

## **Effect of various nutraceuticals on phagocytosis**

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Actual pandemic state of covid-19 infection leads to considerations of the quality of the state of immune system in population and to possible changes of this unfavorable state. We assume that optimal supplementation with minerals and vitamins forms the basis for development of adequate immune responses not only to Covid-19 infection, but also to other bacterial and viral infections. It is necessary, however, to use optimal doses of supplements, as either too low or too high dose might decide between the use of a medicine or a poison. Healthy and balanced diet increases the strength and capacity of the immune system, its cellular components and results in optimal response to pathogens. Ever increasing evidence show the effects of various nutritional components on individual parts of the defense reactions.

Vitamin D is the most important vitamin out of the whole spectrum of vitamins potentially involved in regulation of immune reactions. Dynamics of serum levels of vitamin D is in humans influenced by season, location, quality as well as quantity of sunshine, age, health and social status of an individual (Alexander et al., 2020). Nutritional effects on vitamin D levels in various countries varies from 5 to 35% of normal values. In European countries were the normal levels found in 10-80% of population. Vitamin D insufficiency is not only involved in induction of numerous autoimmune diseases, chronic disease, inflammatory problems of the gastrointestinal tract, asthma bronchiale, diabetes and multiple sclerosis, but also plays a role in increased mortality (Williams and Williams, 2020). This relation of low levels and development of various diseases results in a push for direct supplementation not only in children, but most of all in senior population, with described strong improvement of general health and measurable reduction of the risk of infectious diseases. Supplementation with vitamin D is therefore recommended in population with higher risk of Covid-19 infection (Calder, 2020).

Vitamin C is an essential micronutrient for humans with numerous and pleiotropic functions connected with its ability to donate electrons. Major role of vitamin C in immunity is its antioxidant activity and subsequently defense of respiratory apparatus against oxidative stress caused by pathogens. Vitamin C can stimulate phagocytosis, T cell proliferation, interferon induction and lower viral replication (Garvin et al., 2020). Ability of vitamin C to improve immune response against Covid-19 infection was repeatedly observed (Garvin et al., 2020, Gombart et al., 2020). Low levels of vitamin C result in lower resistance to infections, supplementation on the other hand results in stimulation of phagocytosis, activation of lymphocytes and changes in neutrophil chemotaxis. In addition, vitamin C is necessary for collagen biosynthesis and for sustaining integrity of epithel, which helps to reduce some diseases. Supplementation in a blanket manner in children from highly polluted areas of the Czech Republic resulted in strong reduction of respiratory tract diseases.

Vitamin B6 deficit is involved in insufficient maturation of lymphocytes and in depression of their numbers. In addition, it affects thymus and spleen atrophy (Crespi and Alock, 2021). Vitamin B6 also influences the numbers and function of lymphocytes. In addition, low levels of this vitamin can result in lower activity of NK cells and neutrophils.

Trace elements are essential cofactors important for several important proteins (Garvin et al., 2020, Horowitz and Freeman, 2020). It is necessary to maintain their optimal concentration, as both low and to high levels might be dangerous or even toxic. At the same time, interaction between host and microorganism is dependent on the level of minerals, as trace elements modulate this interaction. In case of disbalance, physiological functions of the organism might be disrupted (Alexander et al., 2020, Zitterman et al., 2016). In addition to physiological functions, trace elements are also involved in regulation of immune reactions. They play role not only in the quality of various barriers, often playing the role of first defense, but also in the whole spectrum of immune reactions, both nonspecific and specific (Garvin et al., 2020, Gombart et al., 2020, Lopez and Skar, 2018).

Zinc deficiency is known since 1960s. Zinc supplementation needs to be carefully done, so it reduces potential insufficiency and at the same time rule off overdose, which might result in dysbiosis and predisposition to various infections due to the compromised salivary immune response (Jawhara, 2020). Zinc supplementation was found to improve antibody response and induction of production of cytotoxic T cells, cytokines and Tregs. Additional roles are reduction of oxidative stress and regeneration of intracellular killing with induction of macrophage-derived cytokines (Chwdhury et al., 2020). Zinc homeostasis-related effects on the activation of key signaling molecules together with epigenetic modification clearly elucidate the role of zinc as a gatekeeper of immune functions (Wessels et al., 2020).

Selenium is a potent micronutrient important for various facets of mammalian health including immune response. Low levels of selenium result in reduction of NK cell activities and higher risk of microbial infections. In addition, mutation of viral particles with increased virulence has been described (Calder, 2020). Long-term deficit results in increase oxidative stress and suppressed numbers of monocytes. In addition, selenium was found to have suppressive effects on breast cancer manifested via epigenetic mechanisms. Recent studies found that addition of selenium improved immunodulation caused by betaglucan. Some studies suggested increased risk of Covid-19 infection.

Betaglucans are natural polysaccharides. Optimal nutritional composition might offer some intake of betaglucan, but current nutritional trend of using industrial type of nutrition constantly lowers the amount of beta glucan in our food. Extensive research of betaglucans and their actions undertaken in last several decades helped to elucidate numerous mechanisms of action and their involvement in improvement of our health, including microbiome, probiotic and prebiotic effects and most of all, immune system (Melvin and Bomberger, 2016, Vetvicka et al., 2019). Our studies suggested the use of orally-given betaglucan both in prevention and in therapeutical interventions in various types of diseases. In these studies the individual fractions

have activity similar to low molecular heparin. In Covid-19 patients is a high risk of development of disseminated intravascular coagulation. Thrombocytopenia and elevated levels of D-dimer induce higher activation of platelets and higher coagulation. Betaglucan effects might be elevated by addition of adequate dose of vitamin C and D. These studies suggest that betaglucan is one of the most prominent immunomodulators. Development of high quality betaglucan helped our laboratory to offer a unique application with excellent effects both in prevention and in therapy.

Phagocytosis is one of the first reaction involved in defense of the organism against invading pathogens. In our current study we compared the effects of 21 days of oral supplementation with betaglucan to several vitamins. For our study, we used yeast-derived insoluble betaglucan (Biorigin, Brazil), vitamin B6 (Life extension, Ft. Lauderdale, USA), zinc (Puritan Pride, USA), vitamin C (Nature Made, USA), sodium selenite (Spectrum, Gardena, USA), and vitamin D (BioTech, USA). Daily doses were 100 µg of betaglucan, 10 µg of selenium, 25 IU of vitamin D, 0.4 mg of vitamin B6, 5 mg of zinc, and 5 mg of vitamin C. All nutrients used in our study have EU declaration of positive effects on immunity.

At the end of supplementation, we evaluate the phagocytic activity of peritoneal macrophages and peripheral blood monocytes and neutrophils. Using the well-established technique employing synthetic HEMA microspheres, we found that betaglucan has significant stimulatory activity. Compared to betaglucan, none of the nutrients alone caused statistically significant stimulation of phagocytosis. When we tested the potential synergistic effects of a combination with betaglucan, only vitamin C + betaglucan and Selenium + betaglucan combinations showed increased activity.

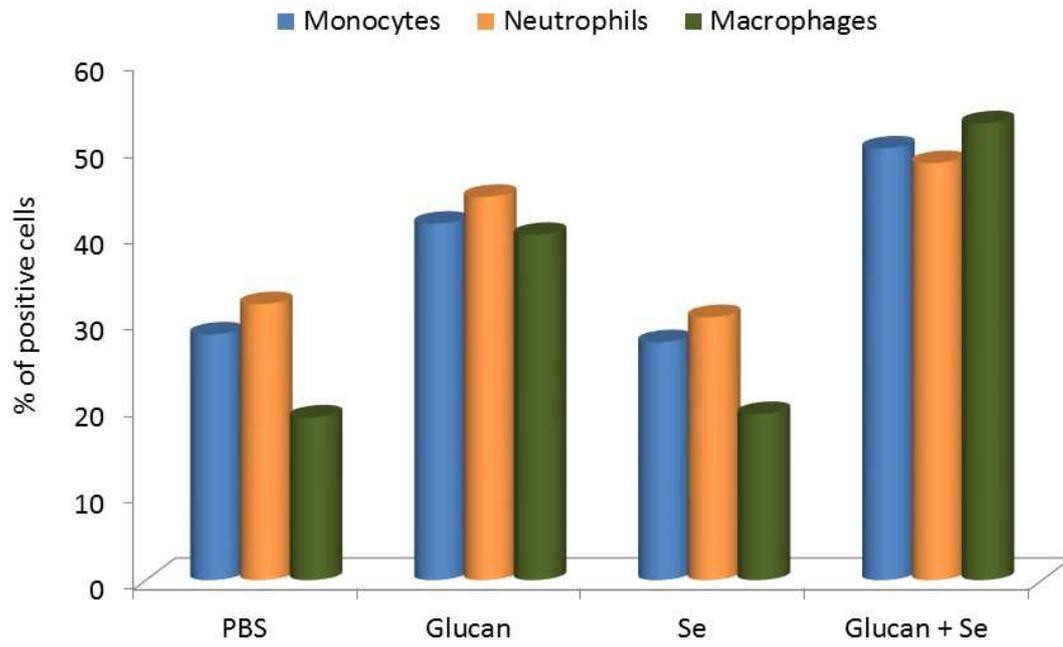
Current time of various lockdowns and home isolations result in serious and long-term lack of physical activity, increased stage of anxiety, depressions and stress. We propose to decrease these risks by preventive supplementation with betaglucans, which results in reduction of corticosteroid production, modulation of cytokines and improved salivary immunity.

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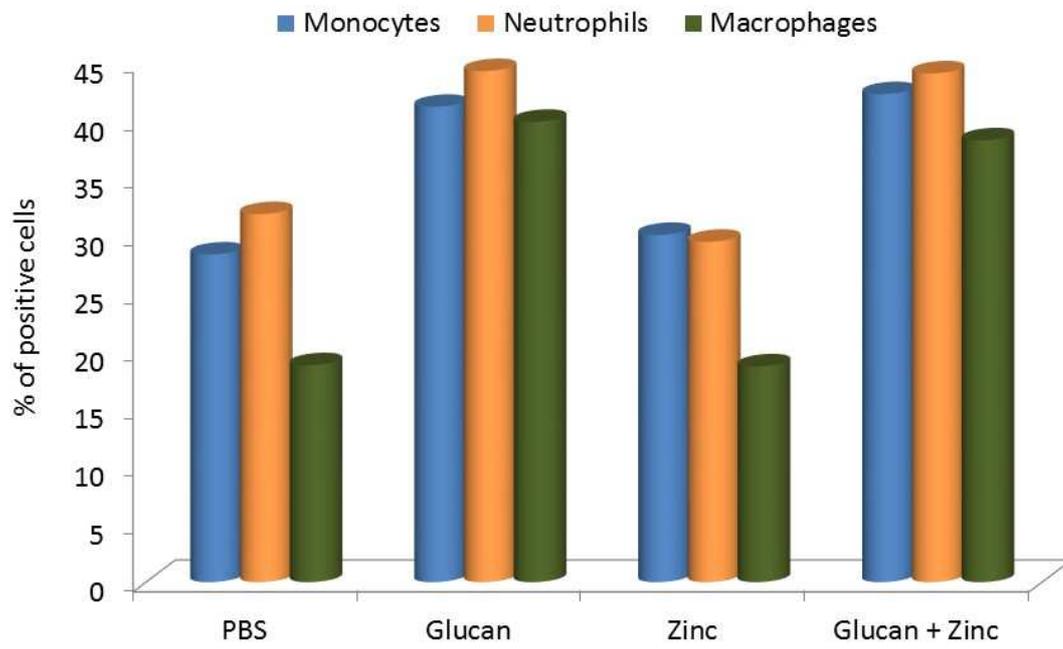
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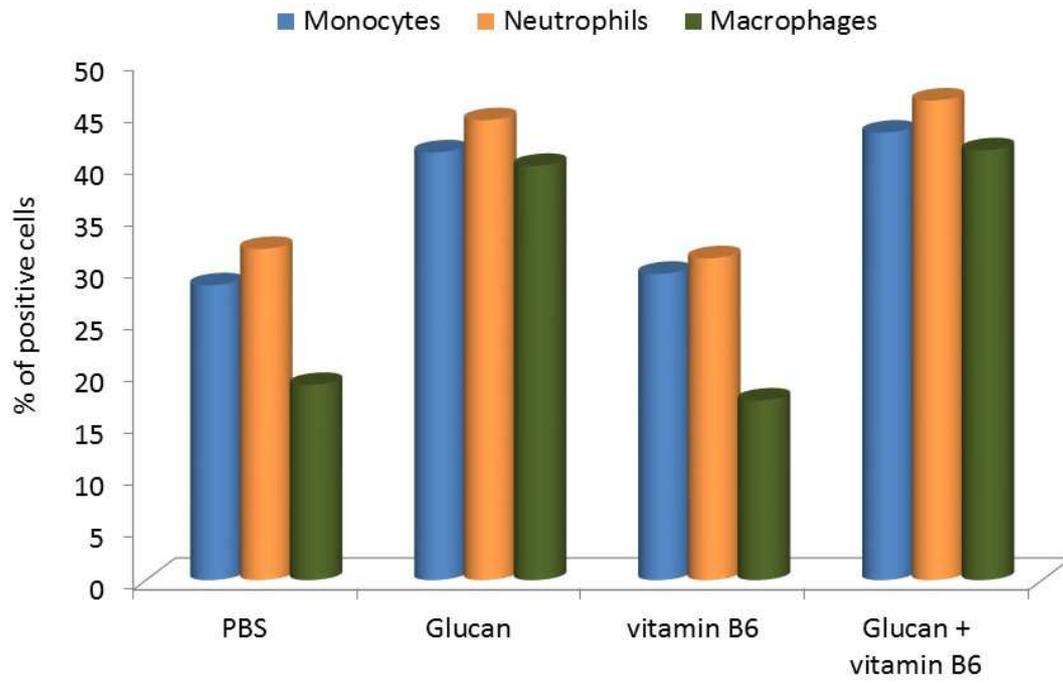
### Effect of glucan and Se on phagocytosis



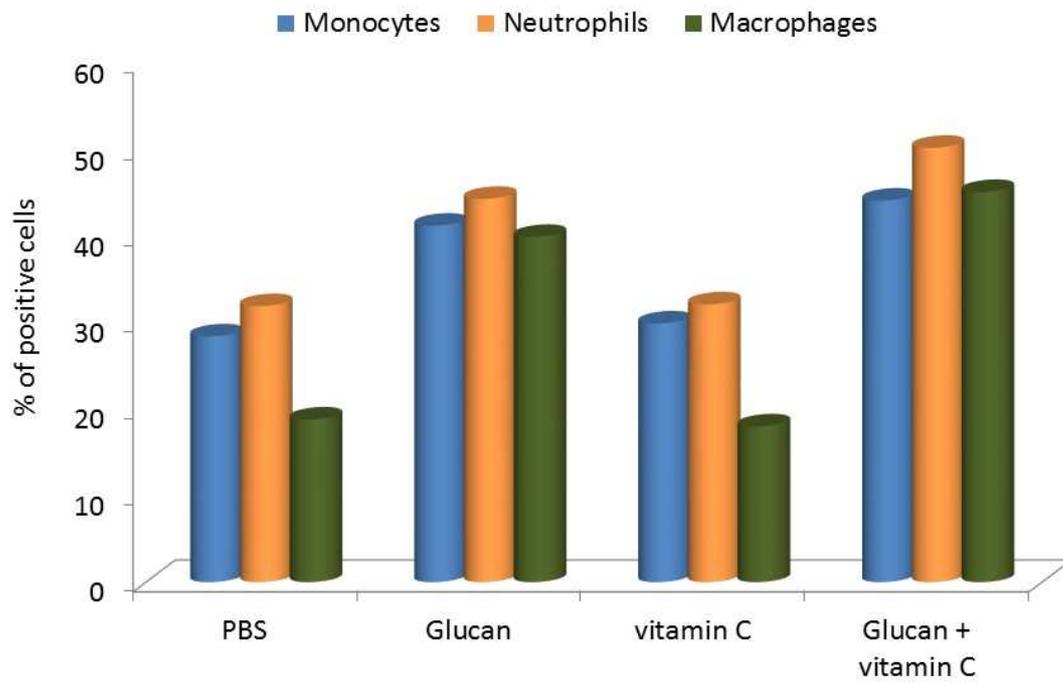
### Effect of glucan and zinc on phagocytosis



### Effect of glucan and vitamin B6 on phagocytosis



### Effect of glucan and vitamin C on phagocytosis



### Effect of glucan and vitamin D on phagocytosis

