ORIGINAL ARTICLE

Effect of vitamin E succinate on inflammatory cytokines induced by high-intensity interval training

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Aim and Scope: The anti-inflammatory effect of vitamin E under moderate exercises has been evaluated. However, the effect of vitamin E succinate, which has more potent anti-inflammatory effect than other isomers of vitamin E has not been evaluated. Therefore, the aim of the present study was to evaluate the effects of vitamin E succinate on tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) production induced by high-intensity interval training (HIIT). **Materials and Methods:** In the present study, 24 rats were randomly divided into control (C), supplementation (S), HIIT, and HIIT + supplementation (HIIT+S) groups. HIIT training protocol on a treadmill (at a speed of 40–54 m/min) and vitamin E succinate supplementation (60 mg/kg/day) was conducted for 6 weeks. **Results:** Serum IL-6 in the HIIT group significantly increased compared with the C group (350.42 ± 123.31 pg/mL vs 158.60 ± 41.96 pg/mL; P = 0.002). Also, serum TNF-α concentrations significantly enhanced (718.15 ± 133.42 pg/mL vs 350.87 ± 64.93 pg/mL; P = 0.001) in the HIIT group compared with the C group. Treatment of the training group with vitamin E numerically reduced IL-6 and TNF-α when compared with the HIIT group (217.31 ± 29.21 and 510.23 ± 217.88, respectively, P > 0.05). However, no significant changes were observed in serum TNF-α (P = 0.31) and IL-6 (P = 0.52) concentrations in the HIIT + S group compared with the C group. **Conclusion:** HIIT-induced IL-6 and TNF-α decreased by administration of Vitamin E succinate.

Key words: High-intensity interval training (HIIT), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), Vitamin E succinate decreased the elevation of IL-6 and TNF-α by HIIT

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INTRODCTION

Cytokines are active, low molecular weight polypeptides that can act in autocrine and paracrine fashion.[1,2] Tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), as two inflammatory cytokines, are expressed by immune cells[1,2] and nonimmune cells including epithelial cells,[3] skeletal myotubes,[4,5] epididymal white adipose tissue,[6] and primary microglia in the brain.[7] IL-6 is pleiotropic and has a different effect on lipid metabolism, insulin resistance, mitochondrial activities, the neuroendocrine system, neurophysiological behavior, and vascular disease.[8] TNF-α enhances proinflammatory cytokines, facilitates antimicrobial activities of phagocytes, and potentiates tissue-damaging properties.[9] These cytokines are upregulated by a different insult, especially oxidative stress.[3,5]

In vitro evidences have shown that superoxide anion ($O_2^{\bullet-}$)[3] and hydrogen peroxide (H_2O_2)[5] increased (TNF-α and IL-6) messenger RNA (mRNA) and protein. Elevated levels of the proinflammatory cytokines IL-6 and TNF-α have been associated with reduced skeletal and cardiac muscle function,[4] and the development of metabolic syndrome.[6,9,10] Therefore, different approaches including exercise training and antioxidant supplementation have been used to reduce inflammatory cytokines. In this context, it has been shown that low to moderate intensity exercise training in animal models on treadmill, wheel running as well as swimming reduced

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IL-6 and TNF-α in the skeletal muscle,[11] adipose tissue,[12] and serum,[10] respectively. It has been shown that 6 weeks of swimming training reduced serum TNF-α, which in turn improved insulin resistance in obese rats.[10] In contrast, Rowsey *et al*. observed no change in circulating IL-6 and TNF-α concentration following low to moderate exercise training in rats.[13] Furthermore, two studies have reported that overtraining[14] and exercise training with 80% maximal oxygen consumption (VO_2 max)[15] increased serum IL-6 and TNF-α. It has been shown that vitamin E supplementation reduced IL-6 mRNA and protein in different tissues by the inhibition of nuclear factor-κB (NF-κB) activation in response to inflammatory insult[4,7,9] and reduced TNF-α mRNA in the gastrocnemius muscle.[4] Additionally, it has been shown that antioxidant prevented translocation of NF-κB proteins to nucleus.[16]

Therefore, low to moderate exercise training and vitamin E supplementation reduce TNF-α and IL-6. However, little is known about circulating levels of TNF-α and IL-6 following high-intensity interval training (HIIT). In recent years, the sports community focused on HIIT to improve performance. However, this type of training applied a lot of pressure on the body, and sufficient insight and understanding about the implications of this type of exercise are not available. HIIT training results in oxidative stress.[17] The anti-inflammatory effect of vitamin E under moderate exercises has been evaluated previously. Furthermore, vitamin E inhibits NF-κB activation, with the greatest inhibition seen with the succinate form.[18]

However, the effect of vitamin E succinate, which has a more potent anti-inflammatory effect than other isomers of vitamin E has not been evaluated in HIT. Therefore, the aim of the present study was to evaluate the effects of vitamin E succinate on TNF-α and IL-6 production induced by HIIT.

MATERIALS AND METHODS

Animals

Twenty-four adult male albino Wistar rats (12 weeks of age, weighing 280 g) were randomly assigned to four groups of control (C), supplementation (S), HIIT, and HIIT + supplementation (HIIT+S). All animal experiments conformed to the guidelines for the use and care of laboratory animals ("Principles of laboratory animal care," NIH publication No. 86-23. Revised 1996),[19] and the study was approved by the Ethics Committee of Birjand University of Medical Sciences (Iran). The animals were kept under controlled conditions with 25 ± 2°C and a 12-h light/12-h dark cycle. The rats had free access to tap water and food. The animals were accustomed to laboratory conditions for 2 weeks prior to the experiment.

High-intensity interval training protocol

The rats were familiarized with running on a motor-driven treadmill for 5 days, 10 min/day at a speed of 10 m/min.[17] Then, HIIT were performed on the basis of an overload principle for 6 weeks, 6 sessions per week at 95-100% VO_2 max. In even and odd days, rats submitted to running at 40 m/min (3 min, two intervals in the first secession and progressively increased to six repetitions in the 6th week) and 54 m/min (30 s, three intervals in the first secession and progressively increased to 20 repetitions in the 6th week), respectively. Active rest was performed between intervals for 60 s at 16 m/min. Also, warm-up and cool-down were performed at 16 m/min (corresponds to 68% VO_2 max) at the beginning and end of HIIT, respectively.[17] The rats were motivated to run by a mild electrical current on the treadmill (0.5 mA, 1 Hz).[14] The rats in C group and S group were exposed to the same environment as the HIIT and HIIT + S group without running.

Vitamin E preparation

Vitamin E succinate (25 g) was purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA) and was dissolved in sesame oil (60 mg/mL in sesame oil). Rats in S and HIIT + S groups were supplemented orally by vitamin E (60 mg/kg body weight) for 6 weeks, 6 days/week, 3 h before exercise training.

Sample collection and cytokine determination

Fasting rats were euthanized under deep anesthesia (ketamine, 60-80 mg/kg and xylazine, 8 mg/kg; IP) 48 h after the last exercise session. Blood samples were taken via cardiac puncture and serum stored at –80°C before determination of levels of cytokines.[13]

We used the commercially 96-well enzyme-linked immunosorbent assay (ELISA) kits to measure the protein levels of IL-6 (Diaclone SAS., Besancon Cedex, France) and TNF-α (Diaclone SAS., Besancon Cedex, France) in the serum. The assay was performed according to the manufacturer's instructions. The absorbance of IL-6 and TNF-α was measured at 620 nm by an Anthos 2020 microplate reader (Biochrom Co., England). Each sample was assayed in duplicate and data were expressed as picograms per milliliter serum.

Statistical analysis

Data were analyzed by Statistical Package for Social Sciences (SPSS Inc., Chicago, USA) software, version 16.0 and expressed as mean ± standard deviation (SD). After determination of normality and homogeneity of variances by Shapiro-Wilk's test and Levene's test, respectively, the data were statistically analyzed by one-way analysis of variance (ANOVA) with Tukey's *post hoc* test used to locate differences ($P < 0.05$).

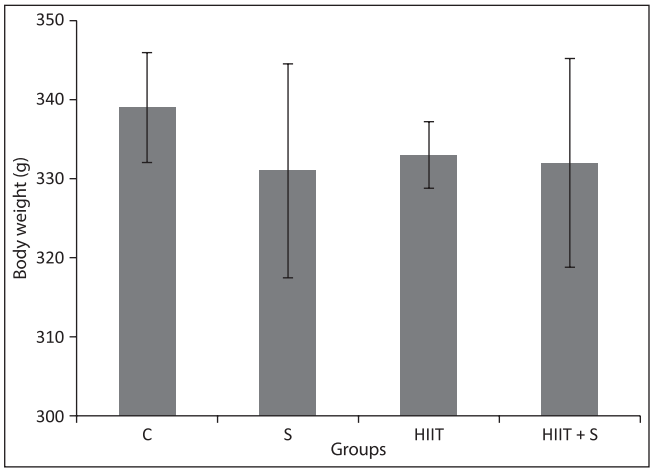

Figure 1: Effect of HIIT and vitamin E on weight. Control (C), supplementation (S), high-intensity interval training (HIIT), and HIIT + supplementation (HIIT+S)

RESULTS

The body weights of rats in C (339 ± 7 g), S (331 ± 14 g), HIIT (331 ± 4 g), and HIIT+S (332 ±13.21 g) groups were not significantly different at the end of protocol [Figure 1].

As depicted in Figure 2, the serum levels of IL-6 increased significantly in the HIIT group (350.42 ± 123.31 pg/mL) compared to C group (158.60 ± 41.96 pg/mL, P = 0.001). Vitamin E supplementation prevented IL-6 from increasing in the HIIT+S group (217.31 ± 29.21 pg/mL, P = 0.521). In contrast, vitamin E supplementation did not have a significant effect on IL-6 level in S group (165.75 ± 29.57 pg/mL, P = 0.998) compared to C group [Figure 2a].

Animals in the HIIT group had a significantly higher TNF-α level (718.15 ± 133.42 pg/mL) compared to C group (350.87 ± 64.93 pg/mL, P = 0.002). Vitamin E supplementation numerically prevented the enhancement of TNF-α in the HIIT+S group (510.23 ± 217.88 pg/mL, P = 0.314). However, vitamin E supplementation did not have a significant effect on TNF-α level in S group (393.93 ± 152.36 pg/mL, P = 0.957) compared to C group [Figure 2b].

DISCUSSION

Low to moderate exercise training is effective in the prevention and treatment of several diseases through reduction in inflammatory cytokines.[10,12,20] IL-6 and TNF-α with 26- and 17-kDa, respectively, synthesized by immune[1,2] and nonimmune cells release into the bloodstream and involve in systemic inflammation.[3-6] It has been demonstrated that skeletal muscles have a major role in elevation of IL-6 in the circulation after exercise.[4,5] In the present study, it was revealed that intensive exercise training resulted in increasing levels of serum IL-6 and TNF-α and administration of vitamin E succinate prevented

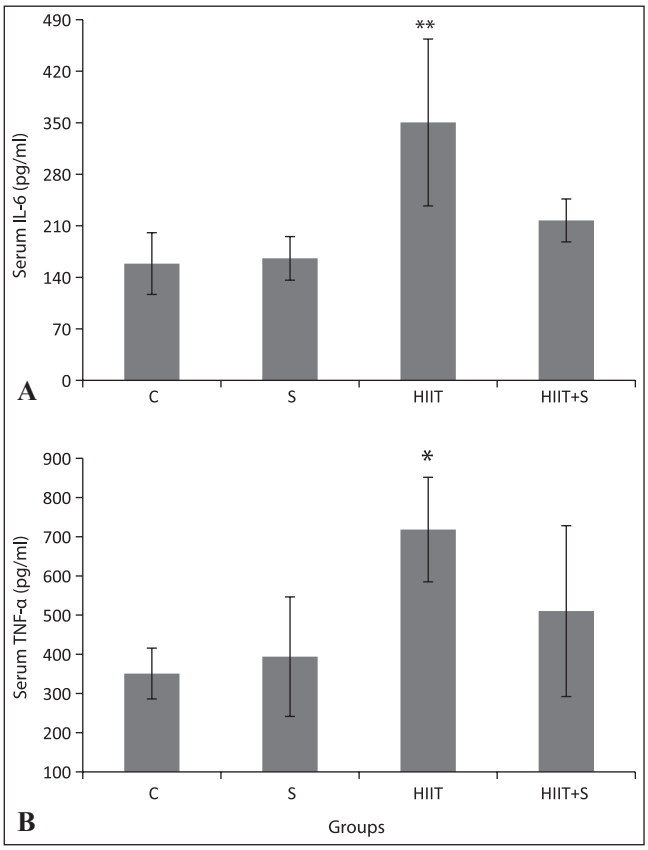

Figure 2: Effect of HIIT and vitamin E on induction of IL-6 (a), and TNF-α (b), Control (C), supplementation (S), high-intensity interval training (HIIT), and HIIT + supplementation (HIIT+S) *P < 0.05 **P < 0.001

the enhancement of these inflammatory cytokines following intensive exercise training.

It has been shown that moderate exercise training on the treadmill resulted in reduction of IL-6 and TNF-α levels in extensor digital longus of healthy rats.[11] Our findings were consistent with other studies that showed an increase in circulating IL-6 and TNF-α of rats following 6 weeks of running on the treadmill with 80% VO_2 max[15] and 11 weeks' overtraining.[14] In addition, Sun *et al*. showed that 6 weeks 'swimming reduced serum TNF-α level.[10] Reduction in inflammatory cytokines in these studies might be attributed to the increase in anti-inflammatory cytokines, especially interleukin-10 (IL-10) and reduction in IκB kinase-β (IKKβ).[12] Animals in the present study ran on the treadmill with maximal capacity; however, they were not overtrained. Muscle damage is one of the candidates for higher levels of IL-6 following intensive exercise training because it has been shown that macrophage and neutrophils penetrate to damage tissue 6-48 h after exercise and releasing IL-6,[14] and animals in this study were euthanized 48 h after the last exercise secession. The other possible reason that has been implicated in contributing to increase in cytokines levels is the recruitment of fast twitch fiber more than slow twitch fiber during intensive

exercise training.[11] IL-6 expressed more in fast twitch than in slow twitch.[21] Augmentation of IL-6 in this study may result from more recruitment of fast twitch fiber during HIIT training, which accompanies increased calcium concentration in the cytosol[11,14] and reduced glycogen content[20,22] in fast twitch fiber that affects IL-6 expression. In addition, high levels of $O_2^{\bullet-}$ and H_2O_2 induced by intensive interval training may influence on levels of IL-6 and TNF-α. Mitochondrial electron transport chain, cytosolic NADH-oxidase, and xanthin oxidase are primary sources of reactive oxygen species (ROS) during exercise training.[4,17] Intensive interval training increases $O_2^{\bullet-}$and H_2O_2 production.[17] Furthermore, high levels of IL-6 and TNF-α result in more production of ROS that causes a positive feedback.[5] Furthermore, it has been demonstrated that TNF-α increased IL-6 expression in a dose-dependent manner through $O_2^{\bullet-}$production in epithelial and fibroblasts,[3] skeletal myotubes,[5] and adipocytes.[23] However, dimethyl sulfoxide, as a hydroxyl radical scavenger,[3] and γ-tocotrienol as an antioxidant[23] inhibit IL-6 induction by TNF-α.

Our results indicate that vitamin E reduced IL-6 and TNF-α levels induced by HIIT. Our findings are in consistence with a number of studies reporting that supplementation of vitamin E may reduced IL-6 and TNF-α.[5,6] Vitamin E is the primary antioxidant that accumulates in the cell membranes. However, adding acetate and succinate to it increases vitamin E penetration to the cytosol and mitochondria of cells.[24] Vitamin E directly scavenges mitochondrial and cytosolic $O_2^{\bullet-}$ species.[24] In addition, it has been proposed that vitamin E downregulates the expression of all relevant exercise-induced ROS generation due to antioxidant activity.[25,26] Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthin oxidase have been previously introduced as primary source of $O_2^{\bullet-}$ and H_2O_2 production during exercise training that leads to inflammatory cytokine production. Furthermore, levels of xanthin oxidase, a $O_2^{\bullet-}$ and H_2O_2 production enzyme, decreases following 4 weeks vitamin E supplementation of rats that were subjected to water immersion restraint stress.[27,28] Taken together, these findings suggest that reduced activity of NADPH oxidase and xanthin oxidase by vitamin E may reduce IL-6 and TNF-α levels. Vitamin E directly inhibits the activation and translocation of NF-κB.[18] In addition, Matsunaga *et al*. (2012) demonstrated that γ-tocotrienol prevented the degradation of inhibitory-κBand and subsequently prevention of the activation of NF-κB, which in turn resulted in reduction of IL-6 production.[23] These evidences suggest that lowering the effects vitamin E on downregulation of IL-6 and TNF-α may be associated with the direct radical scavenging activity of vitamin E.

CONCLUSION

Intensive interval training increases inflammatory cytokines of IL-6 and TNF-α in rat serum, and vitamin E succinate will protect increasing inflammatory cytokines induced by high intensive training.

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Conflicts of interest

There are no conflicts of interest.

AUTHOR'S CONTRIBUTION

EG contributed in designing and conducting the study, data acquisition, manuscript preparation, and agreed with all aspects of the work. SA contributed in the conception of the work, manuscript preparation and editing, approval of the final version of the manuscript, and agreed with all aspects of the work. HF contributed in statistical analysis and agreed with all aspects of the work. TH contributed in conducting the study, manuscript preparation, data analysis, approval of the final version of the manuscript, and agreed with all aspects of the work.

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