

Original Research Paper

Glucan Supplementation Regulates Secretory Immunity and Stress

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Abstract: Sixty-six children were supplemented with beta glucan and dietary nucleotides, another 63 children served as negative control. We evaluated the effects of these supplements on several aspects of secretory immunity such as levels of secretory IgA (SIgA), albumin and SIgA/albumin ratio. In addition, in order to monitor effects of physical challenges and compensatory treatments on stress and reparation of immunity, we measured levels of salivary cortisol. We found significant effects of supplementation with beta glucan on secretory IgA in children with increased physical challenge. To fully explain these findings, we will need to add additional indicators of stress and salivary immunity.

Keywords: Saliva, Salivary IgA, Cortisol, Beta Glucan, Dietary Nucleotides

Introduction

Salivary IgA represents an important component participating in anti-infectious defense of an organism. Its levels fluctuate depending on numerous factors including circadian rhythms with extreme increases during early morning hours (6-8 AM) followed by subsequent decrease during late morning, afternoon stabilization and gradual increase later in the day (Richter *et al.*, 1980). Several studies repeatedly demonstrated relations between SIgA variations and psychological stress of students (Guo *et al.*, 2002). Levels of SIgA are influenced by additional factors such as amount of water consumption (Backes *et al.*, 2015), physical activity and physical challenge (Cieslak *et al.*, 2003; Dwyer *et al.*, 2010; Gleeson and Pyne, 2000), changes in biorhythms during work shift (Guo *et al.*, 2002; Lac and Chamoux, 2003), time of the season, location, altitude above sea level and most of all by stress related to psychological challenge and persisting physical challenge (Cieslak *et al.*, 2003; Cohen *et al.*, 2001; Gleeson and Pyne, 2000; Guo *et al.*, 2002; Hunter *et al.*, 2011; Kidd *et al.*, 2011; Powell *et al.*, 2015; Yang *et al.*, 2002). Two major branches of stress induction include autonomous nervous system and hypothalamus-adrenal gland-pituitary axis. These two

systems coordinate sensitivity of additional physiological systems affecting immunity (Marques *et al.*, 2010).

The salivary albumin level reflects capillary penetration of this protein into saliva and is a dependable indicator of inflammatory response to infectious and noninfectious impulses (Richter *et al.*, 1980; 2015c). Low levels of serum albumin reflect inflammatory reaction of organism and represent negative protein of inflammatory reaction. The situation in saliva is different, as increase albumin levels might suggest actual as well as latent inflammatory reaction. Elevated level finding in saliva usually leads to verification of possible infection including bacterial and viral infections, sinusitis and dental problems including gingivitis. However, there is a possibility of saliva contamination caused by vigorous dental treatment. Changes in capillary penetration during inflammation may influence the levels of salivary IgA and evaluation of the IgA/albumin ratio is important (Cieslak *et al.*, 2003; Dwyer *et al.*, 2010).

Current literature offers clear evidence showing that psychological stress affects human immune reactions (Cieslak *et al.*, 2003; Mizawa *et al.*, 2013). Some studies have demonstrated that stress can significantly influence antibody response of vaccinated individuals (Cohen *et al.*, 2001). This decrease of antibody

formation can result from both actual and/or chronic stress. Psychological stress is defined as a result of direct negative experience or even expectation of problems. Some studies suggested that the relationship between stress and antibody response is regulated mostly by glucocorticoids such as cortisol, catecholamin, epinefrin and norepinefrin (Cohen *et al.*, 2001; Marques *et al.*, 2010). Additional factors known to influence stress reaction, particularly in children, are effects of passive smoking, obesity, nutrition, social-economic conditions and environmental pollution (Backes *et al.*, 2015; Cohen *et al.*, 2001). Depression of ability of antibody response in vaccinated population might be connected with significant changes of salivary IgA (Cohen *et al.*, 2001). Quality of nutrition significantly influences gut microbiome with subsequent effects on nonspecific and specific immune reactions. Microbiome of gastrointestinal tract directly influences afferent neurones of gastrointestinal tract and transfers impulses via nervus vagus. Microbiome thus plays a role in stress regulation of hypopituitary adrenal axis (Cohen *et al.*, 2001). Stress reaction accompanied by an increase of salivary cortisol levels might result in reactivation of some infectious diseases such as Epstein-Barr viral infection (Breivik *et al.*, 1996; Stowe *et al.*, 2001).

Nutritional changes can also influence immune reactions; this includes the use of dietary polysaccharides and other supplements. A solid review summarizes immunomodulatory activities of various botanical extracts in both animal and human models (Ramberg *et al.*, 2010). Beta glucans are known for not only reduction of clinical manifestation of respiratory infections, but also for regulation of the psychological state of patients (Talbot and Talbot, 2012). A large study using dogs showed regulation of general as well as antigen specific IgA and IgM reactions (Stuyven *et al.*, 2010). Lehne *et al.* (2006) confirmed findings of positive effects of glucan supplementation on improvements of immune reactions (Richter *et al.*, 2015a; 2015b; 2015c). It is clear that insoluble dietary fiber plays an important role in the regulation of both immune reactions and psychological state. With beta glucan being an established immunomodulator, more and more studies are focused on food supplementation with beta glucans. However, more studies are necessary to reach clear conclusions (Talbot and Talbot, 2012).

Materials and Methods

Our study used 126 children aged 6 to 12 years. Group 1 represented children supplemented with glucan (BG) and consisted of 6 males and 19 females. Dietary nucleotide (Imuregen-Uniregen Hradec Kralove Czech Republic)-supplemented group involved 41 children (16 males and 25 females). A control group was formed of

63 children (29 males and 34 females). The age differences between individual groups were not statistically significant. All parents were fully informed about the aim of this study and provided signed consent. Children were evaluated during their medical stay at Sanatorium EDEL Zlate Hory. During the 28 to 32-day stay, the children were regularly examined with particular attention to respiratory problems and daily control by a physician and with evaluation of quality of pulmonary functions including spirometry and evaluation of expiratory exhaled nitric oxide.

The medical regime offered several advantages including improved living conditions, optimal nutrition, regular daily physical strain and several hours of stay in cave (fully confirming conditions for speleotherapy). Physical conditions were evaluated before and after the end of the stay using a 6MWT test. All results and final recommendations for subsequent treatment were offered at the end of medical stay. Our study was fully in agreement with a Helsinki declaration (revised version 2000.0901) and was in full compliance with the rules for clinical testing in the Czech Republic.

Saliva was obtained at the same time interval, between 8 and 9 AM. Sample tampons Salivette (Sarstead, Orsay, France) gently chewed in mouth for 2 min were used for all experiments. After subsequent centrifugation at 1000 g for 15 min, the samples were stored at -18°C . IgA and albumin levels were evaluated using a Siemens BM II nephelometer with kits and controls obtained directly from manufacturer. Salivary cortisol was measured using high sensitivity salivary cortisol from Salimetrix (Salimetric LLC, UK) as recommended by manufacturer.

Statistical Analysis

Statistical significance was evaluated by a pair *t* test using GraphPad Prism 5.04 software (GraphPad Software, USA). An average and standard deviation was evaluated after determination of composition of standard values (D'Agostino, Pearson). In case of nonstandard composition, we converted the values into logarithms.

Results

Table 1 summarizes data obtained in this study. The glucan supplemented group had statistically significant decrease of SIgA levels from 302.7 to 182.4 mg L^{-1} . Similarly, the levels of salivary albumin were found to be lower at the end of the study (Fig. 1) However, this decrease was not significant. An increase of cortisol levels from 3.18 to 4.34 ug L^{-1} was statistically significant. The other studied groups showed no significant changes in cortisol levels or SIgA levels (Fig. 2).

Table 1. Effect of supplementation with glucan (group 1), dietary nucleotides (group 2) and placebo (group 3)

Group		S IgA mg/L		Albumin mg/L ⁻¹		SIgA/Albumin		Cortisol µg/L ⁻¹	
		A	B	A	B	A	B	A	B
1	n	25.0000	25.00	25.0000	25.00	25.0000	25.00	25.0000	25.000
	mean	302.7000	182.40	145.5000	103.50	2.8300	2.21	3.1760	4.336
	SD	28.8000	28.90	16.9000	30.20	1.3700	1.28	1.8240	2.516
	P	0.0151		0.1040		0.1550		0.0085	
2	n	41.0000	41.00	41.0000	41.00	41.0000	41.00	41.0000	41.000
	mean	93.1100	68.84	151.5000	61.70	2.2700	2.47	2.9880	2.154
	SD	30.2000	35.70	47.7000	75.00	2.0200	2.38	2.5600	1.890
	P	0.1310		0.0295		0.6911		0.0612	
3	n	63.0000	63.00	63.0000	63.00	63.0000	63.00	63.0000	63.000
	mean	88.9000	76.30	87.4200	85.04	2.6500	2.79	2.3540	2.085
	SD	31.2000	35.80	11.0100	10.89	2.5300	2.51	1.8900	1.950
	P	0.1868		0.4240		0.6892		0.1620	

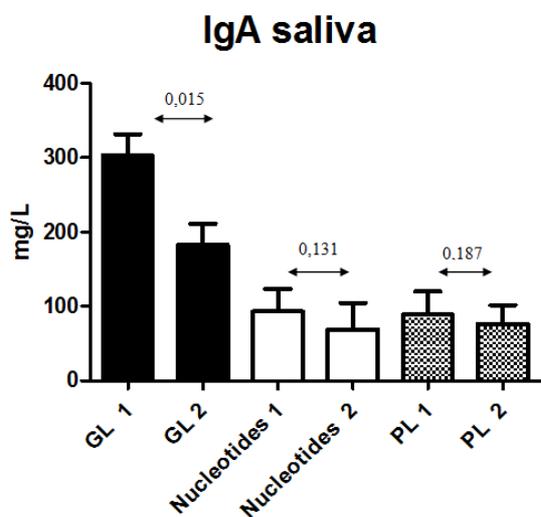


Fig. 1. Effect of supplementation with Glucan (GL), dietary nucleotides (Nucleotides) and Placebo (PL) on salivary IgA

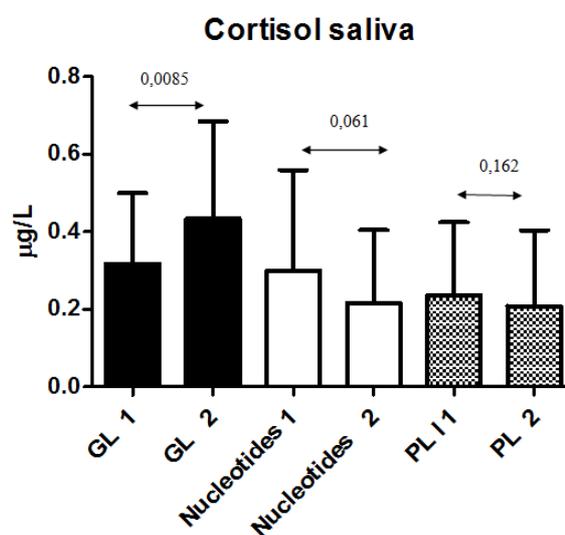


Fig. 3. Effect of supplementation with glucan (GL), dietary nucleotides (Nucleotides) and placebo (PL) on level of cortisol in saliva

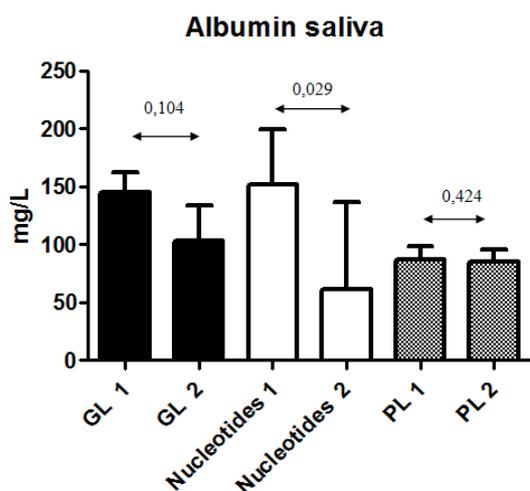


Fig. 2. Effect of supplementation with glucan (GL), dietary nucleotides (Nucleotides) and placebo (PL) on levels of albumin in saliva

The nucleotide-supplemented group showed significant decrease of albumin levels (Fig. 3). A similar, albeit insignificant, trend was found in the glucan-supplemented group, which might be a result of the limited number of children. SIgA/albumin ratio showed no significant differences between beginning and end values.

Discussion

Two supplemented groups were evaluated during their medical stay at Sanatorium EDEL in Zlate Hory. Both groups had differed significantly in examination at the starting point, in basic diagnosis (chronic respiratory problems) and at the time of examination. Glucan-supplemented children were evaluated in March and April, whereas other groups were evaluated at the beginning of fall. In the glucan group we could

clearly observe a clinical picture based on the winter period and location with high level of pollution-high SIgA levels together with indications of inflammation; i.e., high level of albumin mediated by extreme capillary penetration into saliva. SIgA levels in children supplemented with nucleotides and in the control group were lower due to the summer season and location without significant pollution (family summer vacation at sea, etc.). Among the children tested in spring, we found a strong decrease in levels of tested molecules, but it still did not reach normal range described for this age category. We can again confirm that reparation of immune reactions with subsequent significant reduction of bacterial and viral infections needs a long term stay in medical regime. These findings are even more pronounced in children exposed to secondhand smoke. We found that more than 50% of Czech children are exposed to secondhand smoke. We expected to see significant decrease of cortisol levels in the glucan-supplemented group, but conversely we found an increase of this molecule. We hypothesize that this increase was caused by the higher physical load during their stay at the sanatorium-forced physical education, long daily walks and rehabilitative exercise. It is also known that intensive physical exercise can result in lower SIgA levels (Gleeson and Pyne, 2000). It is possible that higher cortisol levels might be, at least partly, influenced by acute stress from sample collection, when expectation of arrival of family members and return home might change the stress level. In children tested after summer holiday we also found higher levels of cortisol, but during the supplementation we found decrease. The control group of children and their optimal levels of all tested parameters were probably caused by after holiday reparation of immune functions and minimal physical burden at the sample collection (usually only 2×45 min of mandatory school exercise at school). In the nucleotide-supplemented group we found nonsignificant decrease of SIgA level, but also significant decrease of inflammatory reaction presented by albumin levels. Dietary nucleotides can significantly help to repair intestinal mucosa and probably positively influence microbiome and subsequently repair inflammatory response.

Conclusion

For additional evaluation and explanation of the relationship between stress and immune response, it is necessary to further evaluate additional factors, particularly seasonal changes, nutritional background and weight of children. As in other developed countries, the number of obese children in the Czech Republic is steadily.

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Author's Contributions

All authors contributed equally.

Ethics

Our study was fully in agreement with a Helsinki declaration (revised version 2000.0901) and was in full compliance with the rules for clinical testing in the Czech Republic.

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