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Authors' Contribution:

A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
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abstract

Background: This study was conducted to determine acute effects of carbohydrate-electrolyte (CHO-E) found in sports drinks, ingested just before an orienteering competition, on the levels of plasma IL-6 and IL-1 β cytokine.

Material and methods: The study was conducted in a randomized, double-blind design, including 23 elite orienteering athletes who volunteered to participate. Following the collection of resting specimens of blood, the experimental group (n = 12) was administered a sports drink containing CHO-E, while the placebo group (n = 11) was administered 500 ml of plain water. The subjects were asked to finish the orienteering competition.

Results: IL-1 β level in the 24th hour after the competition was found to be significantly lower than in pre-competition and 2 hours after the competition in the placebo group ($p < 0.05$). The IL-6 levels of the experimental group in the 24th hour after the competition were significantly lower than those measured at the end of the competition and in the 2nd hour following the competition ($p < 0.05$). In intergroup comparisons, no significant differences were detected in the IL-1 β and IL-6 levels ($p > 0.05$).

Conclusions: A drink containing CHO-E might have stopped muscle contraction-associated cytokine production by increasing the tissue stability of the muscles, compensating the loss of fluid and electrolytes from athletes' bodies.

Key words: sports drink, cytokine, orienteering, IL-6, IL-1 β , carbohydrate.

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INTRODUCTION

Sustainable amount of research has been going on for years to improve and maintain the performance items in sports. Isotonic sports drinks and ergogenic aids are used to improve performance. Sports drinks are developed to compensate for athletes' fluid, electrolyte and energy expenditures, to provide early recovery, or to create positive effects on the immune system [1, 2].

Cytokines, which are in polypeptide and glycoprotein structures, are immunoregulatory molecules, important for the inflammatory and immunological reactions [3]. Cytokines form a different kind of intracellular signaling molecules [4], acting as molecular messengers among the immune system elements, and this way, they coordinate the growth, differentiation, and functional activation of all cells associated with the immune reaction [5, 6, 7]. Cytokines are classified as pro and anti-inflammatory cytokines depending on their physiological effects [8, 9]. Pro-inflammatory cytokines are monitored to determine the inflammatory reactions following muscle injury. Tumor necrosis factor (TNF) consist of TNF- α and interleukine IL-1 β . On the other hand, anti-inflammatory cytokines are the ones blocking the pro-inflammatory cytokines, diminishing their activity. IL-1 (ra), an antagonist of the IL-4, IL-10 and IL-1 receptors, belongs to the class of antiinflammatory cytokines. However, IL-6 acts as both a pro-inflammatory and an anti-inflammatory cytokine, depending on the situation [8, 9, 10, 11]. Production of cytokines IL-1 β and IL-6 starts in the plasma due to injuries to muscles and tissues. Depending on the form and severity of the exercise being performed, muscle injuries develop at the cellular level [12]. As regards to a reaction the exercise, IL-6 is the first cytokine to increase, in varying amounts, depending on the extent of the load and the severity of the exercise [13, 14].

According to some study reports, IL-1 β levels increase two-fold after marathons and strenuous exercises, depending on the distance taken and the level of energy. On the other hand, plasma IL-6 levels increase 100-fold. When the factors which increase IL-6 during exercises are evaluated, it has been demonstrated that the duration of the exercise and the muscle mass used are the major indicators [11, 15, 16].

The aim of the study is to determine the effects of a sports drink containing CHO-E ingested before the competition acutely and in a single dose, on the plasma levels of cytokines IL-6 and IL-1 β in orienteering athletes.

MATERIAL AND METHODS

The study was conducted in a randomized, double-blind design among elite orienteering athletes, aged 14–17 years who voluntarily participated in the study. They were allocated either to the study group (n = 12) (160.25 \pm 7.95 cm, 48.03 \pm 7.93 kg, 19.11 \pm 1.91 kg / cm²) or to the placebo group (n = 11) (165.90 \pm 10.42 cm, 52.21 \pm 7.75 kg, 18.88 \pm 1.38 kg / cm²). Upon reading The Declaration of Helsinki of the World Medical Association to the study participants and informing their families, coaches and themselves on the study in detail, the participants' informed consent was collected.

After taking the first resting blood samples, a sports drink with carbohydrate and electrolyte content (137 kcal, containing 32 g carbohydrates, 120 mg calcium, 248 mg chloride, and 230 mg sodium in 500 ml water) was administered to the

study group. The placebo group received 500 ml of plain water. In order to prevent the psychological effects, the drinks were prepared with care in bottles covered so that the color of the content was hidden. The athletes were asked to consume all of the content, i.e. 500 ml, in the bottles. Due to the fact that the study was within the frame of the camping schedule, the same standard diet was administered to both groups of study participants by the camp dietitian. For this study the approval of the non-interventional ethics committee of the university was obtained with the decision number 2015/3.

The competition took place in an area with 20 obstacles in a distance varying from 7 to 12 km with completion of the target within 60–80 minutes on the blue track, which is one of the advance level categories [17].

Venous blood samples of 15 cc were collected from the athletes' forearms before the competition, after the competition, and in the 2nd and 24th hour after the end of the competition, making a total of 4 blood specimens for 1 study participant. The blood samples were collected into EDTA containing vacutainers and then plasma was separated by centrifugation at 2800 g for 10 min at room temperature. The upper portions of the samples in the tubes were transferred into the Eppendorf tubes and stored at -80°C until they were studied. Plasma concentrations of IL-6, IL-1β were analyzed by the ELISA method (i.e., enzyme-linked immunosorbent assay) with the use of a commercial kit (Immunotech, Marseille, France).

The normality of the distribution was tested using the Kolmogorov-Smirnov test. To perform the inter-group comparisons, a non-parametric Kruskal-Wallis ANOVA was used for between-group analyses. Friedman's ANOVA was used to compare plasma IL-6 and IL-1β levels during the four study time-points. If required, a post-hoc test was applied to detect the source of the differences. The significance level was determined with $p < 0.05$.

RESULTS

There were no statistically significant differences between the groups regarding the age, height, body weight, body mass index and body fat percentage ($p > 0.05$) (Table 1). Although pre- and post-competition IL-6 and IL-1β levels did not significantly differ among the groups ($p > 0.05$), 2 hr and 24 hr post-competition IL-6 and IL-1β levels were significantly different between the groups ($p < 0.05$) (Table 2).

Table 1. The physical properties of the Placebo and CHO-E groups

Group	Height (cm)	Body Weight (kg)	BMI (kg/cm ²)	Body Fat Percentage (%)
Placebo n = 11	165.9 ±10.4	52.21 ±7.8	18.88 ±1.4	16.22 ±6.9
CHO-E n = 12	160.3 ±8.1	48.03 ±7.9	19.11 ±1.9	16.10 ±7.6

Table 2. Plasma IL-6 and IL-1β concentrations

	IL-6 (pg/mL)			IL-1β (pg/mL)	
	Placebo	CHO-E		Placebo	CHO-E
Pre-Competition	1.19 ±1.20	2.07 ±1.96	Pre-Competition	22.75 ±3.13	15.67 ±3.89
Post-Competition	5.75 ±4.56	4.70 ±3.52	Post-Competition	26.88 ±7.63	25.45 ±6.06
2 hr Post-Competition*	4.12 ±2.95	1.83 ±2.15	2 hr Post-Competition*	31.79 ±8.63	13.37 ±4.70
24 hr Post-Competition*	1.44 ±3.15	0.15 ±0.36	24 hr Post-Competition*	29.21 ±10.18	17.49 ±12.60

* $p < 0.05$

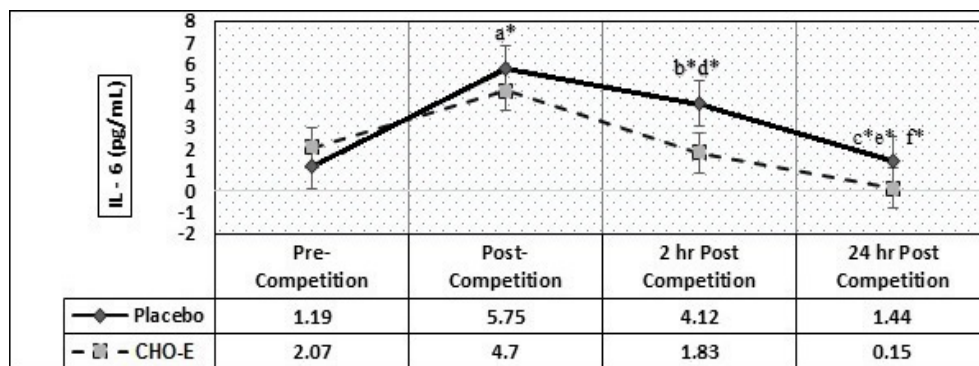


Fig. 1. Plasma interleukin 6 (IL-6) concentration count. Data are mean \pm SD (Placebo n = 11, CHO-E n = 10). ^aSignificantly different from pre-competition values for the placebo group $p = 0.010$. ^bSignificantly different from pre-competition values for the placebo group $p = 0.015$. ^cSignificantly different from post-competition values for the placebo group $p = 0.038$. ^dSignificantly different from pre-competition values for the CHO-E group $p = 0.014$. ^eSignificantly different from post-competition values for the CHO-E group $p = 0.006$. ^fSignificantly different from 2 hr post competition values for the CHO-E group $p = 0.024$; * $p < 0.05$

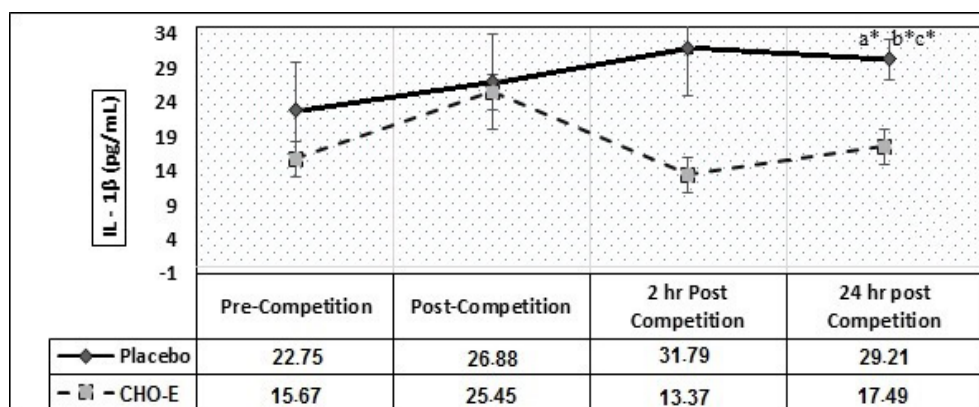


Fig. 2. Plasma interleukin 1 β (IL-1 β) concentration count. Data are mean \pm SD (Placebo n = 9, CHO-E n = 12). ^aSignificantly different from pre-competition values for the placebo group $p = 0.005$. ^bSignificantly different from post-competition values for the placebo group $p = 0.014$. ^cSignificantly different from 2 hr post competition values for the placebo group $p = 0.016$; * $p < 0.05$

Long and strenuous exercise exerts an acute effect on the immunity system. This situation is associated with increased incidences of infections in the following weeks after extreme sports competitions, such as marathons and ultramarathons [18]. As the inflammation commences, leukocytes start the production of cytokines, such as IL-1 β , IL-6 and TNF- α [19]. Study reports emphasize remarkable changes in the blood levels of cytokines IL-1 β , IL-6 and TNF- α following endurance exercises. This is indicative of the magnitude of the increase in the number of blood cytokines in association with the severity of the exercise and cytokine's effect on the development of muscle damage. After the tissue damage, the first cytokines to display increases in the plasma concentrations are demonstrated to be TNF- α , IL-1 β and IL-6 [13, 14].

Due to the fact that orienteering competitions take place both on flat grounds and in inclined fields requiring climbing, muscles undergo both eccentric and concentric contractions. During downward sloping runs, muscle contractions are mainly eccentric, and this causes more muscle damage compared to the runs on the flat grounds, when both concentric and eccentric muscle contractions occur [20]. During the eccentric exercises with skeletal muscle damage when the muscles elongate on contraction, increased interleukin-1 β levels were found in the muscle tissue, and they were demonstrated to be

be maintained at high levels on the 5th day after the exercise [15]. It is recognized that the extreme-level eccentric exercises cause significant increases in the plasma levels of IL-6 [21]. Studies report that the IL-6, which is produced in the early phase of the exercise, is associated with muscle contractions, whereas IL-6, which is produced in the late phase, is associated with muscle damage. IL-6 has been demonstrated to be produced by monocytes which migrate to the areas of the tissue damage following exercises causing muscle damage [16, 21, 22]. The plasma levels of IL-6 have been reported to increase almost 100-fold after long-term challenging exercises, such as marathons, whereas TNF- α and IL-1 β do not change significantly [4, 11, 15]. Anti-inflammatory cytokines (IL-6) are recognized as the ones hindering the pro-inflammatory (IL-1 β) cytokines by diminishing their activity [8, 9, 10, 11]. Our study results demonstrate that increases in IL-6 levels, detected in the blood specimens collected at the end of the exercise, and in the 2nd and 24th hour following the exercise result from the muscle damage thus suggesting that the increase in IL-6 levels decreased the levels of IL-1 β , which is the pro-inflammatory cytokine in the 24th hour of the recovery phase. In the study group receiving the isotonic drink, the muscle damage was not at sufficient levels to enhance IL-6, therefore, no significant changes were detected in the levels of IL-1 β .

Plasma cytokine levels were investigated, and increases in the levels of cytokines such as TNF- α , IL-1 β and IL-6 were observed after challenging exercises [23]. Another study detected two-fold increases in the plasma levels of IL-1 β , compared to the levels before the exercise, in 10 athletes in recreational good condition [24].

Another factor in terms of an athlete's health and performance is the fluid and electrolyte balance. Fluid and electrolyte balance is recognized to be of importance for the optimum exercise performance and for the restoration of health. The increased need for the fluid intake during long-term exercises and the decreases in sodium intake, as well as the marginal insufficiencies in the calcium, magnesium, potassium, phosphorus levels, may lead to decreases in the sportive performances. Therefore, it is reported that the intake of sports drinks by appropriate protocols before, during and after the exercises may prevent the decreases in the performance [25, 26 27]. Studies supporting our results report decreased levels of IL-6 in athletes with the intake of a carbohydrate drink in the 30th minute after a challenging exercise, compared to the placebo group, whereas, the IL-6 release was reported to be maintained in the placebo group during and after the exercise [28]. Another study has demonstrated the increases in the levels of IL-6 by carbohydrate intake [29]. Miles et al. [30], evaluated changes in the plasma levels of IL-6 in athletes who were asked to consume a solution of isoleucine, leucine, valine, sodium, potassium, and magnesium during a mountain trail race of 32 km. When the levels of IL-6 were evaluated in the study and placebo groups, it was reported that the solution suppressed the IL-6 release after the competition compared to the placebo group [30].

In this present study aiming to evaluate the effects of an isotonic sports drink, ingested acutely as a single dose before the competition, on the plasma levels of cytokines IL-6 and IL-1 β , it was observed that the isotonic aids decrease the inflammatory reactions in the circulation, increased the muscle and tissue stability, and prevented cytokine production associated with muscle contractions. Several studies have reported similar results [28, 29, 30].

In addition, it may lead to increases in the sportive performance by its positive effects on the cellular energy metabolism [31].

CONCLUSIONS

In conclusion, the observation of the decreased releases of the IL-1 β and IL-6 in the CHO-E group consuming CHO-E contained in sports drinks following the competition is remarkable.

Considering the results of the study, we recommend the consumption of the sports drinks containing CHO-E before or during the competitions, as they suppress the inflammatory reactions in the circulation.

However, further studies are recommended to evaluate the reactions in varying types and severity of exercises.

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