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Oral supplementation of natural honey and levels of inflammatory and anti- inflammatory plasma cytokines during 10-week of intensive treadmill training in endurance-trained athletes.

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Abstract

The purpose of this study was to determine the effects of natural honey supplementation on levels of plasma cytokines during 10 weeks of treadmill training in endurance-trained athletes. Twenty male endurance-trained athletes (age 20 years, weight 75 kg) participated in this study. The participants were randomly assigned to exercise supplement (E+S, n=10), and exercise control (EC, n=10) groups. All subjects participated in 10 weeks of intensive treadmill training. Venous blood samples were collected immediately after exercise (T1), 1 hour after exercise (T2) and 24 hours after exercise (T3). In the EC group, 10 weeks of training increased the seminal, IL-6, Tumor necrosis factor alpha (TNF- α) immediately after exercise (T1) , 1 h after exercise (T2), 24 hours after exercise (T3)($P<0.05$). However, E+S group showed significant decrease in TNF- α and IL-6 with a corresponding increase in IL1ra immediately after exercise (T1) , 1 h after exercise (T2) and 24 hours after exercise (T3) $p<0.05$. It is proposed that oral supplementation of honey decreases inflammatory cytokine such as IL-6 and TNF- α with a corresponding increase in anti-inflammatory cytokine such as IL1ra.

Keywords: Honey; Inflammatory and anti-inflammatory cytokine; Intensive treadmill training

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Introduction

Inflammation is a response of the innate immune system, and is the reaction of the body to various stresses including cellular damage or infection caused by physical and/or chemical agents .The inflammatory response includes release of various soluble molecules called cytokines and chemokines, which mediate interactions between cells, thus affecting processes such as immunity and protein synthesis. [1,2]. As these inflammatory markers accumulate, they facilitate the infiltration and activation of neutrophils, macrophages, and lymphocytes which are needed to destroy and remove pathogens and damaged tissue. These molecules typically appear within an hour following tissue damage with neutrophils appearing on the scene first. Innate immunity provides the body with a very rapid first line of defense [3]. Myriad studies have linked exercise to a change in cytokine levels. Plasma concentrations of TNF-α, IL-1S, IL-1Ra, IL-6, IL-10, and IFN- γ have been shown to increase in response to exercise in humans [4]. Several factors including type, intensity and duration of physical work have been shown to influence this effect [5]. Strenuous exercise has been shown to increase inflammation as noted by dramatic increases in cytokines such as interleukin (IL-6) and IL-1 receptor agonist (IL-ra) [6]. A significant increase in plasma IL-6 and IL-1ra was observed immediately post-exercise. The mechanism for the effect of exercise on inflammation is unclear and has been suggested to be the result of various triggers such as hypoglycemia, low muscle glycogen, muscle damage, oxidative stress (OS), or stimulation of immune cells [6]. Over 5,000 flavonoids have been identified and are commonly found in varying amounts in fruits, vegetables, herbs, spices, teas, dark chocolate, red wine, and honey. In vitro studies consistently show reductions in inflammatory and inflammation responsive cytokines in cultures of immune cells after treatment with flavonoids [7].

Decreases in the levels of TNF-α, IL-1, IL-6, and nitric oxide (NO) are regularly reported [8]. Increases in the level of the anti-inflammatory cytokine IL-10 have also been shown, but do not follow a dose-response curve (9). One food source that may have ergogenic properties for sports such as running is honey. As a natural product, the composition of honey is highly variable. The major com-

ponents of honey are sugars, of which the monosaccharide's fructose and glucose together make up around 70% of the total product. The remaining 30% consists of disaccharides such as sucrose, and water [10]. Various forms of honey are also sources of antioxidants and have been shown to increase serum antioxidant capacity in humans after just one serving [11] showed that total plasma phenolics and plasma antioxidant capacity were increased within 1 h after ingestion of honey. Research into the effect of honey supplementation on immune system factor in athletics, is extremely limited. So we investigate the effect of honey intake on immune system factors in elite runners.

Materials and Methods

20 male endurance-trained athletes (age 20 years, weight 75 kg) were recruited into study after obtaining an informed contest. Subjects divided randomly in two E groups and E+S group. E performed exercise protocol without honey and E+S group performed exercise with honey.

Subjects in the E + S group received 50 g of honey dissolved in 200 ml tap water for 10 weeks both of groups performed treadmill exercise protocol for 1 hour with 80% vo2max 3 days per week for 10weeks. They were also instructed to consume a diet as similar as possible in each sampling days. Levels of plasma cytokines measurement Venous blood samples were collected immediately after exercise (T1), 1 hour after exercise (T2), 24 hours after exercise (T3).

Venous blood was drawn at each time point using tubes containing no anticoagulant or the anticoagulants ethylenediamine tetra acetic acid or heparin Blood collected in a serum separation tube, stood for 15 min and was centrifuged at 2500 g for 15 min. All serum and plasma samples were then. Stored at -80oC until analysis.Plasma IL-6, TNF- α, and serum IL-1ra were measured in duplicate using high sensitivity ELISA procedures (R & D Systems, Minneapolis, MN.

Results

E group showed increased significantly in levels of TNF-α and IL6 and decreased significantly in IL1ra immediately after exercise (T1), 1 h after exercise (T2), 24 h after exercise (T3) $p<0.05$, but E+S group showed decrease significantly in levels of TNF and IL6 and increased significantly in IL1ra immediately after exercise (T1) ,1 h after exercise (T2),24 hours after exercise (T3) $p<0.05$.

Comparison of plasma cytokines in different groups of the study **TNF**

TNF-α	T3	T2	T1	Group/time
	12.6	11.05	10.2	E
	8.11	7.09	7.01	E+S
	0.004*	0.007*	0.005*	sig

IL6	T3	T2	T1	Group/time
	13.07	12.05	9.05	E
	7.01	8.3	6.2	E+S
	0.002*	0.001*	0.008*	sig

IL1ra	T3	T2	T1	Group/time
	9.13	9.07	9.01	E
	14.09	13.05	12.4	E+S
	0.001*	0.000*	0.001*	sig

Conclusion

Results of the present study indicated that mean values for seminal IL-6, , and TNF- α in the E group increased significantly after 10 weeks of intensive treadmill training compared with E+S group. Increase in the levels of plasma IL-B, IL-6, IL-8, and TNF-α in response to intense and prolonged exercise programs have been reported previously [12, 13]. Previous studies indicate that prolog and training with high intensity to initiate an inflammatory response in whole body and subsequently in cyclists' seminal plasma, and thereby increase the levels of seminal cytokines IL-1B, IL-6, IL-8, and TNF- α, Our finding demonstrate that the honey supplementation lead to significantly decreased the levels of IL-6 and TNF- α Immediately, 1 hour and 24h after exercise in the E + S group. The beneficial effect of honey on immune responses during different types of exercise programs has also been studied [14]. There are less reports to show the effects of honey supplementation on immunological response of

human .also our result showed that intake honey supplementation cause to increase il1 ra that it is anti inflammatory cytokine. Honey is types of falvonoid compounds that act as antioxidants; and has also been reported to increases serum antioxidant capacity in humans. Honey has modulatory effects on immune system of human. Finding showed that doing exercise with high intensity or long duration lead to increase free radical and decrease antioxidant defense [15].

There seems little doubt that relationships between exercise, immune function inflammation, and cytokines exist.Plasma concentrations of TNF- α, IL-1S, IL-1Ra, IL-6, IL-10, and IFN- γ can increase in response to exercise in humans [16, 5]. Several factors including type, intensity and duration of physical work have been shown to influence this effect [17]. Exercise-induced increases in inflammatory cytokines can result In vitro studies consistently show reductions in inflammatory cytokines in cultures of immune cells after treatment with quercetin, a flavonoid found in a variety edible plants including numerous berries, onions, apples, tea leaves, broccoli and honey.Decreases in the levels of TNF-α, IL-1, IL-6, and nitric oxide (NO) are regularly reported [18]. Increases in the level of the anti-inflammatory cytokine IL-10 have also been shown Several factors including type, intensity, and duration of physical work have been shown to influence the effect of exercise on cytokine levels in humans (16). The same discrepancy can be found in studies measuring concentrations of IL-1, with some studies showing increases and others showing no change [19]. In a review paper, Pedersen et al. postulated that these differences are likely due, at least in part, to intensity, type, and duration of the exercise. In the case of IL-6, previously studies demonstrated that eccentric-type exercise was much more likely to cause a detectable IL-6 increase than was concentric exercise, and a recent review offered convincing evidence that IL-6 levels do not rise significantly unless aerobic exercise, such as bicycling or running, is performed for at least 30 – 60 minutes [20] .

In conclusion, we found that 10 weeks of long-term intensity treadmill training significantly increased a decrease anti inflammatory cytokines. Levels of plasma cytokines IL-6 and TNF- α Also we found that intake honey supplementation lead to decrease inflammatory cytokine such as IL6 and TNF- α and increase anti inflammatory cytokine such as IL1ra.Present study is the first research that show the beneficial effects of natural honey supplementation during long-term intensity treadmill training on inflammatory and anti inflammatory cytokines .

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