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Negative effect of high-calorie high-salt diet on spleen oxidant/antioxidants and structure: preventive effects of sprint interval training

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Abstract

Objective: We investigated the effect of a high-calorie high-salt diet and sprint interval training on oxidant/antioxidant status and structural changes in the spleen of male Wistar rats.

Materials and Methods: Eighteen male Wistar rats were randomized into three groups: normal diet (ND), high-calorie high-salt diet (HCSD), and HCSD + sprint interval training (HCSD+SIT). Rats in HCSD and HCSD+SIT groups were under a high-calorie high-salt diet. The SIT (4-9 reps of 10 s duration sprints) performed 3 sessions/week for 8 weeks. Forty-eight hours after the last training session, spleen was removed and used for the assessment of oxidant/antioxidant status and histomorphometric parameters.

Results: Results revealed that compared to the ND group, superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) level, and total antioxidant capacity (TAC) were significantly lower ($P<0.001$) and malondialdehyde (MDA) level was significantly higher in the spleen tissue of the HCSD group ($P<0.001$) and there was no significant difference between the HCSD+SIT and ND groups in none of the assessed variables ($P>0.05$). Moreover, there was a significant increase in the red to white pulp ratio and a significant decrease in the number of lymph cells and splenic trabecular volume in the HCSD group ($P<0.001$). Nonetheless, in none of the assessed morphometric parameters, a significant difference was observed between the HCSD+SIT and ND groups ($P>0.05$).

Conclusion: It seems that SIT training may avert HCSD-induced unfavourable changes in the spleen oxidant/antioxidant status and counteract the deleterious effects of the HCSD on the spleen structure.

Keywords: High-fat diet, High-sugar diet, High-salt diet, High-intensity interval training, Spleen

1. Introduction

A healthy diet is essential for appropriate immune function and maintaining good health (1). High-calorie and high-salt intake are common characteristics of western diets (2). These diets have been shown to lead to hypertension, obesity, diabetes, and other metabolic disorders (3). A long-term intake of western diets may induce oxidative stress by reducing antioxidant defence and/or increasing oxidant formation, which leads to oxidative stress. Oxidative stress, in turn, may cause damage to the cellular structures and trigger inflammatory response (4).

Although several studies have documented diet-induced metabolic dysregulation, oxidative stress, and inflammation in different bodily organs (5), its effects on oxidative stress and morphology of the spleen have yet to be thoroughly known. The spleen is the largest lymphoid organ in the body and there are two main types of tissue in spleen anatomy; the red pulp that is vascular, and has the large, thin-walled sinuses, which are red due to the large number of erythrocytes, and the white pulp that contains lymphoid aggregations, mostly lymphocytes, and macrophages which are arranged around the arteries (6,7). Spleen hosts various cell types and plays a key role in several functions, such as T and B cell activation in response

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to antigens transmission through blood, antibody production, or the clearance of circulating apoptotic cells (8).

High-calorie diet-induced inflammation has been reported to be associated with reduced splenic function through increasing phosphatidylserine externalization in red blood cells, enhancing their interaction with erythrophagocytosis macrophages (9), and inducing extramedullary haematopoiesis of monocyte-like cells, which modulates the secondary response of the immune system to inflammation (10). These changes in function and morphology of the spleen are involved in the pathogenesis of diabetes and obesity-related cardiovascular diseases (11). So, it could be postulated that therapeutic and preventive methods which maintain the normal spleen structure and counteract the harmful effects of western diets could be beneficial in terms of metabolic and general health.

Exercise is a low-cost, safe, preventative/therapeutic approach in reducing the risk of many health conditions (12), and has been shown to have numerous health benefits. However, large population-based studies show that most people fail to achieve the recommended level of physical activity (13), and the lack of time is considered to be the major obstacle to participation and compliance with traditional physical activity and exercise programs (14).

Sprint interval training (SIT), which includes short bursts of very high-intensity activities, is a specific form of high-intensity interval training and requires much lower time in comparison with usual high-intensity interval training protocols, and several studies have documented its potential benefits on health (14–16). It relies mainly on anaerobic energy system, i.e., adenosine triphosphate and creatine phosphate, and does not have the common side effects of conventional exercise methods. Depleted energy reserves are rapidly restored through the glycolysis and aerobic pathways (13,17), so this type of exercise indirectly challenges other energy systems, too. However, there is very limited understanding of the physiological mechanisms underlying the SIT effects on different bodily organs, specifically the spleen as the major lymphoid organ with various physiological functions. To the best of our knowledge, only one study has investigated the effect of exercise (low-intensity treadmill running for 30 min/day, 5 days/week) on spleen morphology in mice fed a high-fat, high-sugar diet and reported a favourable effect of exercise on spleen (6), and no published study has examined the effect of SIT on spleen oxidant/antioxidant status and its structural changes in animals under a high-calorie high-salt diet. So, in the present study, we aimed to investigate the effect of a high-calorie high-salt diet on spleen oxidant/antioxidant status and morphology and to assess the potential benefits of SIT on the deleterious effect of a high-calorie high-salt diet on oxidant/antioxidant status and the spleen morphology.

2. Materials and Methods

2.1. Animals and grouping

Eighteen 6-week-old male Wistar rats (weighing 130-150 g) were obtained from Shahed University Central Laboratory and were housed in separate cages (4 rats in each cage polypropylene, 45 × 28 × 20 cm with wire mesh tops) at an ambient temperature of 21-23°C, humidity between 40–50% and in a 12:12 h light-dark cycle. After the environmental adaptation phase, animals were randomized into three groups (n=6): normal diet (ND) group, high-calorie high-salt diet (HCSD) group, and (3) HCSD + sprint interval training (HCSD+SIT) group. Rats in the ND group received a standard chow, containing 5% fat, 62% carbohydrate, 20% protein, and 0.5% sodium. In HCSD and HCSD+SIT group rats were fed a formulated chow containing 45% fat, 15% protein, 40% carbohydrate, and 1.5% sodium. Animals had ad libitum access to food and water all through the study. Rats in the HCSD+SIT group performed high-intensity interval training three times per week for eight weeks. All the steps of the study were performed according to the "Principles for the Care and Use of Animals in Research" and the study protocol was approved by the Ethical Committee of Shahed University (Code number: IR.Shahed.Rec.1400.005).

2.2. Sprint Interval Training Protocol

Sprint interval training proceeded with a two-weeks of familiarization with the rodent's treadmill, then the animals in the HCSD+SIT group performed the SIT protocol for eight weeks. Every session proceeded with a five-minute warming up (running at 40% of the maximal running speed) and followed by a five-minute cooling down (running at 30-40% of the maximal running speed). Each exercise session consisted of 4-9 bouts of 10-second high-intensity running on the treadmill with 118-166% maximal running speed, which was alternated with 1 min of active recovery at 15-20 m.min-1 (Table 1).

A maximal running speed test (MRST) was performed after the familiarization phase, and the exercise intensity was set based on its results. The test began at an initial speed of 10 m min-1, which was increased by increments of 3 m min-1 every 3 min until the maximal running speed was reached. The test was terminated when the rats were exhausted and remained at the end of the mat on the shock grid for 5 s (18).

Table 1. Exercise training protocol

	General familiarization	Specialized familiarization	Week 1	Week 2	Week 3	Week 4	week 5	Week 6	Week 7	Week 8
Speed (m/m^{-1})	15-20	20-30	50-55	56-60	56-60	60-65	60-65	66-70	66-70	66-70
MRST (%)	_	_	118-130	130-141	130-141	141-153	141-153	153-166	153-166	153-166
Sprint duration	_	10 sec	10 sec							
Resting speed	_	_	15-20 m/min^{-1} -60 s							
Sets	1	3	4	4	5	5	5	7	8	9
Sessions per week	5	3	3							

2.3. Biochemical assessments

Forty-eight hours after the completion of the study protocol and following overnight fasting, rats were anesthetized with ether, then the skin of the abdomen was sterilized with 70% ethanol, and with an incision in the abdomen, the spleen was removed and used for TAC, GPx, SOD, and MDA levels assessments and histomorphological analysis.

Total antioxidant capacity (TAC) assessment protocol was set by the method described by Koracevic et al (19). Initially, 75 µl of the sample was mixed with 500 µl of reagent solution 1 (H2SO4). Then, 20 µl of reagent solution 2 (H2SO4 + CuSO4 + o-Dianisidine) was added. Finally, the absorption of the solution at 630 nm was examined by the microplate reader, and the TAC level was expressed as nanomoles per milligram of protein (nmol/mg pr).

Superoxide dismutase (SOD) enzyme activity was determined using the McCord and Fridovich method (20). Xanthine and xanthine oxidase were used to generate superoxide anion radicals that quantitively react with chloride 2- (4-iodophenyl) -3- (4-nitrophenol) -5-phenyltetrazolium to produce formazan dye. SOD inhibits the reaction by the superoxide radicals' conversion into oxygen. Standard solutions of homogeneous liver tissue were used for SOD measurement. The absorbance was measured with a spectrophotometer at 505 nm for 30 seconds following the addition of xanthine oxidase as a reagent and 3 minutes after the reaction of the duplicate samples (1021 UV / visible Cecil 1021 Cecil Instruments Ltd Milton Technical Centre Cambridge England). The inhibition percentage of the standards and samples was calculated using the following formula: -100 ((tdAStd / min × 100) / (1AS1 / min)). The GPx concentration was reported as Units/mg pr.

Glutathione peroxidase (GPx) levels were assessed according to Ahmadvand et al method (21) . The assessment protocol was as follows: 200 µl of Tris HCl buffer solution, 100 µl of sodium azide, 200 µl of glutathione, 100 µl of hydrogen peroxide (H2O2), and 200 µl of the sample were all combined in a test tube. The test tube was then incubated in a water bath (KTG water bath, Tehran, Iran) at 37 C for 10 minutes. Then, 400 µl of 10% TCA solution was added. The final solution was centrifuged at 3000 rpm for 3 minutes and 25 µl of the supernatant was removed and added to 140 µl of Tris EDTA solution. 30 µl of DTNB solution was added to the mixture. After 30 minutes of incubation at room temperature, the absorbance of the solution at 450 nm was read using an ELISA reader (Awareness Technology Stat Fax 3200 Microplate Reader ،Ramsey ،Minnesota). The GPx concentration was reported as units/mg pr.

Malondialdehyde (MDA) level was assessed using a thiobarbituric acid reaction. Briefly, about 0.2-0.3 g of samples were homogenized in ice-cooled potassium chloride (150 mmol). After centrifugation at 3000 RPM for 10 minutes, 0.5 ml of supernatant was mixed with 3 ml of phosphoric acid (1% V/V). Then, 1 ml of 6.7 g of 1-L TBA was added to the samples. The samples were heated for 45 minutes at 100°C and were then cooled in an ice bath. After adding 3 ml of N-butanol, the samples were centrifuged again at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured by spectrophotometry at 532 nm and the final product of lipid peroxidation was calculated according to the simultaneous calibration curve using MDA standards. MDA was expressed as nanomoles per milligram of protein (nmol/mg pr). The protein content of the samples was assessed based on the Lowry method (22).

2.4. Histomorphometric analysis

For the histomorphometric analysis, a longitudinal midline incision was made on the spleens at a

thickness of 5 µm and the spleens were stained with Hematoxylin and Eosin (H&E) for histomorphometrical evaluation with an optic microscope. Image J (v1.6, NIH) was used to evaluate the histomorphometric status, area of the spleen, and white to red pulp ratio calculated as ((red pulp area - white pulp area) / white pulp area). Mason trichrome staining was used to evaluate spleen capsule, trabeculae and tissue fibrosis (23).

2.5. Statistical analysis

All quantitative data was presented as mean± standard deviation and $p<0.05$ was used as the cut-off for defining statistical significance. The Shapiro–Wilk test was applied to verify the normality of data distribution. One-way analysis of variance and Tukey post hoc tests were used for between-group comparisons. Statistical analysis was conducted using IBM SPSS Statistics 25 (IBM, New York, NY, USA).

3. Results

3.1. The effect of HCSD and SIT on oxidant/antioxidants of the spleen

Results revealed that compared to the ND group, the SOD activity, the GPx and the TAC levels were significantly lower ($P = 0.001$) and the MDA levels were significantly higher in the spleen tissue of the HCSD group ($P = 0.001$). However, results showed that there was not a statistically significant difference between the HCSD+SIT and ND groups in none of the assessed variables ($P > 0.05$) which suggests SIT has prevented the unfavourable alteration in oxidant/antioxidants status induced by the HCSD in the spleen tissue (Figure 1).

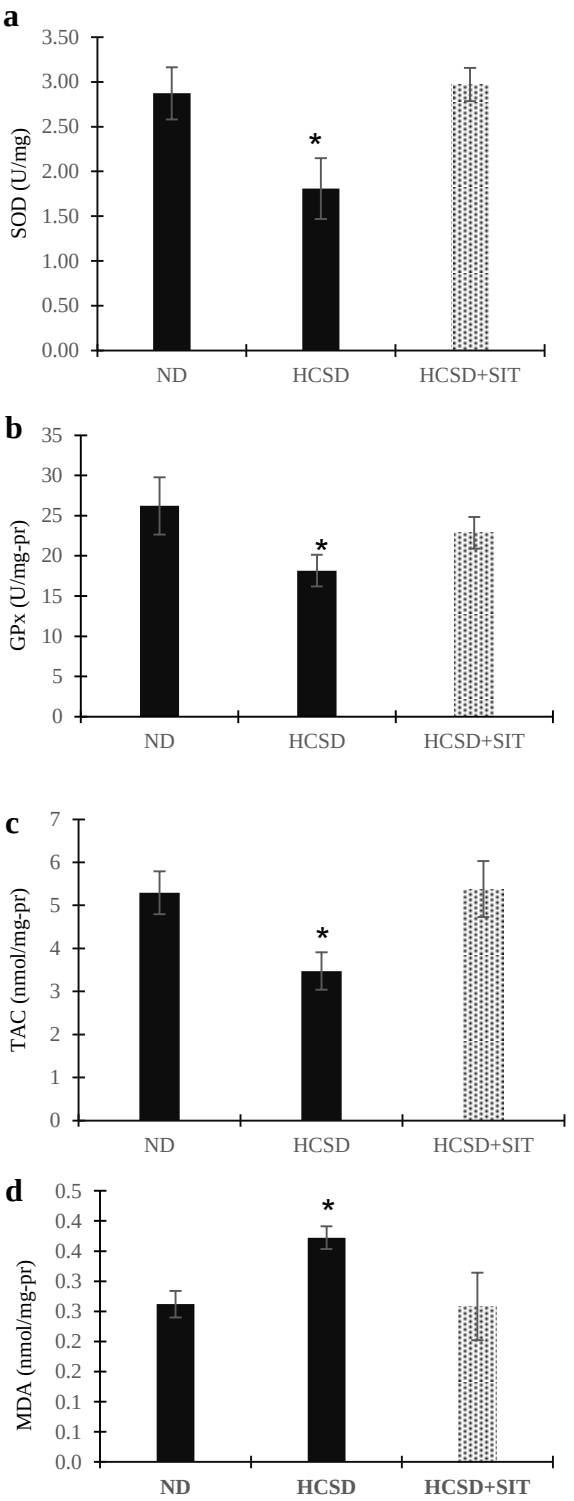
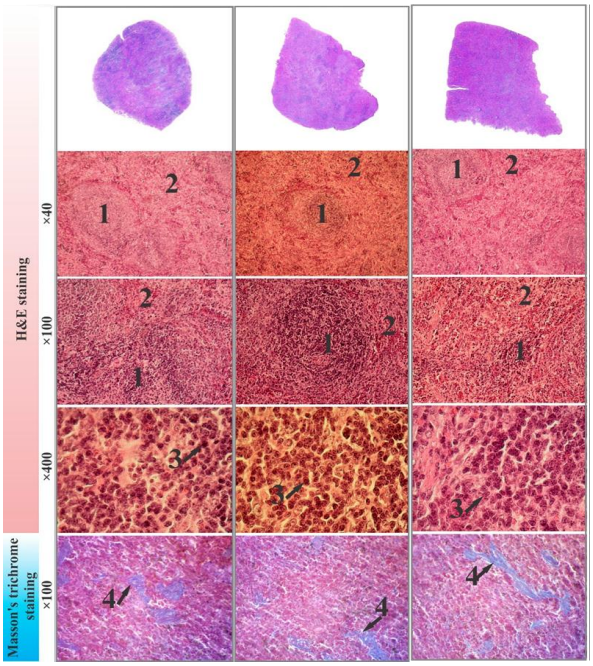


Fig. 1. Spleen tissue SOD activity (a), GPx levels (b), TAC levels (c), and MDA levels (d). All quantities are presented in Mean ± SD with *$P < 0.05$. ND; Normal diet group, HCSD; high-calorie high-salt diet group, HCSD+SIT; high-calorie high-salt diet + sprint interval training group.

3.2. The effect of HCSD and SIT on spleen structure

Histomorphometric examination of the spleen tissue sections showed that in comparison with the ND group, 10 weeks of HCSD significantly increased the red pulp/white pulp ratio (P=0.001), significantly decreased the number of lymphocytes (P=0.001), and the central trabecular volume (P=0.001). However, there was no significant difference in spleen capsule thickness between ND and HCSD groups (P=0.11). Moreover, results revealed that there was not a statistically significant difference between HCSD+SIT and ND group in terms of the red pulp/white pulp ratio (P=0.32), the number of lymphocytes (P=0.06), the central trabecular volume (P=0.06), and the spleen capsule thickness (P=0.23) which implies SIT has prevented the deleterious effects of the HCSD on the spleen structure (Figure 2).



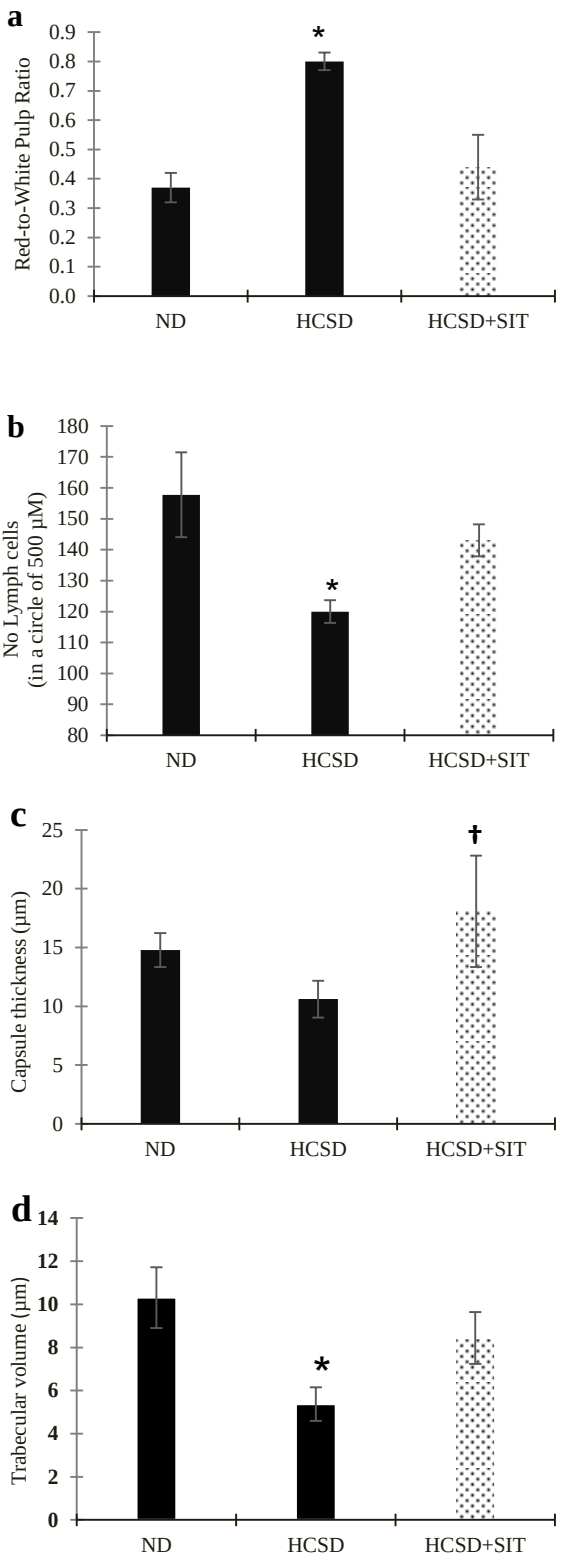


Fig 2. Tissue sections of the spleen in research groups, magnification × 40 to × 400. H&E and Masson's trichrome staining. White pulp (1), the red pulp (2), lymph cells in the white pulp (3), and the central trabeculae (4) are all stained by Masson's trichrome staining. Red pulp to white pulp ratio (a), number of lymph nodes (b), capsule thickness (c), and central trabecular volume (d). Data are expressed as mean ± standard deviation, P <0.05. * Significantly different as compared to the HCSD group, † Significantly different as compared to the ND group.

4. Discussion

In the present study, first we investigated the effect of a diet high in fat, sugar and salt on spleen oxidative/antioxidative status and spleen histomorphometry of Wistar rats. Our findings revealed that a HCSD diet could increase oxidants (MDA) and decrease antioxidants (SOD, GPx, and TAC) in spleen tissue of rats. It is believed that a diet high in saturated fat, glucose, and salt can lead to neuro-hormonal disorders, inflammation, and oxidative stress (24), and some previous studies have reported that a high-fat diet or high-fat high-salt diet could increase oxidative stress and result in reduced antioxidant capacity in different bodily organs (25–27). Increased ROS production and resulting oxidative stress is supposed to be a key player in fibrosis (28); accordingly, our findings showed that HCSD diet led to significant changes in splenic morphology. We found that 10 weeks of HCSD diet led to an increase in the red pulp/white pulp ratio. Moreover, it led to a significant decrease in the number of lymphocytes, and the central trabecular volume, which all suggest that there has been a considerable structural change. Concomitant changes in oxidative stress and morphological parameters supports the notion that ROS are involved in splenic enlargement.

However, the main purpose of this study was to investigate the therapeutic effect of exercise on reducing the diet-induced oxidative stress and deleterious alterations in spleen structure. Our findings indicated that SIT training prevented the diet-induced changes in oxidative/antioxidative status in spleen, as was assessed by MDA, SOD, GPx and TAC level alterations. In line with the findings of the present study swimming training has been shown to increase the SOD and GPx antioxidant enzymes in rat spleen tissue (29). Moreover, Feriani et al (2021) showed that chronic immobility stress led to elevation of pro-oxidant and inflammatory markers, but the exercise training improved the antioxidant mechanisms and decreased inflammatory cytokine content in in the spleen of mice (30). They concluded that exercise training was effective in mitigating the stress induced damages in the spleen and it provides a non-pharmacological therapeutic strategy for stress-evoked pathologies. Additionally, Senna et al (2016) reported that in low-protein fed rats which received lipopolysaccharide (LPS) shot, exercise training (8 weeks, 5 days a week, 60 minutes a day, with 70% VO2max) led to attenuation of splenic lymphocytes apoptosis and TNF-α plasma concentrations (31). Inflammation and oxidative stress are interrelated phenomena; just as inflammation leads to oxidative stress, and vice versa (32). Hence, although not assessed in the present study, exercise-induced favourable changes in oxidative/antioxidative status in the spleen may have been in part due to anti-inflammatory properties of exercise, as exercise has been shown to modulate the function of immune cells that are abundant in the spleen (6). Even, light exercise has been shown to be sufficient to reduce circulating proinflammatory cytokines like TNF secreted by lymphocytes and macrophages (33,34). Moreover, it has been reported that splenic volume decreases during exercise in intensity dependent manner (6,35). This decrease, at least in part, may be related to increased catecholamine levels (35), and resulting vasoconstriction in spleen during sprinting intervals, which may lead to some ischemia in the spleen. The subsequent reperfusion during low intensity phase between sprinting intervals can increase ROS production. This repeated physiologic stress may stimulate the antioxidant enzyme expression, namely SOD and GPx, through the Nrf-2 accumulation and nuclear translocation (36). However, considering the paucity of information about the spleen response and adaptation to various exercise intensities and modalities, further studies are required to confirm these findings.

Our results also showed in rats under HFSD, there is significant changes in splenic morphology. Rats fed a HFSD showed a significant increase in the red to white pulp ratio, and a significant decrease in the number of lymph cells, and splenic trabecular volume. This indicates that HFSD has led to expansion of the red pulp or abridgment of the white pulp. However, considering the significant decrease in the splenic lymph cells, it seems that HFSD has led to specific changes in white pulp rather than morphological alterations in whole spleen. Altukkaynak et al (2007), and Buchan et al (2018) reported that high-fat diet led to expansion of sinusoids and surrounding tissue in both the red and white pulps rather that specific changes in on pulp (6,37). The decrease in the white pulp may be related to diet induced changes in immune cell number and apoptosis in the spleen. A previous study reported that HFD can increase apoptotic rate of splenic lymphocytes (37). Moreover, a diet high in salt has been shown to decrease the percentage and absolute number of natural killer cells in spleen (38).

Interestingly, our results showed that there was no significant difference between the C and HCSD+SIT group in none of the assessed morphometric parameters which indicates SIT has prevented HCSD-induced disturbances in spleen microstructure. This finding is in line with the findings of Buchan et al (2018) who reported regular moderate-intensity exercise reduced high-fat high-sugar diet induced increment in spleen weight and IL-6 levels and reversed diet-induced splenic enlargement (6). The preventative effect of exercise may be related to exercise induced improvement in oxidative/antioxidative status in the spleen. As it has been reported that high-fat diet may induce oxidative stress in different organs including spleen (39), and high-salt diet may increase the production of reactive oxygen species (ROS) by NK cells in the spleen (38),

which in turn they can cause inflammation and microstructural abnormalities in the spleen tissue.

Conclusion

In summary, based on findings of the present study, it seems that a ten-week high-fat, high-sugar, and high-salt diet is sufficient to induce oxidative stress and deleterious alterations in spleen microstructure and the sprint interval training may reverse the diet-induced changes in spleen oxidative/antioxidative status and morphology.

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Declaration of interest

The authors declare that they have no competing interest.

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