ORIGINAL ARTICLE

# The effect of probiotic yoghurt consumption on oxidative stress and inflammatory factors in young females after exhaustive exercise

Mohammad Mazani,[1] Ali Nemati,[2] Abbas Naghizadeh Baghi,[3] Mojtaba Amani,[4] Kobra Haedari,[5] Reza Alipanah-Mogadam[6]

## Abstract

**Objective:** To evaluate the effect of probiotic yoghurt consumption on oxidative stress and inflammatory factors in young females after exhaustive exercise.

**Method:** This study included 27 healthy participants with an age range of 18-25. For two weeks, 450 grams of probiotic yoghurt and 450 grams of ordinary yoghurt were given to the supplement and control groups, respectively. Fasting blood samples were taken at baseline and at the end of study. At the end of the intervention, the participants were given one exhaustive exercise and then fasting blood samples were taken to test for blood antioxidant enzymes, inflammatory markers, and oxidative markers. Data were analyzed using descriptive statistics as well as paired and independent samples t-test.

**Results:** In supplement group, the glutathione peroxidise (GPX) blood levels and serum levels of total antioxidant capacity (TAC) significantly increased at the end of two weeks of intervention ($p<0.05$). After intense physical activity, the blood levels of superoxide dismutase (SOD), GPX and serum levels of TAC significantly increased, whereas the serum level of tumour necrosis factor alpha (TNF-?), matrix metalloproteinase 2 (MMP2), matrix metalloproteinase 9 (MMP9), and malondialdehyde (MDA) significantly decreased in the supplement group compared to the control group ($p<0.05$). Besides, there were no significant changes in other biochemical factors.

**Conclusion:** Regular probiotic yoghurt consumption significantly modulated MMP2, MMP9 and some inflammatory factors, and thus guarded against exhaustive exercise-inducing oxidative injury in young healthy females.

**Keywords:** Probiotic, Oxidative stress, Inflammation, Exhaustive exercise. (JPMA 68: 1748; 2018)

## Introduction

Probiotics are living microbial food supplements which beneficially affect the host body by improving its microbial balance.[1] It has been indicated that the intake of probiotic product supplements have potential health benefits for healthy persons and also in disease prevention.[2] Some probiotic strains exert antioxidant activity and may be useful in reducing systemic oxidative stress and also stimulating the immune system, reducing inflammation and preventing cytokine-induced oxidative stress properties.[3] Oxidative stress is considered an imbalance between oxidant and antioxidant levels and is believed to be related to several conditions such as heart disease,[4] rheumatoid arthritis,[5] hypertension,[6] Alzheimer's disease,[7] Parkinson's disease,[8] atherosclerosis[9] and aging.[10] Athletes with exhausted physical activity are often exposed to an excess of circulating reactive oxygen species (ROS). A single session of intense training is sufficient to produce significant amounts of ROS. Prolonged exercise increases oxygen uptake during the exhaustive activity, and ROS production results in oxidative stress.[11] During physical activity, oxygen consumption may rise up to 200 times in activating muscle fibres compared to resting time,[12] leading to production of superoxide[13] and inflammation induced by tissue damage. Probiotic supplementations could act as protective factors against oxidative damage in athletes to furnish an appropriate antioxidant barrier essential for preventing dangerous levels of oxidative stress.[14] A recent study in trained men suggests that probiotic supplementation could beneficially affect tumour necrosis factor alpha (TNF-$\alpha$) and exercise-induced protein oxidation.[15] Recently, clinical trials in non-pregnant women have revealed that the expenditure of probiotics can decrease oxidative stress.[16,17] However, these studies are limited and report conflicting findings.[18,19] The current study was planned to test the effect of probiotic yoghurt consumption in comparison to ordinary yogurt on oxidative stress and inflammatory factors in young women after an intense physical activity.

## Subjects and Method

This experimental study was conducted from 2013 to 2014 in Ardabil University of Medical Sciences, Ardabil, Iran. The proposal was approved by Medical Ethical

[1,2,4-6]Department of Clinical Biochemistry, School of Medicine, Ardabil University of Medical Sciences, [3]Department of Exercise Physiology, University of Mohaghegh Ardabili, Ardabil, Iran.

**Correspondence:** Ali Nemati. Email: nutrition1391@gmail.com

Committee of the Ardabil University of Medical Sciences (Ethical code: Arums.1018) and recorded in the Iranian Registry of Clinical Trials identification code of IRCT201307105144N4. Sample size was determined according to the following formula for each group: $n= [(Z_{1-\alpha/2}) + (Z_{1-\beta})] 2 \times (\delta_1^2 + \delta 22) :(\mu_2 - \mu_1)^2$ using PASS11 software.[20] Thirty healthy females were randomly allocated into two groups of the same size (control group = 15, supplement group= 15) at the beginning of study. Two participants from the control group and one from the supplement group were excluded due to non-cooperation and abuse of probiotic supplements. Finally, 27 healthy females with moderate physical activity aging from 18 to 25 years old were recruited and randomly divided into two groups [supplement (n = 14) and control (n = 13) groups] at the beginning of study. The aims of the current survey were explained to the participants and a written consent was signed prior to filling in a questionnaire. Subjects were prohibited from taking any supplements, anti-inflammatory drugs and severe exercise for 3 days before the start of the study.

Participants with certain diseases, non-athletes, smokers, alcohol and other supplement-consumers, certain diets, professional training, sedentary, body mass index (BMI) greater than 25, and less than 18.5 kg/m$^2$ were excluded.

After getting the consent, general information, anthropometric factors (height and weight) and fasting blood samples, two cups (450 grams) of probiotic yoghurt and the same amount of regular yogurt were given to the supplement and control groups on a daily basis for two weeks, respectively.Fasting blood samples were taken from participants at baseline and at the end of study by venepuncture and the sera were immediately separated. At the end of the intervention, the participants were given one exhaustive exercise according to Bruce test until fatigue and then after exhaustion the fasting blood samples were immediately taken. Bruce test was performed on both the groups. Bruce protocol is a maximal exercise test where the athlete works to complete exhaustion as the treadmill speed and incline is increased every three minutes[21] (Table-1). The length of time on the treadmill is the test score and can be used to estimate the VO2 max value. From the total run time, an estimate of the athlete's VO2 max can be calculated with Pollock's formula (VO2 max = 4.38 × T - 3.9).[22] Collected sera were kept frozen at -80ºC for further analysis.

**Table-1:** Incline and treadmill speeds in the Bruce test.

| Stage | Time (min) | km/hr | Incline |
|---|---|---|---|
| 1 | 0 | 2.74 | 10% |
| 2 | 3 | 4.02 | 12% |
| 3 | 6 | 5.47 | 14% |
| 4 | 9 | 6.76 | 16% |
| 5 | 12 | 8.05 | 18% |
| 6 | 15 | 8.85 | 20% |
| 7 | 18 | 9.65 | 22% |
| 8 | 21 | 10.46 | 24% |
| 9 | 24 | 11.26 | 26% |
| 10 | 27 | 12.07 | 28% |

## Measurement of biochemical factors

### Serum total antioxidant capacity (TAC)

Serum TAC was measured by colorimetric method using Randox Kit (Randox Laboratories Ltd., UK).[23]

### Malondialdehyde (MDA)

MDA levels were assessed using the thiobarbituric acid reactive substances (TBARS) method. Samples were heated with 0.6% thriobarbituric acid under an acidic condition. After cooling; the colored product was extracted into n-butanol. The pink colour absorbance was measured at 530 nm. MDA standards were prepared with 1, 1, 3, 3-tetraethoxypropane.[24]

### Glutathione

Total serum glutathione levels were assayed using Glutathione Assay Kit (Cayman Chemical Co., USA).

### Superoxide dismutase (SOD) and Glutathione peroxidase (GPX) activity

Erythrocyte antioxidant enzymes activity of superoxide dismutase (SOD) and glutathione peroxidase (GPX) were determined by spectrometric method using Ransod and Ransel kit, respectively. For the accuracy of assessment, duplicate assays were performed.

### Tumour necrosis factor alpha (TNF-α) and Interleukin 6 (IL6)

Commercial cytokine enzyme-linked immunosorbent kits for measurements of serum or plasma levels of circulating TNF-α and Interleukin 6 (IL6) were used (Bender Med Systems, Vienna, Austria) and analyses were performed according to the manufacturer's instructions. For the measurement of TNF-α, highly sensitive kits were used (measurement ranged 0.5 to 32 pg/mL and 0.125 to 8 pg/mL of serum, respectively). Levels of IL6 differed considerably across participants. Therefore, high-sensitivity kits (0.156 to 10 pg/mL) were used alternatively with standard-sensitivity kits (3.12 to 300 pg/mL), with all sera investigated in parallel on the same plate.

### High-sensitivity C-reactive Protein; hs-CRP

## Test (hs-CRP)

High-sensitivity C-reactive protein (hs-CRP) was measured using photometric method test and devices by AutoAnalyzer (Hitachi 911, Japan).

## Matrix metalloproteinase 2 and 9 (MMP2 and MMP9)

MMP-9 and MMP-2 enzymes linked immunosorbent assay (ELISA) kits were used (Bender Med Systems, Vienna, Austria). Serum samples were diluted 1:10 according to the manufacturer's specifications for MMP9 and MMP2 assay. For the accuracy of assessment, duplicate assays were performed. Quantification of immunoreactive MMP2 and MMP9 was carried out on 96-well Microtiter ELISA plates using standard protocols. The colour formation was measured at 450nm and 630nm on Anthos 2000 microplate reader and the sample concentration of MMP9 and MMP2 was estimated using MultiCalc program (Wallac, Turku, Finland).

## Dietary Method

The food intake was estimated for energy and other nutrients by 24-hour recall method for three days in a week during the study. Mean daily dietary intake and food composition were estimated using Nutritionist IV software.

## Data Analysis

In our study, after 2 weeks probiotic supplementation, exhaustive exercise was given in next stage, so according to the opinion of the statistical consultant, data was analysed using descriptive statistics as well as paired and independent samples t-test. Statistically significant level for all tests was considered as $p<0.05$.

## Results

There was no significant relationship among age, weight, calorie, and nutrients intake in control and supplement groups (Table-2). Although mean of VO2 max in the supplement group was more than control group (36.34±9.73 vs. 32.76±8.17 ml/kg/min), but this difference was not significant (p= 0.313). After intense physical activity, the blood levels of SOD, GPX and serum levels of TAC significantly increased, whereas the serum level of TNF-α, MMP2, MMP9, MDA significantly decreased in the supplement group compared to the control group ($p<0.05$). There were no significant differences between the two groups in the serum levels of other biochemical factors such as hs-CRP, IL6, and TNF-α after intense physical activity ($p>0.05$).

In the supplement group, GPX blood levels and serum levels of TAC significantly increased at the end of two-week intervention ($p<0.05$), but the increase in serum levels of High-density lipoprotein (HDL), SOD and reduction of MMP2, MMP9, MDA, hs-CRP, IL6, and TNF-α was not significant ($p>0.05$) (Tables-3-4). The data also supported that probiotic yoghurt consumption did not affect the serum lipid profiles significantly ($p>0.05$). Although the serum level of low-density lipoprotein

**Table-2:** The comparison of VO2 max, anthropometric factors and nutritional intake in control and supplement groups.

| Variables | Groups | | P value | Variables | Groups | | P value |
|---|---|---|---|---|---|---|---|
| | Supplement (N=14) M±SD | Control (N=13) M±SD | | | Supplement (N=14) M±SD | Control (N=13) M±SD | |
| Weight (kg) | 64 ± 9.49 | 59.8 ± 11.1 | 0.22 | V. B1 (mg) | 1.32 ± 0.23 | 1.22 ± 0.23 | 0.35 |
| BMI (kg/m$^2$) | 24.13 ± 3.57 | 22.3 ± 3.39 | 0.21 | V. B2 (mg) | 1.46 ± 0.52 | 1.64 ± 0.39 | 0.32 |
| Height (cm) | 162.93 ± 4.71 | 162.69 ± 3.94 | 0.88 | V. B3 (mg) | 21.65 ± 10.84 | 26.23 ± 13.69 | 0.33 |
| VO2 max (ml/kg/min) | 36.34 ± 9.73 | 32.76 ± 8.17 | 0.313 | V. B6 (mg) | 1.22 ± 0.39 | 1.37 ± 0.54 | 0.4 |
| Cal (Kcal/day) | 2222.6 ± 230 | 2273.2 ± 203.2 | 0.28 | V. B9 (µg) | 142.6 ± 70.86 | 152.71 ± 66.3 | 0.7 |
| Protein (g) | 82.15 ± 23 | 92 ± 23.4 | 0.52 | V. C (mg) | 64.51 ± 49.61 | 34.33 ± 30.85 | 0.07 |
| CHO (g) | 292.1 ± 34.2 | 283.5 ± 35 | 0.63 | Ca (mg) | 771.76 ± 200.33 | 909.81 ± 282 | 0.15 |
| Total fat (g) | 87.5 ± 16.3 | 90.2 ± 11.9 | 0.24 | Fe (mg) | 12.3 ± 1.98 | 11.95 ± 2.23 | 0.66 |
| SF (g) | 31 ± 4.7 | 34.24 ± 8.72 | 0.08 | Zn (mg) | 8.6 ± 1.42 | 9 ± 1.28 | 0.43 |
| PUFA (g) | 23.5 ± 8.1 | 17.8 ± 8.1 | 0.1 | Cu (mg) | 1.27 ± 0.28 | 1.32 ± 0.22 | 0.62 |
| MUFA (g) | 24.5 ± 4.78 | 27.1 ± 3..2 | 0.27 | Mg (mg) | 233.44 ± 56.48 | 234.58 ± 32.59 | 0.93 |
| Omega 3 (g) | 0.01 ± 0.01 | 0.02 ± 0.03 | 0.57 | Se (µg) | 0.06 ± 0.03 | 0.08 ± 0.04 | 0.18 |
| Oleic acid (g) | 11.7 ± 3.6 | 12.6 ± 7 | 0.07 | Biotin (µg) | 10.62 ± 9.38 | 10.24 ± 9.38 | 0.99 |
| Linoleic acid (g) | 21.6 ± 7.7 | 16.4 ± 7 | 0.55 | V. B12 (µg) | 2.55 ± 0.85 | 3.1 ± 0.92 | 0.11 |
| V. E (mg) | 13.58 ± 6.28 | 15.91 ± 10.95 | 0.25 | V.K (µg) | 101.56 ± 59.56 | 102.85 ± 79.3 | 0.62 |
| V.D (µg) | 1.26 ± 0.93 | 1.5 ± 1.27 | | | | | |

CHO = Carbohydrate, BMI = Body mass index, V = Vitamin, V.B9= Folic acid, Ca = Calcium, Mg = Magnesium, Cal = Calorie, MUFA = Mono unsaturated fat (g), PUFA = Poly unsaturated fat, SF= Saturated fat, Se = Selenium, Fe = Iron, Cu = Copper, Zn = Zinc.

**Table-3:** The comparison of antioxidant enzymes activities and lipid profiles before and after intense physical activity in both groups.

| Variables | Measurement stage | Supplement group (N=14) | Control group (N=13) | P value |
|---|---|---|---|---|
| Glutathione peroxidase (U/gHb) | Before | 45.2 ± 2.99 | 44.42 ± 4.45 | 0.59 |
| | After 2 weeks | 50.36 ± 6.11* | 45.3 ± 4.46 | 0.02◆ |
| | After exhausted exercise | 52.47 ± 6.38** | 47.21 ± 3.41 | 0.01◆ |
| Superoxide dismutase(U/gHb) | Before | 1480.9 ± 108.67 | 1556.9 ± 174.88 | 0.1 |
| | After 2 weeks | 1509.41 ± 210.21 | 1526.3 ± 158.65 | 0.1 |
| | After exhausted exercise | 1701.8 ± 146.25** | 1558.2 ± 163.14 | 0.03◆ |
| Total antioxidant capacity (mmol/l) | Before | 1.31 ± 0.13 | 1.25 ± 0.23 | 0.3 |
| | After 2 weeks | 1.54 ± 0.38* | 1.28 ± 0.25 | 0.04◆ |
| | After exhausted exercise | 1.72 ± 0.43** | 1.31 ± 0.25 | 0.006◆ |
| Cholesterol (mg/dl) | Before | 176.57 ± 31.66 | 165.38 ± 33.24 | 0.37 |
| | After 2 weeks | 183 ± 24.4 | 175.69 ± 26.86 | 0.46 |
| | After exhausted exercise | 187.64 ± 27.15 | 179.46 ± 30.3 | 0.46 |
| HDL- Cholesterol (mg/dl) | Before | 45.85 ± 10.42 | 41 ± 6.46 | 0.16 |
| | After 2 weeks | 49.85 ± 10.24 | 43.53 ± 7.27 | 0.07 |
| | After exhausted exercise | 51.42 ± 9.65 | 50.46 ± 6.43 | 0.76 |
| Triglyceride (mg/dl) | Before | 93.57 ± 60.63 | 90.76 ± 23.8 | 0.87 |
| | After 2 weeks | 98.78 ± 48.96 | 101.15 ± 34.87 | 0.88 |
| | After exhausted exercise | 103.79 ± 52.15 | 100 ± 31.24 | 0.83 |
| LDL(mg/dl) | Before | 112 ±23.6 | 106.15 ± 30.47 | 0.58 |
| | After 2 weeks | 113.39 ± 18 | 111.92 ± 24.6 | 0.86 |
| | After exhausted exercise | 128.74 ± 29.57** | 135.87 ± 25.67** | 0.5 |

Values are mean ±SD, *P <.05 vs baseline, **P<.05 vs after 2 weeks.
◆P <0.05 in Independent Sample T Test between two groups.

**Table-4:** The comparison of inflammatory and oxidant parameters before and after intense physical activity in both groups.

| Variables | Measurement stage | Supplement group (N=14) | Control group (N=13) | P value |
|---|---|---|---|---|
| MMP2 (pg/ml) | Before | 3948.5 ± 1126.71 | 4918.1 ± 2395.23 | 0.18 |
| | After 2 weeks | 3859.8 ± 1651.39 | 5037 ± 2414.57 | 0.04◆ |
| | After exhausted exercise | 3985.6 ± 1331 | 5614 ± 1981.95 | 0.01◆ |
| MMP9 (ng/ml) | Before | 19.46 ± 0.16 | 19.51 ± 0.21 | 0.53 |
| | After 2 weeks | 19.31 ± 0. 2 | 19.58 ± 0. 3 | 0.04◆ |
| | After exhausted exercise | 19.36 ± 0.2 | 20.19 ± 1.27 | 0.03◆ |
| TNF-α (pg/ml) | Before | 275.95 ± 207.99 | 261.33 ± 176.98 | 0.84 |
| | After 2 weeks | 221.96 ± 158.72 | 347.35 ± 202.17 | 0.08 |
| | After exhausted exercise | 285.57 ± 207.84** | 408.86 ± 257.71 | 0.18 |
| hs-CRP (mg/L) | Before | 1.22 ± 0.91 | 1.02 ± 0.94 | 0.58 |
| | After 2 weeks | 1.18 ± 0.98 | 1.12 ± 0.87 | 0.86 |
| | After exhausted exercise | 1.96 ± 1.24 | 1.57 ± 1.45 | 0.62 |
| IL-6 (pg/ml) | Before | 0.52 ± 0.29 | 0.6 ± 0.42 | 0.54 |
| | After 2 weeks | 0.47 ± 0.3 | 0.73 ± 0.65 | 0.16 |
| | After exhausted exercise | 0.59 ± 0.34 | 0.85 ± 0.4 | 0.07 |
| MDA(nmol/l) | Before | 1.87 ± 0.84 | 1.71 ± 0.65 | 0.6 |
| | After 2 weeks | 1.76 ± 0.66 | 1.93 ± 0.82 | 0.5 |
| | After exhausted exercise | 1.92 ± 0.66 | 2.49 ± 0.68 | 0.03◆ |

Values are mean ±SD, *P <.05 vs baseline, **P<.05 vs after 2 weeks.
◆P <.05 in Independent Sample T Test between two groups.
MMP2: Matrix metalloproteinase 2.
MMP9: Matrix metalloproteinase 9.
TNF-α: Tumour necrosis factor alpha.
Hs-CRP: High-sensitivity C-reactive protein.
IL6: Interleukin 6.
MDA: Malondialdehyde.

cholesterol (LDL-C) significantly increased after intense training in both groups ($p<0.05$), these changes were within normal range.

## Discussion

Our findings showed that the administration of 450g probiotic yoghurt to the supplement group for two weeks modulated the serum levels of TNF-$\alpha$, MMP2, MMP9, MDA, and lipid peroxidation consistent with the previous studies.[8,25,26] Several studies indicated that some strains of probiotics positively prevent and correct oxidative stress in humans through their direct antioxidative activity and modulate the immune system.[3,27,28] Probiotic yoghurt administration improved GPX and TAC at the end of supplementation and exhaustive exercise. The present study showed that probiotic yoghurt consumption significantly decreased some inflammatory factors such as MMP2 in accordance with an increase of antioxidants levels compared with regular yoghurt consumption. The present study reports the first evidence of improvement in some antioxidants levels in healthy females and the results are in line with the findings of another study.[29] However, no statistically significant changes were observed in MDA and enzymes of antioxidant activity in the supplement group compared to the control group during the intervention which might be explained by the short duration of the study. The precise mechanisms of antioxidant effects of probiotics remain largely unknown. These effects may be partly related to a probiotic-mediated decrease in oxidative stress. Moreover, the immune-modulatory and anti-inflammatory effects of probiotics and the modification of intestinal micro-flora could be other probable mechanisms. The MDA concentration insignificantly decreased after probiotic yoghurt administration. Although both the yoghurts (regular and probiotic) given to both groups had bioactive peptides and antioxidant properties, the results showed that probiotic yoghurt was more effective in increasing antioxidative activity than the regular one.

It has been documented that physical activity can cause reactive oxygen species (ROS) generation in skeletal muscles.[30] Administration of antioxidant supplementation may play a positive role in metabolism of physical activity.[31-33] Probiotics are defined as living microbial food ingredients beneficial to health.[34] Probiotic supplementation has antioxidant property and can prevent risky levels of oxidative stress.[14] Consumption of probiotics producing glutathione and having complete glutathione redox cycle enzymes, GPX and glutathione reductase (GRed), may contribute to the reduction of lipid hydroperoxides in the gastrointestinal tract and in hepatocytes and prevent them from entering the circulation.[35] The hs-CRP, TNF-$\alpha$, and MMPs are biomarkers of inflammation.[36] Serum levels of TNF-$\alpha$, MMP2, MMP9, and MDA were significantly lower in the control group than the supplement group. Exhaustive exercise caused an increase in the serum levels of MDA, MMP2, and MMP9 in both groups, but these increases were significantly higher in the control group. It was found that exercise induces increase in MMP9 messenger ribonucleic acid (mRNA) levels and its activity in the skeletal muscles.[37] Consistent with the previous studies, our findings showed antioxidant enzyme activity and MDA increase during severe physical activity.[32] Physical exercise is a complex process constituting several factors affecting the expression levels of MMP2 and MMP9 in skeletal muscles in a condition such as local ischaemia and increases in muscle stretching, in shear stress, and in the wall tension of blood vessels.[38] Our results strongly support the anti-inflammatory and antioxidative activity of probiotic yoghurt, which is in response to the preventative effect of the controlling oxidative stress during intensive training. This study showed that the antioxidant activities increased in the supplement group in comparison with the control group at the end of the intervention and after intense physical activity. Therefore, it is suggested that there is a synergy between endogenous antioxidant enzyme and exogenous antioxidant mixture in the diet to reduce oxidative stress rather than individual antioxidants. In order to guard against the destructive effects of oxidative stress, our bodies have a complex system of endogenous antioxidant protection in the form of enzymes such as SOD, catalase, and GPX.[39] Supplementation with antioxidants, either through an increased consumption in the diet or from supplementation, has become extremely popular as a means to improve one's health or physical performance. It has been suggested that increased circulating levels of certain antioxidants will help to prevent the accumulation of free radicals inside our cells thereby reducing oxidative stress.[40]

Our results revealed significantly elevated serum levels of SOD and GPX in the supplement group in comparison with the control group during the intense training. In the supplement group, increases of MMP2, MMP9, and MDA were less than the control group following exhaustive exercise. In a similar vein, a study by Lamprecht et al. reported that the probiotic yoghurt consumption has positive effects on some inflammatory factors due to exercise-induced oxidative stress.[15] On the other hand, reportedly the probiotic supplementation did not affect oxidative stress indices in women with rheumatoid arthritis.[41] This discrepancy may be related to the participants of the study.

The limitations of this study included its short duration, low sample size, low cost for measuring most antioxidant agents and other inflammatory parameters and the absence of proper cooperation on the part of some participants during the study. The absence of proper cooperation on the part of some participants during the study was also a deterrent. Further investigations are proposed with longer duration and a larger sample to confirm the positive effect of probiotic yoghurt on the management of oxidative stress during exhaustive exercises.

## Conclusion

Regular intake of probiotic yoghurt significantly modulates MMP9, MMP2 and some inflammatory factors, and thus guards against exhaustive exercise inducing oxidative injury in young healthy females. Probiotic yoghurt consumption seems to exert beneficial effects on reducing MMP2 and MMP9 serum levels and increasing antioxidant enzyme activities before and after exhaustive exercises. Further large-scale studies are recommended in this domain.

**Acknowledgments:** We are grateful to Ardabil University of Medical Sciences for financial support as well as to all the participants.

**Disclaimer:** None.

**Conflict of Interest:** None.

**Source of Funding:** The office of the Vice-Chancellor, Ardabil University of Medical Sciences, Ardabil, Iran, financed the study.

## References

1. Fuller R. Probiotics in man and animals J Appl Bacteriol; 66: 365-78.
2. Parvez S, Malik KA, Ah Kang S, Kim HY. Probiotics and their fermented food products are beneficial for health. J Appl Microb 2006; 100: 1171-85.
3. Mikelsaar M, Zilmer M. Lactobacillus fermentum ME-3–an antimicrobial and antioxidative probiotic. Microb Ecol Health Dis 2009; 21: 1-27.
4. Molavi B, Mehta JL. Oxidative stress in cardiovascular disease: molecular basis of its deleterious effects, its detection, and therapeutic considerations. Curr Opin Cardiol 2004; 19: 488-93.
5. Filippin LI, Vercelino R, Marroni N, Xavier RM. Redox signalling and the inflammatory response in rheumatoid arthritis. Clin Exp Immunol 2008; 152: 415-22.
6. Ong SL, Zhang Y, Whitworth JA. Reactive oxygen species and glucocorticoid-induced hypertension. Clin Exp Pharmacol Physiol 2008; 35: 477-82.
7. Chen Z, Zhong C. Oxidative stress in Alzheimer's disease. Neurosc Bull 2014; 30: 271-81.
8. Kullisaar T, Songisepp E, Mikelsaar M, Zilmer K, Vihalemm T, Zilmer M. Antioxidative probiotic fermented goats' milk decreases oxidative stress-mediated atherogenicity in human subjects. Br J Nutr 2003; 90: 449-56.
9. Wang Y, Wang GZ, Rabinovitch PS, Tabas I. Macrophage Mitochondrial Oxidative Stress Promotes Atherosclerosis and Nuclear Factor-κB–Mediated Inflammation in MacrophagesNovelty and Significance. Circ Res 2014; 114: 421-33.
10. Yan LJ. Positive oxidative stress in aging and aging-related disease tolerance. Redox Biol 2014; 2: 165-9.
11. Deaton CM, Marlin DJ. Exercise-associated oxidative stress. Clin Tech Equine Pract 2003; 2: 278-91.
12. Doll E, Keppler D, Keul J. Energy metabolism of human muscle. Med Sports Sci 1972; 179: 273-4
13. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. USA: Oxford University Press, 2015.
14. Verdenelli MC, Ghelfi F, Silvi S, Orpianesi C, Cecchini C, Cresci A. Probiotic properties of Lactobacillus rhamnosus and Lactobacillus paracasei isolated from human faeces. Eur J Nutr 2009; 48: 355-63.
15. Lamprecht M, Bogner S, Schippinger G, Steinbauer K, Fankhauser F, Hallstroem S, et al. Probiotic supplementation affects markers of intestinal barrier, oxidation, and inflammation in trained men; a randomized, double-blinded, placebo-controlled trial. J Int Soc Sports Nutr 2012; 9: 45.
16. D'souza A, Fordjour L, Ahmad A, Cai C, Kumar D, Valencia G, et al. Effects of probiotics, prebiotics, and synbiotics on messenger RNA expression of caveolin-1, NOS, and genes regulating oxidative stress in the terminal ileum of formula-fed neonatal rats. Pediatr Res 2010; 67: 526-31.
17. Songisepp E, Kals J, Kullisaar T, Mändar R, Hütt P, Zilmer M, et al. Evaluation of the functional efficacy of an antioxidative probiotic in healthy volunteers. Nutr J 2005; 4: 22.
18. Capcarova M, Hascik P, Kolesarova A, Kacaniova M, Mihok M, Pal G. The effect of selected microbial strains on internal milieu of broiler chickens after peroral administration. Res Veterinary Sci 2011; 91: 132-7.
19. Awney HA. The effects of Bifidobacteria on the lipid profile and oxidative stress biomarkers of male rats fed thermally oxidized soybean oil. Biomarkers 2011; 16: 445-52.
20. Machin D, Campbell MJ, Tan SB, Tan SH. Sample size tables for clinical studies. 3rd ed. United Kingdon: John Wiley & Sons; 2011.
21. Bruce R. Multi-stage treadmill test of submaximal and maximal exercise. In: American Heart Association. Committee on Exercise & Kattus, Albert A. Exercise testing and training of apparently healthy individuals: a handbook for physicians. AHA, Dallas, Tex. ; New York; 1972, pp 32-4.
22. Pollock ML, Foster C, Schmidt D, Hellman C, Linnerud AC, Ward A. Comparative analysis of physiologic responses to three different maximal graded exercise test protocols in healthy women. Am Heart J 1982; 103: 363-73.
23. Wiid I, Seaman T, Hoal EG, Benade AJ, Van Helden PD. Total antioxidant levels are low during active TB and rise with anti-tuberculosis therapy. IUBMB Life 2004; 56: 101-6.
24. Yagi K. Lipid Peroxides and Related Radicals in Clinical Medicine. In: Armstrong D. (eds) Free Radicals in Diagnostic Medicine. Advances in Experimental Medicine and Biology. Boston: Springer, 1994; 366
25. Kullisaar T, Shepetova J, Zilmer K, Songisepp E, Rehema A, Mikelsaar M, et al. An antioxidant probiotic reduces postprandial lipemia and oxidative stress. Open Life Sci 2011; 6: 32-40.
26. Amaretti A, Di Nunzio M, Pompei A, Raimondi S, Rossi M, Bordoni A. Antioxidant properties of potentially probiotic bacteria: in vitro and in vivo activities. Appl Microbiol Biotechnol 2013; 97: 809-17.
27. An H, Zhou H, Huang Y, Wang G, Luan C, Mou J, et al. High-level expression of heme-dependent catalase gene katA from Lactobacillus sakei protects Lactobacillus rhamnosus from oxidative stress. Mol Biotechnol 2010; 45: 155-60.
28. Uskova MA, Kravchenko L. Antioxidant properties of lactic acid bacteria--probiotic and yogurt strains. Vopr Pitan 2008; 78: 18-23.

29. Martarelli D, Verdenelli MC, Scuri S, Cocchioni M, Silvi S, Cecchini C, et al. Effect of a probiotic intake on oxidant and antioxidant parameters in plasma of athletes during intense exercise training. Curr Microbiol 2011; 62: 1689-96.
30. Vainshtein A, Grumati P, Sandri M, Bonaldo P. Skeletal muscle, autophagy, and physical activity: the ménage à trois of metabolic regulation in health and disease. J Mol Med (Berl) 2014; 92: 127-37.
31. Ristow M, Zarse K, Oberbach A, Klöting N, Birringer M, Kiehntopf M, et al. Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci 2009; 106: 8665-70.
32. Abbas Naghizadeh-Baghi MM, Shadman-Fard A, Nemati A. Punica granatum juice effects on oxidative stress in severe physical activity. Mater Sociomed 2015; 27: 48-51
33. Mazani M, Fard AS, Baghi AN, Nemati A, Mogadam RA. Effect of pomegranate juice supplementation on matrix metalloproteinases 2 and 9 following exhaustive exercise in young healthy males. J Pak Med Assoc 2014; 64: 785-90.
34. Salminen S, Bouley C, Boutron MC, Cummings J, Franck A, Gibson G, et al. Functional food science and gastrointestinal physiology and function. Br J Nutr 1998; 80: S147-S71.
35. Navab M, Anantharamaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Fogelman AM. Mechanisms of disease: proatherogenic HDL—an evolving field. Nature Rev Endocrinol 2006; 2: 504-11.
36. Mirtaheri E, Pourghassem Gargari B, Kolahi S, Dehghan P, Asghari-Jafarabadi M, Hajalilou M, et al. Effects of Alpha-Lipoic Acid Supplementation on Inflammatory Biomarkers and Matrix Metalloproteinase-3 in Rheumatoid Arthritis Patients. J Am Coll Nutr 2015; 34: 310-7.
37. Koskinen S, Wang W, Ahtikoski A, Kjaer M, Han X, Komulainen J, et al. Acute exercise induced changes in rat skeletal muscle mRNAs and proteins regulating type IV collagen content. Am J Physiol Regul Integr Comp Physiol 2001; 280: R1292-R300.
38. Kjaer M. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. Physiol Rev 2004; 84: 649-98.
39. Kerksick C, Willoughby D. The antioxidant role of glutathione and N-acetyl-cysteine supplements and exercise-induced oxidative stress. J Int Soc Sports Nutr 2005; 2: 38-44.
40. Poljsak B, Šuput D, Milisav I. Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. Oxidative Medicine Cellular Longevity 2013; 2013: Article ID 956792
41. Vaghef-Mehrabany E, Homayouni-Rad A, Alipour B, Sharif SK, Vaghef-Mehrabany L, Alipour-Ajiry S. Effects of probiotic supplementation on oxidative stress indices in women with rheumatoid arthritis: a randomized double-blind clinical trial. J Am Coll Nutr 2016; 35: 291-9.