Effects of Pectin Polysaccharides on Leukocyte Migration into the Oral Cavity during Exercise

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The effects of pectin polysaccharides on leukocyte migration into the oral cavity during exercise were studied. The migration and hence, counts of neutrophils in the oral cavity increased during exercise. Pectins reduced neutrophil migration into the oral cavity during exercise because of hyperstimulation of their functional activity.

Key Words: *pectin; exercise; neutrophils*

Pectin polysaccharides, plant cell wall components, are present in human nutrition rations. Many of them have been isolated and purified and are used as sources of alimentary fibers, prebiotics, and bactericidal. Oral pectins are characterized by anti-inflammatory [6,8] and immunostimulatory [5,7] effects. Intensive exercise can cause an inflammatory reaction: increase in functional activity of leukocytes and blood concentrations of proinflammatory cytokines and LPS [2,3,11]. Leukocyte migration to and accumulation in the focus of inflammation is one of the key stages of inflammation. The neutrophil counts and levels of their migration to the oral cavity depend on the blood neutrophil counts and presence of inflammation in the oral cavity tissues [13].

However, the effects of pectin polysaccharides on neutrophil migration to the oral cavity in humans have never been studied.

We studied the effects of pectin polysaccharides on the leukocyte migration to the oral cavity in exercise.

MATERIALS AND METHODS

The study was carried out in 8 male volunteers (Table 1). None of them had inflammatory processes in the periodontium or other body tissues by the moment of the study. All volunteers have been informed about the study and signed consent about their voluntary participation in the experiment.

Pactovit, a pectin drink (manufactured by R. Zh. Tkhakarov in collaboration with the Pectin Foodstuffs Company), served as the source of pectins. The components of the drink are beet and apple pectins, fructose, and citric acid. The volunteers received the pectin drink 3 times during one day (during breakfast, lunch, and dinner), 1.2 g pectin per meals (a total of 3.6 g/day).

The exercise inducing the immune system response was performed on an Ergometer X1 device (Kettler). The exercise intensity during the first 5 min was 60 W, after which it increased by 30 W every 5 min (Table 1) [10]. The pedal rotation velocity had to be maintained at the level of 60-65 rpm. The exercise was discontinued after heart rate surpassed 170 bpm or it was impossible to maintain cycling velocity during more than 1 min after the next increase of exercise intensity. It is known that exercise gradually increasing in intensity causes an immune response consisting in elevation of the blood cytokine concentrations and leukocyte count [4,9,12].

The blood was collected and oral fluid was washed off during the morning hours. Venous blood was collected into sterile vacutainers (Becton Dickinson). Washing from the oral cavity was made with a sterile 0.9% NaCl solution: the volunteers took 5 ml of the solution into the mouth, rinsed the oral cavity during 3-5 sec, and spat out the fluid into tubes. In order to evalu-

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TABLE 1. Anthropometric Characteristics of Volunteers and Exercise Levels

Parameter	Median	Min-Max
Age, years	26	24-34
Height, kg	80	67-103
Body length, cm	178	170-189
BWI, kg/m^2	24.7	21.6-33.3
Maximum exercise intensity, W	195	120-270
Exercise duration, min	23	14-36
Maximum heart rate, bpm	158	135-170

Note. BWI: body weight index; Min-Max: minimum and maximum values.

ate the leukocyte migration, the volunteers collected the oral washout fluid every minute during several minutes. Blood specimens were centrifuged at 400*g* for 15 min, plasma was collected and frozen at -40°C.

Leukocytes were counted in specimens of the blood and oral washout fluid in Goryaev chamber. The concentration of TNF-α was measured by EIA with ProCon TNF-α kits (Protein Contour).

The results were statistically processed by non-parametric paired Wilcoxon test using Statistica 9.0 software. The values in the same volunteers before pectin drink intake served as the control for paired comparison in the related sample. The differences were considered significant at $p<0.05$.

RESULTS

Blood neutrophil count and plasma TNF-α concentration changed negligibly under conditions of exercise used in our study. The neutrophil count in the oral cavity increased significantly after exercise: by 4.7 times after 1 h and by 5.1 times after 2 h (Table 2). The increase in the cell count was due to a significant increase of cell migration into the oral cavity: by 3 and 3.1 times 1 and 2 h after exercise, respectively.

The blood neutrophil count increased significantly after pectin drink: from 4050 cell/μl before exercise to 5686 cell/μl 2 h after it. However, pectins caused no appreciable changes in the cell counts in comparison with their levels before the drink. The concentration of TNF-α increased significantly during exercise after the pectin drink intake (Table 3). The neutrophil counts in the oral cavity did not change after exercise following pectin drink intake, as the level of cell migration to the oral cavity did not change. However, 2 h after exercise the neutrophil migration and count in the oral cavity were significantly lower after pectins than before the drink.

These data indicate that pectins present in the bio-additive reduce the neutrophil migration to the oral cavity during exercise. It is known that the functional activity of the oral neutrophils is low, while that of blood neutrophils is high during inflammatory processes in the oral cavity, caused by microorganisms or their vital activity products. Restimulation of oral neutrophils in patients with inflammatory processes

TABLE 2. Neutrophil Counts before and after Exercise

Neutrophils	Pectin	Exercise				
		before exercise	directly after exercise	after 1 h	after 2 h	after 24 h
Blood, cell/μl	Before	5000 (2950-6150)	3852 (3481-5337)	4585 (4285-6322)	5147 (4217-6328)	3535 (3100-3941)
	After	4050 (3300-4575)	4012 (3643-5436)	4535 (3006-6197)	5686$^+$ (3899-6324)	5476 (2977-5938)
Oral cavity, cell/μl	Before	90 (50-140)	280 (70-450)	420$^+$ (285-865)	460$^+$ (290-1380)	80 (50-100)
	After	120 (80-165)	430 (270-550)	310 (145-445)	260* (105-385)	60$^+$ (40-130)
Migration to oral cavity, cell/μl/min	Before	60 (50-120)	115 (78-150)	180$^+$ (125-248)	185$^+$ (165-323)	90 (50-230)
	After	100 (50-153)	170 (123-335)	150 (90-288)	135* (60-225)	110 (65-135)

Note. Here and in Table 3: the median is presented (lower and upper quartiles are shown in parentheses). $p<0.05$ in comparison with the corresponding parameter *before pectin, $^+$before exercise.

TABLE 3. Plasma Concentrations of TNF-α before and after Exercise

TNF-α, pg/ml	Exercise				
	before	after	after 1 h	after 2 h	after 24 h
Before pectin intake	84 (40-141)	78 (41-120)	92 (42-113)	112 (47-137)	111 (39-162)
After pectin intake	99 (43-142)	184*+ (113-374)	126 (54-153)	121 (50-260)	146 (42-169)

in the oral cavity leads to their hyporeactivity [1]. The immunostimulatory effect of pectins has been described [5,7]. Blood leukocyte reactivity increases, as the concentration of TNF-α increases during exercise after pectin intake and virtually does not change before the bioadditive. Comparison of blood neutrophil counts before and after pectin intake showed no difference in these blood cell counts during exercise before pectins and their counts after pectin drink. These data indicate that neutrophil reactivity increases after pectin drink, but exercise stimulates these cells as well, and due to this double stimulation the cells are hyperstimulated, which leads to reduction of their activity. In the control, neutrophil migration and their counts in the oral cavity increased 1 h after exercise, while after 2 h (recovery after exercise) these parameters started to decrease; after pectin intake, leukocyte migration into the oral cavity did not increase during exercise.

Hence, pectin polysaccharides reduce the leukocyte migration into the oral cavity at the expense of their reactivity increase, this leading to inhibition of cell activity during exercise.

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