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ORIGINAL ARTICLE

Pleuran (β-glucan from *Pleurotus ostreatus*) supplementation, cellular immune response and respiratory tract infections in athletes

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Abstract Prolonged and exhausting physical activity causes numerous changes in immunity and sometimes transient increases the risk of upper respiratory tract infections (URTIs). Nutritional supplements as countermeasures to exercise-induced changes have increasingly been studied in the last decade. One of the most promising nutritional supplements is β-glucan, a well-known immunomodulator with positive effects on the function of immunocompetent cells. In this double blind, placebo-controlled study, we investigated the effect of pleuran, an insoluble β-(1,3/1,6) glucan from mushroom *Pleurotus ostreatus*, on selected cellular immune responses and incidence of URTI symptoms in athletes. Fifty athletes were randomized to pleuran or placebo group, taking pleuran (commercial name Imunoglukan®) or placebo supplements during 3 months. Venous whole blood was collected before and after 3 months of supplementation and additionally 3 months after supplementation period was completed. Incidence of URTI symptoms together with characterization of changes in phagocytosis and natural killer (NK) cell count was monitored during the study. We found that pleuran significantly reduced the incidence of URTI symptoms and increased the number of circulating NK cells. In addition, the phagocytosis process remained stable in pleuran group during the study in contrast to placebo group where significant reduction of phagocytosis was observed. These findings indicate that pleuran may serve as an effective nutritional supplement for athletes under heavy physical training. Additional research is needed to determine the mechanisms of pleuran function.

Keywords Beta-glucan · Pleuran · Natural killer cells · Exercise · Innate immunity · Athletes

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Introduction

Prolonged and exhausting physical activity sometimes causes immunosuppression, whereas moderate physical load improves immune function and reduces the risk of respiratory tract infections. In regularly exercising active athletes, physical stress may impair immune function and induce depression of immune response (Nieman 1997). In addition, during prolonged exercise, exposure to airborne pathogens is increased due to higher rate and depth of breathing. Exercise-induced perturbations in cellular and humoral aspects of immunity are related to upper respiratory tract infection (URTI) susceptibility (Gleeson 2006b). The relationship between exercise and susceptibility to infection has been modeled in the form of a "J"-shaped curve (Nieman 1994). This model assumes that the risk of URTIs increases with the intensity of the physical load.

Athletes must train hard for competition and it is important to keep their immune systems robust and avoid illness. Exercise-induced immune dysfunction does not put athletes

in danger of serious illness, but it could be sufficient to increase the risk of URTI or influenza development. The appearance of URTI symptoms at a crucial time in the athlete's training or competition schedule can have adverse effects on performance. It has been suggested that immunonutrition for heavily exercising athletes is needed and helps maintain immunity and health (Nieman 2008). The influence of nutritional supplements on the immune response and incidence of URTI to heavy physical load are an interesting area of research (Nieman and Bishop 2006; Nieman et al. 2003, 2005, 2006, 2007a, b). The vast majority of studies have focused on carbohydrate (CHO) supplementation. CHO supplementation has been shown to influence cell distribution, lymphocyte proliferation, natural cell-mediated cytotoxicity and distribution of selected cytokines (Gleeson 2006a; Lancaster et al. 2003; Bishop et al. 2001; Nieman 1998; Nieman et al. 1998; Rohde et al. 1998). Antioxidants, another promising countermeasures, do not alter exercise-induced changes in immune function but they are able to reduce URTI symptoms in athletes (Peters et al. 1993, 1996; Nieman et al. 2002, 2007b). Except for carbohydrate supplementation, the available data for other nutritional agents do not provide sufficient evidence to support their role in preventing exercise-induced immune suppression.

β-glucans, glucose polymers, are derived from a variety of sources including yeast, grain, or fungus, belong to the class of drugs known as biological response modifiers (Bohn and BeMiller 1995) and numerous studies have shown that (1,3)-β-D-glucans enhance the immune functions, such as anti-infective, antitumor, and immunostimulatory activity (Pretus et al. 1991; Williams et al. 1996; Browder et al. 1990; Sherwood et al. 1986, 1987). A few human clinical studies demonstrated that β-glucan decreased infection incidence in surgical patients, need for antibiotics and it shortened intensive care unit length stay (Babineau et al. 1994a, b; Dellinger et al. 1999). Furthermore, rodent studies indicated that oat β-glucan supplements offset the increased risk of infection associated with exercise stress (Davis et al. 2004a, b; Murphy et al. 2007, 2008, 2009).

The aim of this double blind, placebo-controlled study was to investigate the effects of pleuran (commercial name Imunoglukan®) supplementation on URTIs incidence and on changes in selected immunological parameters, particularly those of non-specific cellular immunity, in athletes over a training period.

Methods

Subjects

In total, 50 healthy men ($n = 26$) and women ($n = 24$) top-level athletes in different sport disciplines from the National Sport Centre were recruited for the study. The experimental pleuran group consisted of athletes ($n = 25$) engaged in canoeing ($n = 8$), mountain biking ($n = 3$), swimming ($n = 6$), shooting ($n = 2$), running ($n = 5$), and cycling ($n = 1$). The placebo group consisted of athletes ($n = 25$) engaged in canoeing ($n = 7$), mountain biking ($n = 3$), swimming ($n = 5$), shooting ($n = 2$), running ($n = 2$), cycling ($n = 3$), and cross-country skiing ($n = 3$).

The experimental procedures were performed in accordance with the Helsinki Declaration, as revised, for clinical research involving humans. Before the entrance examination, the athletes were randomly divided into two groups, placebo and pleuran group. No significant differences regarding age, body composition, or maximal performance measure during the study were found between the groups (Table 1). Subjects signed informed consent documents after the study details were explained. Subjects were examined before the administration of Imunoglukan® or placebo, after 3 months of administration and after the next 3 months without using Imunoglukan® or placebo. Athletes that met entrance criteria were required to take two capsules of Imunoglukan® (100 mg of β-glucan and 100 mg of vitamin C) or placebo (vitamin C 100 mg only) in the morning on an empty stomach for 3 months. No side effects were recorded by the participants during the study, and none of the athletes had to be excluded because of medical conditions or other reasons.

Research design

To evaluate the effect of pleuran, venous blood samples were collected into an evacuated tube and treated with either EDTA or sodium heparin. Subjects were monitored before and after the supplementation period for symptom score, which included occurrence of fever, increased fatigue, use of antibiotics, occurrence of cold, cough, and herpes. Among laboratory parameters, phagocytosis and natural killer (NK) cell count were monitored.

Table 1 Demographic basic characteristics for athletes in pleuran and placebo groups

Characteristic	Pleuran ($n = 25$)	Placebo ($n = 25$)	P
Male:female (n)	14:11	12:13	
Age (years)	23.6 ± 0.8	24.0 ± 0.9	0.742
BMI (kg m^{-2})	22.8 ± 0.6	23.7 ± 0.5	0.284
$VO_{2\ peak}$ (mL kg^{-1} min^{-1})	59.5 ± 1.5	58.7 ± 1.4	0.708

Data represent mean ± SEM

Incidence of URTI symptoms

Self-reporting of URTI symptoms of using an invalidated physical health questionnaire before and after the supplementation period was used in this study. Questionnaires addressed overall health status and URTI symptoms. The URTI-related symptoms measured included nasal congestion, runny nose, sore throat, sneezing, and cough. In addition, non-specific URTI symptoms, such as fatigue, headache, general malaise, and body aches, were also included in questionnaires.

Preparation of *Staphylococcus aureus* bioparticles

Two milligrams of FITC-labeled *Staphylococcus aureus* (*S. aureus*) bioparticles (Invitrogen, UK) was resuspended and thoroughly mixed in the small amount of sterile Dulbecco's phosphate-buffered saline (D-PBS). The equal volume of *S. aureus* BioParticles opsonizing reagent (Invitrogen, UK) was added and thoroughly mixed. The suspension was incubated in the dark at 37°C for 60 min and subsequently incubated at room temperature for a period of 45 min under shaking condition. After incubation, bioparticles were washed fourth with sterile D-PBS and finally re-suspended in D-PBS to a final concentration of 10^8 cells/ml.

Phagocytosis

The test of polymorphonuclear leukocytes (PMNs) function was performed according to (Heinzelmann et al. 1997) with some modifications. Briefly, heparinized whole blood (100 µl) was mixed with FITC-labeled *S. aureus* bioparticles at a final concentration 10^7 cells/tube. The mixture was incubated at 37°C. After 30-min incubation, samples were placed immediately on ice to stop phagocytosis. The red blood cells were lysed on ice with cold IOTest 3 lysing solution (Beckmann Coulter, Brea, CA, USA) for 30 min according to the manufacturer's instructions. The samples were immediately subjected to flow cytometry analysis. Trypan blue was used to quench the extracellular fluorescence to eliminate non-specific *S. aureus* binding to the PMNs cell surface.

Flow cytometry

The fluorescence from FITC-labeled *S. aureus* was measured by flow cytometry (BeckmanCoulter FC500, USA). Fluorescence values of the granulocytes population were collected after defining this region by right and forward-angle light scatter signals. The percentage of FL1-positive (FITC) PMNs determined the percentage of phagocytosis.

NK cells

NK cells from whole blood were counted on a flow cytometer FC500 (Beckmann Coulter, USA) after staining with monoclonal antibodies marked with fluorescent dye (CD45-FITC/CD16CD56-RD1/CD19-ECD/CD3-PC5) (Immunotech, Prague, Czech Republic) according to the manufacturer's instructions. Absolute and percentage counts of NK cells (CD3-CD16/56+) were determined.

Statistical analysis

Data are expressed as the mean with the standard error of the mean (SEM) of the measured parameters calculated for the whole group (pleuran or placebo). Data in all figures were analyzed using two groups and three time points' repeated-measures ANOVA. When interaction effect was significant, changes within and between both groups were analyzed using Student's *t* test. *P* values <0.05 were considered significant. Analyses were performed using GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA).

Results

URTI symptoms

Figure 1 shows the changes in reported URTI symptoms in both therapeutic groups before and after 3 months of

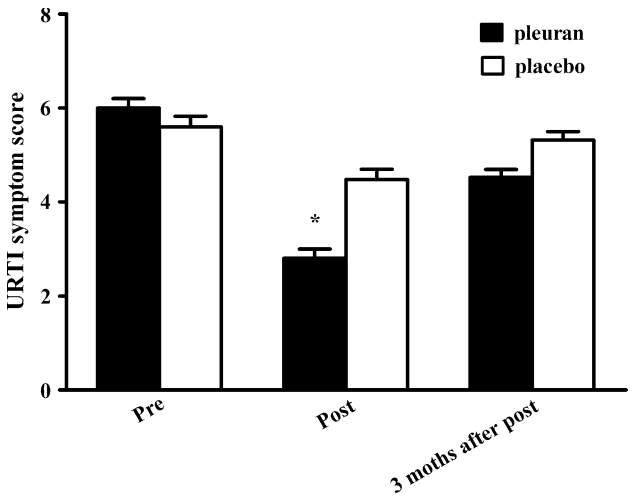

Fig. 1 Upper respiratory tract infection symptom score in both therapeutic group of athletes before (Pre) and 3 months after (Post) supplementation, and after additional 3 months without supplementation (3 months after post). Significant difference ($P < 0.001$) in pattern of change in frequency of URTI symptoms between pleuran and placebo group. *Significant decrease ($P < 0.001$) in URTI symptoms in pleuran group before and after supplementation. Data represent mean ± SEM

supplementation and after additional 3 months without supplementation. Significant time effects ($P < 0.001$) were measured for URTI symptoms. There was a significant difference ($P < 0.001$) in pattern of change in frequency of URTI symptoms between pleuran and placebo group. After 3 months of supplementation, 3 (12%) and 21 (84%) of the athletes in the pleuran and placebo groups, respectively, reported at least four symptoms associated with URTIs. In total 65 and 117 episodes of URTIs in pleuran and placebo group, respectively, were reported during the supplementation period. In addition, a significant decrease ($P < 0.001$) in URTI symptoms was observed in pleuran group after supplementation.

Phagocytosis

Significant time effects were measured for phagocytosis ($P < 0.001$) and the groups showed different patterns of changes ($P < 0.05$). A significant reduction of phagocytosis ($P < 0.001$) below the baseline value of 5% was observed in the placebo group after 3 months of supplementation (Fig. 2). No reduction of phagocytosis was found within pleuran group. After 3 months of supplementation, there was a significant difference ($P < 0.01$) in phagocytosis between pleuran and placebo group.

NK cell count

The changes in the number of NK cells before and after supplementation period and after additional 3 months period without supplementation within both therapeutic groups are shown in Fig. 3. Significant time effects ($P < 0.001$) and different patterns of changes for groups ($P < 0.001$) were measured for number of NK cells. Significant increases ($P < 0.001$) in the number of NK cells were observed in the pleuran group after 3 months of supplementation and additional 3 months of non-supplementation compared to baseline value. No changes in the number of NK cells were measured in placebo group over the 6-month study period (Fig. 3).

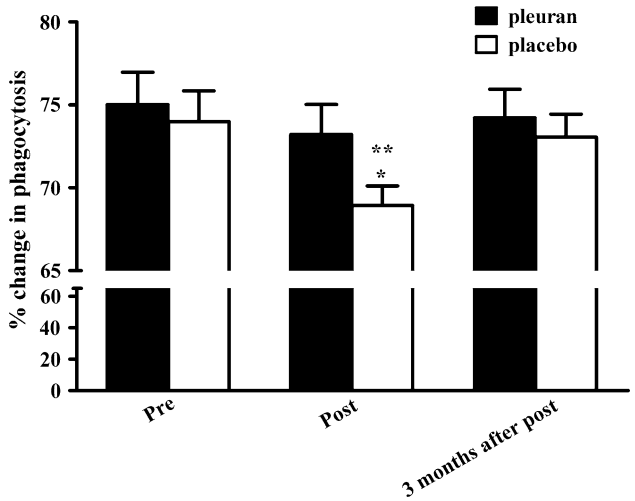

Fig. 2 Percentage changes in phagocytosis observed in both treatment groups of athletes before (Pre) and 3 months after (Post) supplementation, and after additional 3 months without supplementation (3 months after post). *Significant reduction of phagocytosis ($P < 0.001$) below the baseline value by 5% in the placebo group. **Significant difference ($P < 0.01$) in phagocytosis between pleuran and placebo group after 3 months of supplementation. Data represent mean ± SEM

Discussion

The primary finding of this double blind, placebo-controlled study was that 200 mg/day for 3 months of pleuran, a mushroom-derived β-glucan compared to a placebo supplementation decreased incidence of symptoms associated to URTIs, suppressed the changes in phagocytosis process and increased the number of NK cells in regularly training athletes.

Various different nutritional agents have been tested for their capacity to help restore a weak immune system and decrease incidence of URTIs after intensive exercise (Nieman and Bishop 2006; Nieman 2008). The most commonly used nutritional supplements in athletes include zinc, antioxidants, glutamine, plant sterols, n-3 polyunsatured fatty acids, and carbohydrates. With possible exception of carbohydrates and vitamin C, there is insufficient clinical evidence to support traditional nutritional supplements as protecting agents against URTIs or exercise-

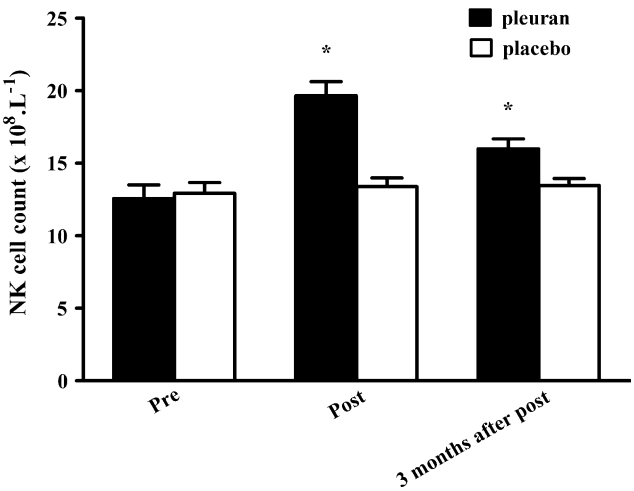

Fig. 3 Changes in number of natural killer cells in both treatment groups of athletes before (Pre) and 3 months after (Post) supplementation, and after additional 3 months without supplementation (3 months after post). *Significant increases ($P < 0.001$) in the number of NK cells compared to baseline value. Data represent mean ± SEM

induced immune changes. The failure of nutritional agents to demonstrate their beneficial effect in humans has shifted attention to new advanced classes of food supplements such as β-glucan, quercetin, and curcumin (Nieman 2008).

The most promising nutritional supplement, β-glucan has attracted attention in recent years due to its wide biological and pharmacological activities. The effects of β-glucan supplementation on exercise-induced changes in immune function and upper-respiratory tract symptoms have been investigated in several animal studies (Davis et al. 2004a, b; Murphy et al. 2004, 2007, 2008, 2009) and in three recent human studies of top-level athletes (Nieman et al. 2008; Talbott and Talbott 2009; Bobovcak et al. 2010). In a very recent human study, Nieman et al. (2008) investigated the effect of an oat β-glucan supplement on exercise-induced suppression of immune system in athletes. They found that oat β-glucan did not alter exercise-induced changes, in particular, suppression of NKCA in the endurance athletes during 18 days of supplementation. Furthermore, there was no reduction of URTI incidence in athletes during 2-week period after intensive exercise. These data contrast with findings from studies investigating the effect of oat β-glucan on immunosuppression in exercised mice (Murphy et al. 2004, 2007, 2008, 2009; Davis et al. 2004b). It may be related to differences in the ability of oat β-glucan to interact with immune cells through the proximal small intestine in humans. However, the exact mechanism by which β-glucans interact with the human immune system is still undetermined, the hypothesized mechanisms of action of β-glucan in humans show high similarity with the animal model (Chan et al. 2009).

The recognition and response to β-glucans is mediated primarily by cell surface receptors. To date, four receptors have been identified, including scavenger receptors, complement receptor 3, lactosylceramide, and more recently, dectin-1. Based on mostly animal data, β-glucans enter the proximal small intestine rapidly and are captured by the macrophages after oral administration (Hong et al. 2004). The β-glucans are then fragmented into smaller size β-glucans and are carried to the marrow and endothelial reticular system. How β-glucans mediate their effects after ingestion in humans remains to be defined. Although, data collected from the first human study (Nieman et al. 2008) do not support soluble β-glucan as a promising immunonutrition supplement (200 mg/subject/day) and an agent reducing URTI incidence, results from present double-blind study suggested that insoluble β-glucan significantly reduced the incidence of URTI symptoms and increased cellular immune responses in athletes after 3 months of supplementation.

Similarly, in our very recent study, we found that pleuran supplementation (100 mg/subject/day) improves the rate of restoration of normal immune functions in athletes after heavy exertion (Bobovcak et al. 2010). Additionally, pleuran was able to alter exercise-induced suppression of NKCA in athletes after 2 months of supplementation. These results show a discrepancy with the results of Nieman et al. (2008), where soluble oat β-glucan neither augmented resting immune functions nor altered URTI incidence in athletes. In addition to these comparisons, insoluble yeast-derived β-glucan supplement administered at two different doses (250 and 500 mg) significantly decreased URTIs incidence and improved mood state in marathon athletes (Talbott and Talbott 2009). These findings suggest that insoluble β-glucans, rather than their soluble forms, may be more effective in suppression of exercise-induced changes in immune functions and in preventing respiratory tract infections in humans.

We speculate that the differences in immunomodulatory activity between soluble and particulate β-glucans could be explained by the need of cross-linking of membrane receptors (Michalek et al. 1998). Moreover, data from the recent studies showed that also origin, molecular weight and structure of β-glucans may influence their immunomodulatory effects (Sonck et al. 2010; Rice et al. 2005; Adams et al. 2008). It has been demonstrated that β-glucans with higher molecular weight exhibit higher binding affinities for the β-glucan receptor (Mueller et al. 2000). The primary structure of pleuran, particulate β-glucan with high molecular weight of 750 kDa, is similar to that of β-D-glucans commonly found in basidiomycetes and ascomycetes (Karacsonyi and Kuniak 1994).

Another important factor for immunological potency of β-glucans is the degree of branching. Pleuran has a branched structure consisting of a backbone of (1,3)-linked β-D-glucopyranosyl residues, every fourth being substituted with single D-glucopyranosyl groups (Karacsonyi and Kuniak 1994). Dectin-1 is highly specific for glucans that have a (1,3)-β-D-glucopyranosyl backbone and did not interact with a mixed-linkage polymer backbone in plant-derived glucans (e.g. barley and oat glucans) characterized by alternating regions of (1,3) and (1,4)-β-linkages (Adams et al. 2008). Mueller et al. (2000) suggested that the presence of (1,6)-β-linked side-chain branches increases the affinity of pattern recognition receptors for glucan. In addition, the increased affinity of dectin-1 for β-glucans with side-chain branches suggests that the binding pocket may have a unique structure that preferentially interacts with branched glucans (Adams et al. 2008). These findings suggest that immunomodulatory capacity of β-glucans is strongly dependent on solubility and structural factors such as backbone structure and degree of branching. Thus, unbranched soluble oat or barley β-glucans may have ineffectual influences on exercise-induced immune changes.

Long-term intensive training in elite athletes has immunosuppressive effects and is mainly manifested by decreased resistance to URTIs or by persisting viral or parasitic infections. The J-shaped model describing relationship between exercise and susceptibility to infections is based on the examination of symptoms associated to URTIs and not clinically diagnosed infections with a particular pathogen. Due to this limitation, a few studies have examined the effects of exercise based on clinical outcomes attributed to specific respiratory pathogen using a human model (Cox et al. 2008; Spence et al. 2007). According to a study by Spence et al. (2007), causative agents of URTI symptoms in athletes were found only in 30% of cases using a variety of isolation techniques including serology, culture, and polymerase chain reaction from nasopharyngeal, saliva and blood samples. Similarly, Cox et al. (2008) found that viral or bacterial pathogen associated with URTI was detected only in 57% of athletes with URTI symptoms. This finding suggested that although the symptoms were consistent with URTIs, they may not be caused by common respiratory pathogens but instead to other phenomena such as allergic reactions or hypersensitivity. Therefore, self-reporting of symptoms of URTI using physical health questionnaires represents weakness of our study.

Another weakness of the present study is that we have used the combination of pleuran with vitamin C instead of pleuran itself, however, most randomized placebo-controlled studies have been unable to demonstrate that vitamin C supplements modulate immune responses following heavy exertion (Nieman et al. 1997, 2000, 2002). In addition, according to a recent systematic review (Moreira et al. 2007), prevention of URTI infections after exercise by vitamin C was only supported by 2 of 13 performed trials. The combination of vitamin C and pleuran may have a beneficial effect on preventing exercise-induced immunodepression. This possible synergistic effect merits further investigation.

In addition to these limitations, subjects participated in this study were athletes from different sport disciplines with differences in training patterns. Due to this limitation, the athletes were randomly divided into two groups, with comparable representation of each discipline in both groups.

In conclusion, pleuran or placebo was taken on daily basis by regularly trained elite athletes. After 3 months of supplementation, subjects were monitored for an additional 3 months without daily supplementation. Water insoluble pleuran significantly reduced the incidence of URTI symptoms and increased the number of NK cells. In addition, pleuran significantly improved the phagocytosis process. The results from this double-blind study suggest that pleuran, insoluble β-glucan from the *Pleurotus ostreatus* mushroom, supplementation may have the capacity to attenuate immune changes and decrease URTI symptoms in intensively training athletes.

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