ROS scavengers (Sang and Chu 2017). Previous studies have shown that oat supplementation can reduce ROS production, suppress NF-κB activation, and enhance antioxidant capacity in response to acute exercise (Koenig et al. 2016). Zeng et al., (2020) demonstrated that oatmeal supplementation approximately three hours before high-intensity interval exercise (HIIE) may alleviate ROS production (Zeng et al. 2020). However, prior studies have not examined the effects of prolonged oatmeal supplementation on NRF2-related antioxidants, and immune cell profiles. In this regard, we need to use oatmeal to check for changes in NRF2 pathway antioxidants. Since oatmeal is often studied in exercise, we can easily compare it to other aids like oxygen-ozone (O_2-O_3).

Recent research indicates that ozone (O_3), an inorganic substance with potent oxidant properties, can activate NRF2 within cells, leading to the transcription of various antioxidant response elements (AREs) in human cells (Sagai and Bocci 2011; Smith et al. 2017). However, no studies have compared the effects of oxygen–ozone (O_2-O_3) administration and dietary interventions on NRF2 pathway-mediated antioxidant responses (including superoxide dismutase (SOD), glutathione peroxidase (GPX), xanthine oxidase (XO), total antioxidant capacity (TAC), and HO-1) following intense exercise in both women and men.

Therefore, we aimed to determine how O2-O3 administration and oatmeal supplementation affect oxidant and antioxidant biofactors after an HIIE session. This preliminary pilot study evaluated stress-related responses to interventions by assessing metabolic, cardiovascular, antioxidant, and immune markers, including lactate, blood glucose, heart rate, blood pressure, NRF2-related antioxidants, and immune cell profiles.

Materials and Methods

Type of study

This preliminary pilot study employed a single-blind, repeated-measures design with two washout periods.

Participants

The study consisted of 14 young men (n = 5) and women (n = 5) who met the following criteria: no metabolic, orthopedic, or cardiovascular conditions; involvement in physical education classes no more than twice per week (ACSM 2013); ages 20-36 years; and a body mass index (BMI) between 20 and 30 kg/m². Additionally, four participants voluntarily withdrew for personal reasons.

Sample size

To determine the sample size for each gender ($\alpha = 0.05$, power = 0.8), changes in

SOD and GPX levels were considered, based on data from a previous study (Koenig et al. 2016).

Study design and blood sampling procedures

Before the initial assessments, participants rested for 30 min; the first blood sample was then collected to establish baseline oxidant and antioxidant biofactor levels (BASELINE, Figure 1). Trials began at 8 a.m. after a 12-hr overnight fast. Following clinical interview and baseline assessments, participants completed the HIIE protocol, and a second blood sample was taken immediately to assess the acute effects of HIIE on oxidant and antioxidant levels (HIIE, Figure 1).

After a seven-day washout period, participants underwent six consecutive days of oxygen–ozone (O_2-O_3) exposure in a sauna cabin (25 min per session). Immediately after the final session, they performed the HIIE protocol, and a third blood sample was collected to evaluate the impact of ozone on oxidant and antioxidant capacity (SAUNA, Figure 1).

Following another seven-day washout, participants consumed oatmeal daily for six days. On the sixth day, they rested for two hours after oatmeal consumption before completing the HIIE protocol. The fourth blood sample was taken immediately after training to assess the effect of pre-workout oatmeal supplementation on oxidant and antioxidant biofactors (OATMEAL, Figure 1).

For female participants, the study lasted four and a half weeks to account for menstrual cycle phases, ensuring that assessments were conducted at optimal times for accuracy (Schouwenberg et al. 2011).

Oxygen and ozone (O_2-O_3) administration

Participants were instructed to remove all clothing and enter a sauna cabin made of laminated plastic (Fiberglass, Pishro Sanat Bonyan Salamat Shargh, Iran), keeping their heads outside to avoid inhaling ozone (O3). A gas mixture of oxygen (O2) and O3 was circulated at a rate of 1 l/min using an Aerozone generator (SOPS100, Pishro Sanat Bonyan Salamat Shargh, Iran). A 2000 W heater (Pishro Sanat Bonyan Salamat Shargh, Iran) created steam, maintaining a temperature of 37-40°C. The heater was preheated for 5 min, achieving 39-40°C for 3 min and 36-37°C for 2 min. A thick towel and thin polythene sheet were placed around the participants' neck to prevent O3 leakage, despite the cabin door being sealed with O3-resistant material (Bocci et al. 1999). Each participant completed six consecutive ozone administration sessions over one week, with each session lasting approximately 60 min and sauna temperature maintained at 36-37°C. The ozone concentration increased from 50% in the first session to 60%, 70%, and 80% in subsequent sessions (Merhi et al. 2019). Additionally, participants' blood pressure, heart rate (HR), and weight were assessed before and after each session (Figure 1).

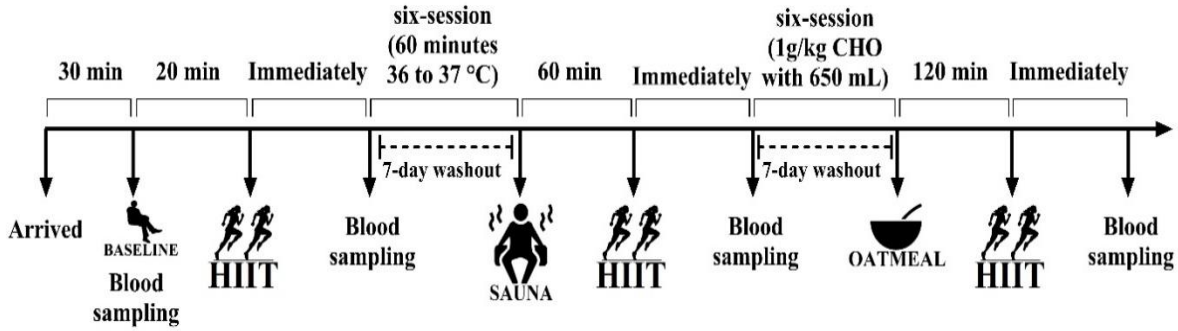

Figure 1. Flowchart illustrating participant progression through the study phases. HIIT: high-intensity interval training; CHO: carbohydrate.

Oatmeal administration

Following the ozone intervention, participants were instructed to include oatmeal in their daily meals for six days following a seven-day washout period. The oatmeal comprised oat flakes (© ABO Co., Tehran, Iran) and semi-skimmed milk (1.3% fat; © Alis Co., Mashhad, Iran), providing 1 g of carbohydrates per kg of body mass. Participants also consumed a standardized amount of fluid (650 ml of water and milk) with their oatmeal (Moore et al. 2009). The oatmeal contained 58.7 g of carbohydrate, 6.7 g of fat, 13.5 g of protein, and 10 g of fiber per 100 g of oats (OAB Available online: https://oabshop.ir/), while the milk provided 3.5 g of carbohydrates, 1.3 g of fat, 3.5 g of protein, and 0 g of fiber per 100 ml (Alis, available online: https://alis.ir/en/). On the sixth day of oatmeal consumption and following the HIIE protocol, the fourth blood sampling was done (Figure 1).

HIIE protocol

The primary objective of the HIIE sessions was to determine the maximum aerobic speed of untrained participants. Each participant completed a running session on a motorized treadmill (TF100, Turbo Fitness, Taiwan) at a speed of 8 km/hr after a 10-min warm-up. The maximum aerobic speed was established by increasing the treadmill speed by 1 km/hr every two minutes until the participant could no longer continue. The HIIE protocol was then set at 120% of this final speed (Dupont et al. 2004). Participants performed 15 intervals of HIIE, each lasting 30 sec, with 15 sec of recovery between intervals. The HIIE protocol was conducted before and after the ozone and oatmeal interventions (Figure 1).

Physiological and biochemical assessments

Body fat percentage (BFP) was measured using the U.S. Navy body composition method (Peterson 2015). Blood samples were collected from the median cubital vein, and white blood cell count (WBC) was assessed by a hematology analyzer (SYSMEX KX-21, Japan). Serum levels of NRF2 were measured using a commercial ELISA kit (#RK11523, Zellbio, Germany), along with SOD, GPX, XO, TAC, and HO-1 levels (Zellbio, Germany). Lactate levels were determined using a commercial ELISA kit with lactate oxidase (Boehringer Diagnostika, Germany). No significant changes in plasma volume were observed between pre- and post-exercise (Matomäki et al. 2018).

Statistical analysis

The results are presented as mean ± standard deviation (SD). Statistical analysis was conducted using STATA software, version 12 (StataCorp, USA). A two-tailed repeated measures ANOVA was performed, using pre-test values as covariates, to assess variations in oxidant and antioxidant levels after ozone and oatmeal administrations across genders and time, including time × gender interactions. Statistical significance was set at $p < 0.05$.

Results

Weight and cardiovascular variability

Table 1 presents the baseline laboratory assessment values for each gender among all participants.

Our findings indicate no significant difference in weight variability between men and women over time ($F = 1.692$, $p>0.05$). However, systolic blood pressure (SBP) showed significant increases and decreases over time ($F = 10.603$, $p<0.001$), with notable changes in both genders ($F = 1.815$, $p<0.05$). Similarly, diastolic blood pressure (DBP) and mean arterial pressure (MAP) exhibited significant fluctuations over time ($F = 1.749$, $p < 0.05$; $F = 4.227$, $p<0.001$, respectively) and significant gender differences ($F = 1.818$, $p<0.05$; $F = 1.731$, $p<0.05$, respectively). Moreover, HR demonstrated significant changes over time

(F = 53.456, p<0.001), and between genders (F = 6.985, p<0.001; see the supplementary material).

Post-hoc analysis revealed significant differences between various time points, including 2 min after oatmeal session-related HIIE compared to before the fourth, third, and first ozone sessions, 1 min after the oatmeal session-related HIIE compared to 1 min after ozone session-related HIIE, and immediately after the oatmeal session-related HIIE compared to 2 min after the ozone session-related HIIE. Besides, a significant difference was noted in men between immediately after the oatmeal session-related HIIE and before the third ozone session (p<0.05; see the supplementary material).

Overall, significant differences in DBP were observed before the fourth versus the third ozone session (p<0.05). Moreover, MAP significantly changed between 2-min after the oatmeal session-related HIIE and before the fourth ozone session, as well as immediately after the oatmeal session-related HIIE compared to 2-min after the ozone session-related HIIE and after the fourth ozone session (p<0.05).

Furthermore, significant differences were found between 2-min after the oatmeal session-related HIIE and several other time points, including before the third, fourth, fifth, and sixth ozone sessions, immediately after ozone session-related HIIE, 2-min after ozone session-related HIIE, before oatmeal session-related HIIE, and immediately after oatmeal session-related HIIE (p<0.05; see the supplementary material).

Table 1. Baseline laboratory assessment data for study participants

Variables	Overall (n =10)	Men (n =5)	Women (n =5)
Age (year)	31.00±4.59	30.6±4.92	31.4±4.77
Height (m)	1.71±0.089	1.77±0.06	1.64±0.04 *
Body mass (kg)	76.59±13.88	87.58±7.76	65.6±8.45 *
BMI (kg/m^2)	26.09±3.55	27.82±5.7	24.36±2.0
BFP (%)	26.88±7.8	20.82±5.7	32.94±3.76 *
SBP (mmHg)	114.7±15.29	125.6±6.3	103.8±13.75 *
DBP (mmHg)	75.00±10.6	83.4±3.64	66.6±7.95 *
MAP (mmHg)	88.1±12.02	97.3±4.3	66.6±7.95 *
HR (beat/min)	90.9±14.13	94.8±18.2	87.0±8.91
Glucose (mg/dl)	81.9±9.7	80.6±13.5	83.2±4.9
Lactate (mg/dl)	28.6±5.89	26±5.91	31.2±5.11
Platelets (×10^3/cum)	249±47	250±65	248±29
PCT (%)	0.24±0.04	0.23±0.05	0.25±0.03
MPV (fL)	9.94±0.95	9.7±1	10.1±0.87
PDW (%)	16.4±1.1	16.3±1.5	16.65±0.7
WBC (×10^3/cum)	7.9±2.48	9.66±1.73	6.14±1.76 *
Neutrophil (×10^3/cum)	58.2±7	61.3±7.2	55.1±5.8
Lymphocyte (×10^3/cum)	34.4±5.9	31.6±5.4	37.3±5.4
Monocyte (×10^3/cum)	5.21±1.25	5.28±1.7	5.14±0.69
Eosinophil (×10^3/cum)	1.72±0.74	1.4±0.86	2.04±0.49
Basophil (×10^3/cum)	0.34±0.12	0.34±0.09	0.34±0.16
NRF2 (ng/ml)	9.2±1.35	9.4±1.58	9.02±1.23
SOD (ng/ml)	17.69±7.3	20.4±9.11	14.9±4.5
GPX (U/ml)	129±88.5	95.4±68	164±100
XO (ng/ml)	45.3±16.1	48.3±23.6	42.4±2.7
HO-1 (pg/ml)	1.74±0.46	1.58±0.19	1.9±0.62

Results are mean ± standard deviation. *p<0.001 compared with the men. BFP: body fat percent; BMI: body mass index; DBP: diastolic blood pressure; GPX: glutathione peroxidase; HO-1: heme oxygenase-1; HR: heart rate; MAP: mean atrial pressure; MPV: mean platelet volume; NRF2: nuclear factor erythroid 2-related factor 2; PCT: procalcitonin; PDW: platelet distribution width; SBP: systolic blood pressure; SOD: superoxide dismutase; WBC: white blood cell; XO: xanthine oxidase.

Blood glucose, lactate, and WBCs

Our results indicated that glucose levels did not change over time (F = 0.85, p > 0.05). In contrast, lactate levels significantly increased over time (F = 34.51, p < 0.001), with a significant Factor × Gender interaction (F = 4.8, p < 0.01; Table 2).

Additionally, platelet levels rose over time ($F = 11.56$, $p < 0.001$), while neutrophil levels showed a significant decrease ($F = 9.11$, $p < 0.05$). Post-hoc analysis revealed that neutrophil levels were significantly lower following the sauna-related HIIE compared to baseline and the oatmeal-related HIIE due to ozone administration ($p = 0.011$, $p < 0.05$, respectively). Lymphocyte levels also significantly increased over time ($F = 9.43$, $p < 0.001$) with a significant Factor × Gender interaction ($F = 4.69$, $p < 0.01$).

Furthermore, WBC levels changed significantly over time ($F = 44.43$, $p < 0.001$). Post-hoc analysis showed that WBC levels increased in both women and men, but women experienced a more significant reduction in WBC levels following oatmeal administration compared to oxygen-ozone (O_2-O_3) administration ($p < 0.05$, 95% CI 0.19 – 3.17; Table 2).

NRF2 pathway-related antioxidants

This study found that NRF2 levels did not change over time ($F = 1.678$, $p > 0.05$); Figure 2). Moreover, SOD levels remained constant throughout the study period, although a significant interaction between factor × gender indicated a notable increase over time ($F = 3.86$, $p < 0.05$); Figure 4). Furthermore, there were no significant changes in GPX (Figure 3), XO (Figure 5), or HO-1 (Figure 6) levels over time ($F = 0.937$, $p > 0.05$); $F = 0.445$, $p > 0.05$); $F = 1.576$, $p > 0.05$); respectively).

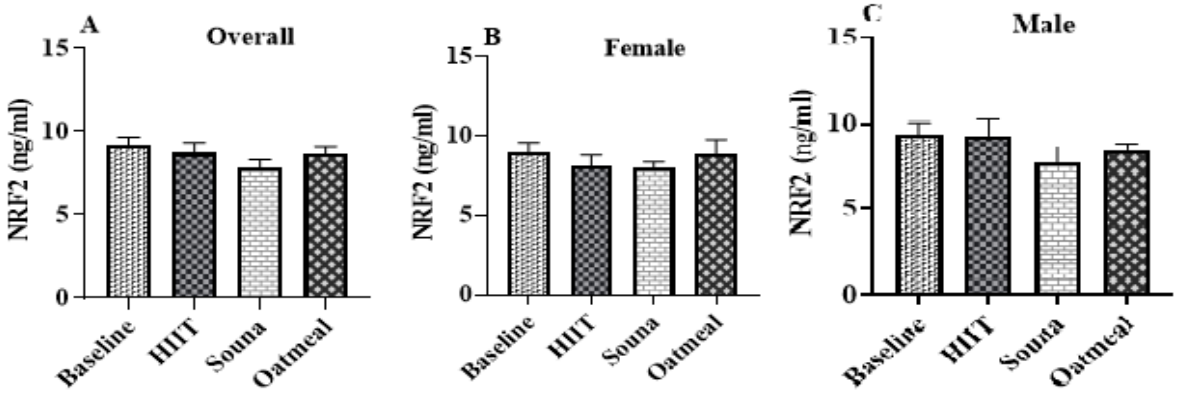

Figure 2. Levels of NRF2 in A: overall (n = 10), B: females (n = 5), and C: males (n = 5) during four session of the study, presented as mean ± SEM. There were no significant differences in overall levels of NRF2 (A), among females (B) and among males (C) following the study's interventions ($p > 0.05$). The X-axis represents the time points for blood sampling, including baseline, immediately HIIE, immediately after oxygen–ozone (O_2-O_3) session-related HIIE, and immediately after oatmeal session-related HIIE. HIIE: high-intensity interval exercise; NRF2: Nuclear factor erythroid 2-related factor 2.

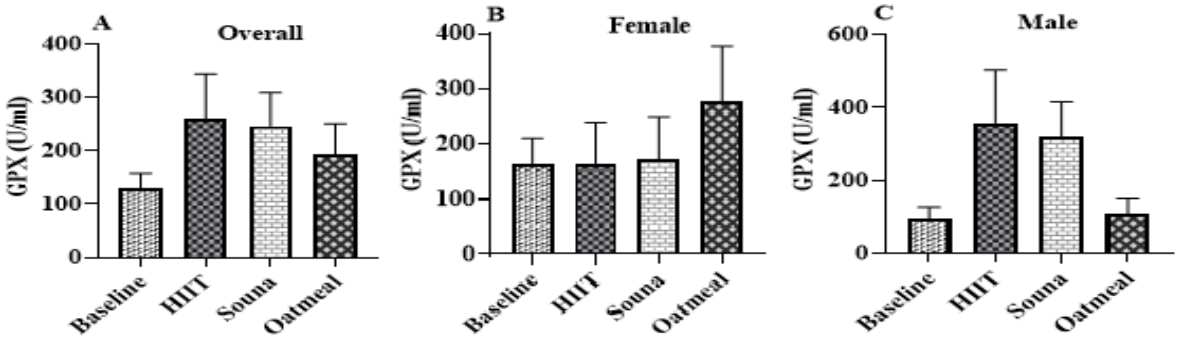

Figure 3. Levels of GPX in A: overall (n = 10), B: females (n = 5), and C: males (n = 5) during four session of the study, presented as mean ± SEM. There were no significant differences in overall levels of GPX (A), among females (B) and among males (C) following the study's interventions ($p > 0.05$). The X-axis represents the time points for blood sampling, including baseline, immediately HIIE, immediately after oxygen–ozone (O_2-O_3) session-related HIIE, and immediately after oatmeal session-related HIIE. GPX: glutathione peroxidase; HIIE: high-intensity interval exercise.

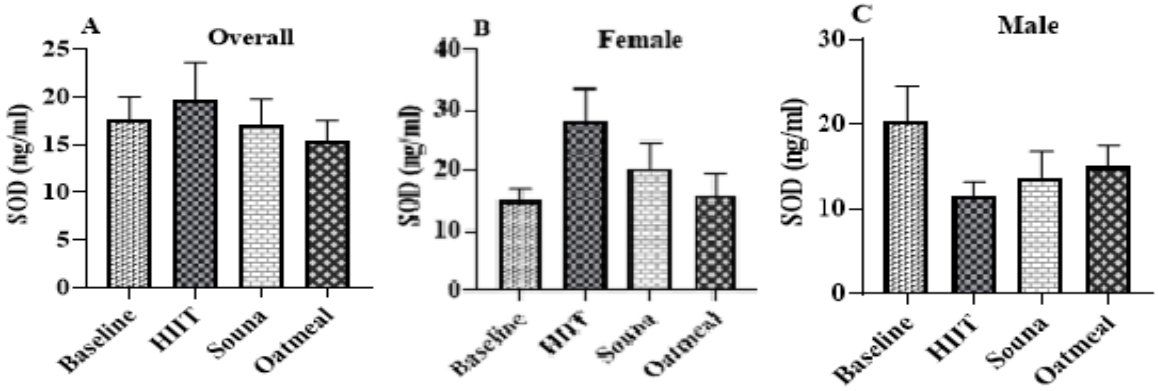

Figure 4. Levels of SOD in A: overall (n = 10), B: females (n = 5), and C: males (n = 5) during four session of the study, presented as mean ± SEM. There were no significant differences in overall levels of SOD (A), among females (B) and among males (C) following the study's interventions ($p > 0.05$). However, there was a significant Factor × Gender change in overall levels of SOD ($p = 0.022$). The X-axis represents the time points for blood sampling, including baseline, immediately HIIE, immediately after oxygen–ozone (O_2-O_3) session-related HIIE, and immediately after oatmeal session-related HIIE. HIIE: high-intensity interval exercise; SOD: superoxide dismutase.

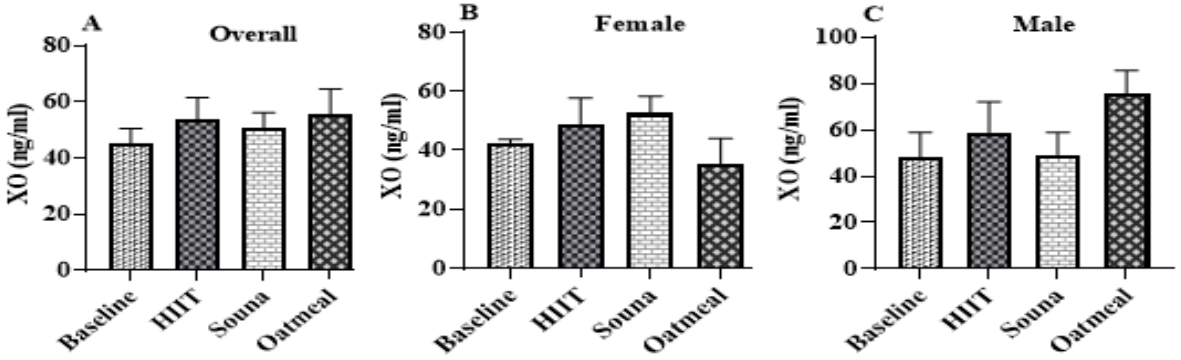

Figure 5. Levels of XO in A: overall (n = 10), B: females (n = 5), and C: males (n = 5) during four session of the study, presented as mean ± SEM. There were no significant differences in overall levels of XO (A), among females (B) and among males (C) following the study's interventions ($p > 0.05$). The X-axis represents the time points for blood sampling, including baseline, immediately HIIE, immediately after oxygen–ozone (O_2-O_3) session-related HIIE, and immediately after oatmeal session-related HIIE. HIIE: high-intensity interval exercise; XO: xanthine oxidase.

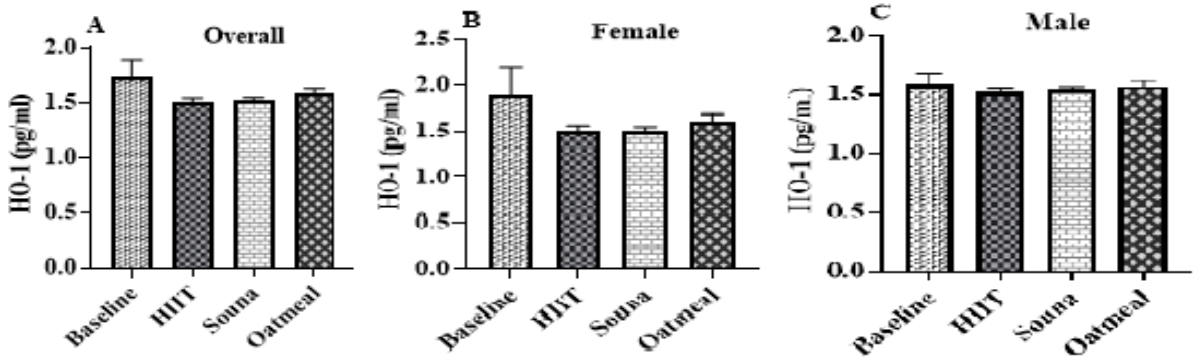

Figure 6. Levels of HO-1 **A:** overall (n = 10), **B:** females (n = 5), and **C:** males (n = 5) during four session of the study, presented as mean ± SEM. There were no significant differences in overall levels of HO-1 (A), among females (B) and among males (C) following the study's interventions ($p > 0.05$). The X-axis represents the time points for blood sampling, including baseline, immediately HIIE, immediately after oxygen–ozone (O_2-O_3) session-related HIIE, and immediately after oatmeal session-related HIIE. HIIE: high-intensity interval exercise; HO-1: heme oxygenase.

Table 2. Analysis of laboratory findings in study participants

Variable	Overall (n =10)				Men (n =5)				Women (n =5)			
	Baseline	**HIIE**	**Sauna**	**Oatmeal**	**Baseline**	**HIIE**	**Sauna**	**Oatmeal**	**Baseline**	**HIIE**	**Sauna**	**Oatmeal**
Glucose (mg/dl)	81.9±9.7	81.7±12	79.6±10	75.6±8	80.6±13.5	84.8±8	76±11	73.2±8	83.2±4.9	78.6±16	83.2±9	78±7
Lactate (mg/dl)	28.6±5.89	89±27*	81±27*	74.9±22*	26±5.91	104±21*	101±21*	90±19*	31.2±5.11	73±24	60±14	59±12
Platelets (×10^3/cum)	249±47	291±50*	268±42†	281±49	250±65	290±73*	258±58	274±70	248±29	292±21	279±16	287±24
PCT (%)	0.24±0.04	0.29±0.04*	0.28±0.03*	0.29±0.04*	0.23±0.05	0.28±0.05*	0.26±0.05	0.28±0.05	0.25±0.03	0.3±0.03	0.3±0.01	0.3±0.03
MPV (fl)	9.94±0.95	10±0.9	10.3±1	10.5±1*†	9.7±1	9.7±1.2	9.8±1.2	10.4±1.5	10.1±0.87	10.1±0.7	10.7±0.7	10.6±0.6
PDW (%)	16.4±1.1	15.8±2.1	16.7±2	15.9±1.8	16.3±1.5	15.5±2.5	15.4±2.2	15.4±2.1	16.65±0.7	16.1±1.9	17.4±2	16.5±1.5
WBC (×10^3/cum)	7.9±2.48	12.6±3.2*	12.9±2.6*	12±2.6*	9.66±1.73	14.4±2.5*	14±2.8*	13.8±2.1*	6.1±1.76	10.8±3*	11.9±2 *	10.2±1.7
Neutrophil (×10^3/cum)	58.2±7	52.4±6.5*	46.8±7*‡	51.8±7	61.3±7.2	52.9±8*	43.7±7	47.6±7.9	55.1±5.8	51.8±5	49.9±6	56±2.7
Lymphocyte(×10^3/cum)	34.4±5.9	38.75±5.8	44±6.8*	39.2±6.3 ±	31.6±5.4	38.1±7	46.4±7.2	42.4±7.3	37.3±5.4	39.3±5	41.6±6	36±3.2
Monocyte (×10^3/cum)	5.21±1.25	7±0.7*	6.9±0.9*	6.8±1.4*	5.28±1.7	7±0.9	7.5±0.8	7.5±1.7	5.14±0.69	7±0.5*	6.3±0.5*	6.12±0.9
Eosinophil (×10^3/cum)	1.72±0.74	1.42±0.6	1.85±0.7	1.9±1.2	1.4±0.86	1.34±0.7	2±0.8	2.3±1.4	2.04±0.49	1.5±0.5	1.7±0.5	1.5±0.9
Basophil (×10^3/cum)	0.34±0.12	0.38±0.1	0.33±0.1	0.24±0.09	0.34±0.09	0.48±0.1	0.32±0.1	0.2±0.07*	0.34±0.16	0.28±0.1	0.34±0.2	0.28±0.1

Note. Based on mean ± standard deviation. *p<0.001 compared with Baseline; †p<0.001 compared with HIIE; ±p<0.001 compared with Sauna; ‡p<0.001 compared with Oatmeal. MPV: mean platelet volume; PCT: procalcitonin; PDW: platelet distribution width; WBC: white blood cell.

Discussion

In this study, we evaluated the effects of oatmeal and oxygen–ozone (O_2-O_3) administrations on the NRF2 pathway and related antioxidants after intense exercise. Our results showed that NRF2 levels remained unchanged, but SOD levels significantly increased. Moreover, lactate, platelet, procalcitonin (PCT), lymphocyte, and WBC levels increased only in men, with SOD concentrations rose in women and decreased in men following HIIE. Although, neutrophil and platelet counts were reduced after ozone administration, oatmeal administration increased lactate, mean platelet volume (MPV), monocyte, and WBC levels in both genders. However, circulatory levels of lymphocytes, eosinophils, and basophils decreased after oatmeal consumption, while weight changes after oatmeal supplementation were not significant.

Recent studies have shown that high-intensity exercise can increase ROS production, placing additional stress on antioxidant capacity and triggering oxidative stress (Pingitore et al. 2015; Zeng et al. 2020). Thus, the primary aim of this study was to assess the impact of prior oxygen–ozone (O_2-O_3) and dietary interventions on the NRF2 pathway-mediated antioxidant biofactors (SOD, GPX, XO, TAC, and HO-1) following intense exercise in both women and men.

Previous research has shown that repeated oxidative stresses can activate the transcription factor NRF2 which triggers the AREs (Smith et al. 2017) to combat oxidative stress by activating endogenous radical scavengers and antioxidants (Inal et al. 2011; Sagai and Bocci 2011; Smith et al. 2017). Additionally, the NRF2 complex plays a crucial role in inflammation signaling pathways, with evidence suggesting that activating NRF2-dependent antioxidant signaling pathway may help to mitigate NF-κB-dependent inflammation and muscle loss (Buhrmann et al. 2011; de Sire et al. 2020; Li et al. 2008; Saha et al. 2020).

Recent findings suggest that administering large doses of drug-dependent antioxidants and anti-inflammatory substances to alleviate exercise-induced oxidative stress may be detrimental (Ji et al. 2016). Conversely, high-intensity exercise is known to cause excessive ROS production, leading to muscle damage (Owens et al. 2019). Our results showed an increase in SOD concentrations in women and a decrease in men following the HIIE intervention. SODs are key enzymes in the defense system that initiate the breakdown of superoxide anions (O2•−), serving as the primary defense against ROS. Additionally, research indicates that not only does a single session of endurance exercise enhances extracellular superoxide dismutase (EcSOD) gene activity, but exercise training also elevates EcSOD protein levels in mouse skeletal muscle (Zelko et al. 2002).

Furthermore, platelet, PCT, and WBC levels increased only in men following the HIIE intervention. Studies indicate that moderate-intensity exercise can reduce platelet activity, while strenuous exercise increases platelet activation, aggregation, and hyperreactivity (Kestin et al. 1993), thereby enhancing thrombotic tendencies (Cadroy et al. 2002). Strenuous exercise has been shown to cause platelet aggregation due to higher shear stress and the release of platelet agonists in plasma (El-Sayed et al. 2004). Additionally, intense physical activity elevates catecholamine secretion, with epinephrine and ADP working together to enhance platelet adhesion, aggregation, and bonding to fibrinogen (Barale et al. 2023; Blandini et al. 1995). Indeed, acute high-intensity exercise results in higher oxidative stress and lower antioxidant capacity compared to moderate exercise (Barale et al. 2023).

In this context, the literature suggests that ozone therapy induces the release of various biologically active compounds due to the moderate oxidative stress it generates (Bocci et al. 2005). Hydrogen peroxide

(H_2O_2), a ROS, is formed in plasma when ozone reacts with polyunsaturated fatty acids (PUFAs) and water. Concurrently, ozone produces lipid ozonation products (LOPs) such as hydroperoxides, 4-hydroxynonenal (4-HNE), isoprostanes, malondialdehyde, lipoperoxyl radicals, ozonides, and alkenals (Inal et al. 2011). This moderate oxidative stress enhances the activation of the transcription factor NRF2 which is crucial for initiating the transcription of AREs. By activating AREs, ozone-induced oxidative stress leads to an increase in the levels of antioxidant enzymes in the body (Inal et al. 2011). High-intensity exercise appears to elevate oxidative stress levels beyond the threshold necessary for the production of antioxidant enzymes (Sagai and Bocci 2011). Similarly, the six consecutive days of oxygen–ozone (O_2-O_3) administration did not positively influence the activation of antioxidant enzyme production. Our results conflict with other studies that did not investigate the effects of O_2-O_3 administration on HIIE-induced oxidative stress (Bocci et al. 1999; Merhi et al. 2019; Serra et al. 2023). Additionally, our findings confirm that oxygen–ozone (O_2-O_3) has no detrimental effects on body composition, the cardiovascular system, or metabolite levels (Bocci 1999; Bocci et al. 1999; Merhi et al. 2019; Sagai and Bocci 2011).

Our findings confirmed that six consecutive days of oxygen–ozone (O_2-O_3) treatment reduced exercise-induced platelet-neutrophil aggregates, potentially suppressing thrombus formation by platelet activation (Aldemir and Kiliç 2005). Furthermore, previous studies indicated that female soccer players experience elevated CXCL1/interleukin (IL)-8 levels in the bloodstream during exercise (Timmons et al. 2006). This elevation may result from increased blood flow and significant catecholamine release during intense short-term exercise (Sand et al. 2013), which we demonstrated through variations in heart rate, blood pressure, and lactate concentration (Breuer et al. 1993).

The results of this study indicated that weight changes following oatmeal supplementation were minimal. Thus, our findings suggest that oatmeal supplementation does not negatively impact body composition or cardiovascular function (Zeng et al. 2020). However, oatmeal administration increased lactate accumulation, MPV, monocytes, and WBC in both men and women after intense exercise. Interestingly, levels of lymphocytes, eosinophils, and basophils decreased following oatmeal administration. Oat supplements are known for their nutritional value, containing various AVAs and being rich in antioxidants such as tocotrienols, tocopherols, and flavonoids (Peterson 2001). The presence of AVA-related antioxidants, anti-inflammatory properties, and NF-κB inhibition in this supplement classifies it as an antioxidant and anti-inflammatory agent. Previous research has shown that AVA supplementation can enhance the body's natural antioxidant defenses, reduce NF-κB-DNA binding in WBCs, lower overall inflammation, and decrease muscle damage (Koenig et al. 2014). Additionally, various studies have emphasized the role of polyphenolic phytochemicals, particularly AVA, in boosting antioxidant capacity during exercise-induced ROS generation (Chang et al. 2010; Lafay et al. 2009; McAnulty et al. 2004). While few studies have assessed the impact of AVA supplementation on eccentric contractions (Koenig et al. 2016; Zhang et al. 2020), a recent study demonstrated that oatmeal supplementation prior to HIIE can reduce ROS production triggered by such exercise. However, the study focused solely on the short-term effects of oatmeal consumption before HIIE in women (Zeng et al. 2020). Previous research has shown that these nutrients can influence the immune system through various mechanisms, including the production of antimicrobial proteins and the enhancement of phagocytic activity, as well as the essential functions of neutrophils and

macrophages (Chen et al. 2021). For instance, oat-derived polysaccharides bind to specific membrane receptors on immune cells, particularly antigen-presenting cells (macrophages, dendritic cells, and B lymphocytes), leading to a reduction in inflammatory responses (Kopiasz et al. 2020).

Moreover, regarding the gender differences, evidence suggests that men may experience oxidative stress more frequently than women. This disparity may be linked to higher estrogen levels in women, lower levels of p47 required for enzyme assembly, and reduced superoxide production, even in the absence of estrogen (Allegra et al. 2023). Furthermore, studies indicate that men show greater platelet aggregation in response to thromboxane A2 compared to women (Gasecka et al. 2023). Besides, previous research has established that women possess stronger antioxidant and anti-inflammatory properties than men, attributed to their higher estrogen levels. Therefore, it is essential to consider sex differences in experimental studies related to ROS production, antioxidant efficacy, and aggregatory reactions (Allegra et al. 2023; Gasecka et al. 2023).

This study offers several practical advantages over previous research, including the use of HIIE to analyze the effects of oxygen–ozone (O_2-O_3) and oatmeal treatments on oxidant and antioxidant levels, evaluating both male and female participants, controlling variables to measure HIIE intensity and research interventions, and introducing novel biomarkers related to oxidant and antioxidant activity that have not been previously studied. Additionally, female participants were not measured during their menstrual cycles to avoid hormonal fluctuation-related variations (Kander et al. 2017).

However, the study has limitations, including a small sample size which reduces the ability to detect statistical differences and limits the generalizability of the findings. Moreover, since this investigation was a preliminary pilot study, more research is needed to confirm the findings.

In summary, our findings indicate that oxygen-ozone (O_2-O_3) therapy and oatmeal supplementation providing 1 g/kg body mass of carbohydrates may not negatively impact body composition, cardiovascular health, or metabolite accumulation. O_2-O_3 treatment probably resulted in a negligible decrease in NRF2 levels and lowered platelet counts in men, while oatmeal intake decreased lymphocytes, eosinophils, and basophils. Although it may appear that women exhibited increased SOD concentrations, it is important to note that men showed a decrease after HIIE. Additionally, men experienced elevations in platelet, PCT, and WBC levels post-HIIE, suggesting that strenuous exercise probably promotes platelet aggregation due to increased shear stress and the release of platelet agonists. Notably, neutrophil levels decreased only in men following HIIE. Overall, women probably demonstrated more pronounced anti-inflammatory and anti-aggregatory responses than men after intense exercise, though further research is necessary to confirm these observations.

Acknowledgment

We express our gratitude to all participants for their valuable contributions. We also extend our thanks to all the collaborating centers for their prompt response to our study requirements, along with the co-investigators for their support.

Conflicts of interest

The authors state that there is no financial or other conflict of interest.

Funding

This study was funded by the authors.

Ethics approval and consent to participate

All participants were fully informed of the study procedures and provided written prior to interview and data collection. The

study was conducted in accordance with internationally accepted ethical standards and was approved by the ethical and scientific committee of the authors' institution (No: IR.REC.1395007).

Authors' Contributions

SA and MAS: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. ZM, MKM, and HAA: Writing – review & editing, Visualization, Validation, Methodology, Formal analysis, Data curation. MGR: Writing – review & editing.

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Supplementary Table 1. Weight and cardiovascular variability between women (n = 5) and men (n = 5) over time

Variable	Weight (kg)			Systolic blood pressure (mmHg)			Diastolic blood pressure (mmHg)			Mean arterial pressure (mmHg)			Heart rate (beat/min)		
	Overall	**Men**	**Women**	**Overall**	**Men**	**Women**	**Overall**	**Men**	**Women**	**Overall**	**Men**	**Women**	**Overall**	**Men**	**Women**
Baseline	76±13	87±7	65±8	115±15	126±6	104±13	75±10	83±3	66±7	88±12	97±4	78±9	90±14	94±18	87±8
Immediately after HIIE	76±130	87±7	65±8	130±17	139±14	120±14	75±9	78±7	71±10	92±10	98±8	87±10	141±25†	160±11	122±21
1-min after HIIE	N/A	N/A	N/A	122±21	136±20	108±11	73±9	76±12	71±6	89±11	96±12	83±8	120±16***	133±3	104±12
2-min after HIIE	N/A	N/A	N/A	118±19	133±17	104±6	72±10	76±13	69±7	87±12	94±13	80±6	133±13***	123±3	104±12
Before first session of ozone	76±13	87±8	65±8	109±12	116±7	102±12	71±9	75±2	67±12	84±9	89±2	79±11	81±12***	79±17	82±7
After first session of ozone	76±13	87±8	65±8	118±13	124±8	111±15	73±9	78±6	68±10	87±10	93±6	82±11	81±12***	79±17	82±7
Before second session of ozone	76±13	87±7	65±8	114±16	116±15	111±19	71±8	72±2	71±12	85±9	86±3	84±14	83±12***	76±12	90±9
After second session of ozone	76±13	87±7	65±8	113±12	120±9	106±11	72±9	77±8	68±9	86±10	91±7	80±9	89±13***	93±16	86±9
Before third session of ozone	76±13	87±7	65±8	109±10	115±7	103±10	71±7	75±4	66±7	83±8	88±4	78±8	77±11***	69±9	84±7
After third session of ozone	76±13	86±7	65±8	116±10	120±7	113±11	71±6	75±3	67±7	86±6	90±2	82±7	91±10***	87±12	95±6
Before fourth session of ozone	76±13	86±7	65±8	104±11***	111±10	96±5	65±6***	69±5	60±5	77±7	83±6	72±5	81±10***	82±10	80±11
After fourth session of ozone	76±13	86±7	65±8	111±11	115±8	106±12	70±11	74±6	66±15	83±11	88±6	79±14	97±15***	105±12*	90±15
Before fifth session of ozone	76±13	86±8	65±8	115±16	118±14	111±19	74±10	78±8	70±11	87±11	91±9	83±13	76±10***	74±13	78±5
After fifth session of ozone	76±13	86±7	65±8	115±14	121±11	109±16	70±9	72±2	67±14	84±10	88±3	81±14	94±12***	96±13	93±13
Before sixth session of ozone	76±13	87±8	65±8	115±17	127±12	103±13	74±12	82±2	66±12	88±13	97±5	78±12	84±12	87±17	82±4
After sixth session of ozone	76±13	87±7	65±8	119±12	125±9	113±13	75±11	76±4	74±16	89±10	92±5	87±14	98±15	100±21	96±7
Immediately after ozone session-related HIIE	76±13	86±8	65±8	135±11***	135±7	134±15	71±13	66±9	76±16	92±11	89±7	95±15	143±20***	160±16*	127±4*
1-min after ozone session-related HIIE	N/A	N/A	N/A	128±10***	132±5	124±13	71±9	71±6	71±12	90±9	91±5	88±12	121±16***	135±7*	107±4*
2-min after ozone session-related HIIE	N/A	N/A	N/A	124±12***	131±7	118±13	70±10	71±3	70±10	88±8	91±3	85±11	133±11***	123±6*	103±4
Before oatmeal session-related HIIE	76±14	87±8	65±8	119±11	127±8	112±9	76±10	84±6	68±7	90±10	98±6	82±8	89±7***	91±6*	88±9*
Immediately after oatmeal session-related HIIE	76±14	87±8	65±9	140±16***	146±6	134±21	77±7	79±2	74±9	97±10***†	101±2*	93±13	145±21***	163±9*	127±11*
1-min after oatmeal session-related HIIE	N/A	N/A	N/A	130±15***	141±7	118±13	77±6	81±3	73±7	94±8	101±2	88±8	123±15***	137±2*	109±7*
2-min after oatmeal session-related HIIE	N/A	N/A	N/A	125±15***†	137±8*	113±9	75±6	78±4	71±6	91±8***	98±3	85±6	117±11***	126±3*	108±9*

Data are presented as mean ± standard deviation (SD). † Indicates significant overall differences between genders ($p<0.05$); *Indicates significant differences within each gender over time ($p<0.05$). ***Indicates significant differences within each gender over time ($p < 0.001$). Session definitions: Baseline: Measurements taken before any exercise or intervention. HIIE: performed immediately after the baseline measurement. Ozone Sessions: Measurements taken before and after each of the six ozone therapy sessions, which were conducted for 25 minutes each. Oatmeal Session-related HIIE: Measurements taken before and after the oatmeal consumption followed by HIIE. HIIE: High-Intensity Interval Exercise; N/A: Not Applicable.