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Dietary intake of *Agaricus bisporus* white button mushroom accelerates salivary immunoglobulin A secretion in healthy volunteers

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ABSTRACT

Objective: Secretory immunoglobulin A (SIgA) acts as the first line of adaptive humoral immune defense at mucosal surfaces. A lack of SIgA or the inability to produce antigen-specific SIgA can lead to an increased risk of infections. Dietary intake may improve mucosal immunity by accelerating SIgA secretion. This study investigated the effect of dietary intake of *Agaricus bisporus* white button mushroom (WBM) on salivary IgA (sIgA) secretion in healthy subjects.

Methods: Twenty-four healthy volunteers were randomly assigned to a normal daily diet (control group) or a normal diet with WBM. The subjects in the active group ($n = 12$, 41.4 ± 11.3 y old) consumed 100 g of blanched WBM daily with their normal diet for 1 wk, whereas those in the control group consumed their normal diet ($n = 12$, 43.5 ± 12.5 y old) without WBM. Saliva was collected before and after commencement of the study and every week thereafter for 3 wk. Saliva flow rate, sIgA concentration, and osmolality were determined and the sIgA:osmolality ratio and the sIgA secretion rate were calculated.

Results: There was no significant difference between pre- and postdietary mushroom intakes for all indices in the control group ($P > 0.05$). In contrast, the mean sIgA secretion rate increased significantly at weeks 1 and 2 by 53% and 56%, respectively, compared with that at week 0 ($P < 0.0005$) in the WBM intake group and then returned to a baseline level at week 3. Changes in sIgA secretion rate over the intervention period were greater in the WBM group than in the control group without WBM. In both groups, no significant changes in osmolality and saliva IgG were noted. There was, however, a significant increase in the sIgA:osmolality ratio ($P < 0.0012$), confirming the postdietary WBM-induced sIgA increase.

Conclusion: The dietary intake of *A. bisporus* WBM significantly accelerates sIgA secretion, thereby indicating its potential health benefits for improving mucosal immunity.

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Introduction

The mucosal membrane of the oral cavity, the gastrointestinal, genitourinary, and respiratory tracts, and the mammary gland occupies the largest area of the human body [1]. Its continuous exposure to the environment is vulnerable to attack by pathogenic microorganisms that cause epithelial cell dysfunction and/or cell death [2]. Protection against infectious agents is carried out by the body's specialized innate and adaptive immunity system [3]. The adaptive mucosal immune defense is largely mediated by secretory immunoglobulin A (SIgA), which is the predominant immunoglobulin class in human secretions [2–4]. Evidence for a gut contribution to adaptive mucosal immune responses derives largely from animal studies [3,5]. Antigen-sensitized mucosal immunocytes (e.g., IgA B cells) initiated in the gut-associated mucosal tissue migrate with the blood to mucosal tissues to form the mucosa-associated lymphoid tissue, where they differentiate into plasma cells producing antigen-specific IgA antibodies for defense of the mucosal surfaces against invasive pathogens. A similar mechanism appears to occur in humans to ensure that SIgA antibodies are produced at every mucosal site, which includes the intestine, respiratory tract, salivary and mammary glands, and genitourinary tract [6–10]. For example, oral intake of killed bacteria [7] or bacterial antigen [8] has led to the generation of IgA-committed

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B cells in peripheral blood before their appearance in mucosal sites to secrete the specific IgA antibody and establish mucosal immunity.

The function of the SIgA antibody in mucosal defense is to perform "immune exclusion" by preventing potentially harmful pathogens and antigens from adhering to and penetrating through the secretory epithelia, containing the gut microbiota, reinforcing the epithelial barrier function and contributing to the immunologic homeostasis [3,5]. Thus, changes in the protective capacity of SIgA to perform these functions can lead to infections and inflammatory diseases [5,11]. SIgA in saliva has been used to monitor the status of the mucosal immune system (reviewed by Albers et al. [12]). The salivary glands (parotid, sublingual, and submandibular) are an important source of SIgA in the upper respiratory tract [13]. Numerous studies of saliva composition have found decreases in salivary SIgA secretion with age [14], psychological and occupational stresses [15–18], nutritional deficiencies [19,20], strenuous physical exercise [21,22], and immune-compromised individuals [23] and may lead to an increased risk of respiratory infections [19,20,24]. These examples illustrate the importance of measuring salivary SIgA and point to the potential benefit of dietary intervention for improving or slowing the decline of salivary IgA (sIgA) in susceptible populations.

Agaricus bisporus white button mushroom (WBM) constitutes the bulk of all mushrooms consumed, especially in Western countries. The mushroom contains bioactive compounds that have been shown to exhibit immunomodulating and anticancer properties [25–27]. To our knowledge, no studies have been conducted to determine the effect of mushroom consumption on sIgA secretion. The objective of this study was to test the hypothesis that the dietary intake of *A. bisporus* WBM is effective in enhancing sIgA secretion in healthy subjects.

Materials and methods

Subjects

Ethics approval was obtained from the University of Western Sydney human research ethics committee. The subjects were recruited by a local advertisement placed on the university campus. The participants included researchers, laboratory staff, and employees. They were informed of the experimental procedures and gave their written informed consent. Participants also completed a medical questionnaire before participating. Twenty-four healthy subjects (24–56 y old, 12 male and 12 female) participated in the recruitment phase and met the following exclusion criteria: 1) smokers, 2) subjects with recurrent respiratory infections, 3) subjects taking antibiotics, 4) subjects taking immunosuppressive drugs, 5) subjects taking medications or probiotics that may affect saliva secretion, 6) subjects actively participating in strenuous exercises, 7) subjects having symptoms affecting the digestive tract and the immune system, and 8) alcohol and drug abuse. At each visit, a participant provided a saliva sample for the determination of baseline sIgA. The same methods for saliva collection and sIgA determination were used throughout the study, as described below.

Study protocol

Twenty-four eligible subjects were randomly assigned to one of the two groups: a normal daily diet with an intake of WBM ($n = 12$) and a normal daily diet without an intake of WBM ($n = 12$) as a control, matched for age (41.4 ± 11.3 versus 43.5 ± 12.5 y) and gender (six men and six women in each group). A block randomization procedure was used to produce the balanced study arms. The participants were instructed to consume a serving of 100 g of fresh WBM blanched in boiling water for 5 min before taking it with their meals at dinner daily for 7 d. Before saliva collection, subjects were not allowed to drink for at least 10 min. Unstimulated whole saliva samples were collected at the same time of the day (week 0) and every week (weeks 1 to 3) after study initiation. The participants were asked to record any illness or symptoms experienced and/or medications or probiotic bacteria intake during the 3-wk study period. This information was used to evaluate the safety and eligibility for saliva collection at follow-up.

Saliva collection and sIgA detection

The saliva samples were collected under supervision from 10:00 to 12:00 at week 0 and every week after study initiation. Participants were asked not to drink 10 min before saliva collection. The participants were rested in a seated position for 2 to 3 min with minimum movement before the sample collection. They were asked to swallow to empty their mouths to void the mouth of saliva. Whole saliva was then collected by passive dribble with the head leaning forward, keeping the mouth slightly open to allow the saliva to drain into a preweighed sterile tube for a period of 2 min. The tube was reweighed after collection of the sample to estimate the saliva flow rate. The amount of saliva in grams was converted to milliliters assuming the density of saliva to be 1 g/mL. Aliquots of the saliva sample were frozen at $-80°C$ for later analysis. Total immunoglobulin G (IgG) and IgA concentrations in saliva were measured by commercial enzyme-linked immunoassay kits (Mabtech AB, Nacka Strand, Sweden). The lower and higher intra-assay coefficients of variation for IgA and IgG were 2.1% and 2.5% and 2.6% and 2.9%, respectively. The interassay coefficients of variations were 3.3% and 4.7% and 3.1% and 4.3%, respectively. Data were expressed as IgG and sIgA concentrations, IgG and sIgA secretion rates, and saliva flow rate per 2 min.

Statistical analyses

Salivary IgA data based on literature values from intervention studies were used to estimate the sample size needed for the study [28–30]. A sample of 12 subjects per group has an 80% power to detect differences between pre- and post-mushroom intake indices including osmolality, saliva flow rate, sIgA concentration, and sIgA secretion flow rate. All data, except sIgA secretion rate and sIgA:osmolality ratio, were normally distributed. Data that were not normally distributed were log-transformed before analysis. The differences between the control (non-WBM intake) and the intervention (WBM intake) groups for each variable were analyzed by analysis of variance with post hoc tests for multiple comparisons. $P < 0.05$ was accepted as statistically significant for all tests. Data were analyzed using InStat 3.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Four of 12 subjects from the control group were not eligible for saliva collection at follow-up because of poor compliance (absence or refused). The remaining participants met the exclusion criteria throughout the test period. The mean absolute values for sIgA secretion rate, sIgA concentration, saliva flow rate, osmolality, sIgG concentration, and IgG secretion rate over time are listed in Table 1. There was no significant difference between the pre- and postdietary intervention for all indices in the control non-WBM intake group ($P > 0.05$). In marked contrast, the differences were highly significant for sIgA secretion rate ($P < 0.0005$) and IgA concentration ($P < 0.0005$) at weeks 1 and 2 compared with week 0 in the WBM intake group. Mean sIgA concentration remained relatively unchanged in the control group, whereas it increased in the WBM group until 3 wk and returned to a baseline level. The saliva flow rate was subject to a wide interindividual variation (range 0.3–2.6 μg/2 min). The means of individual changes in salivary flow rates over time were not statistically significant in the two groups. Although the saliva flow rates were similar in both groups, the sIgA concentration and sIgA secretion rate were found to be significantly higher in the WBM group than in the control group at weeks 1 and 2 ($P < 0.0005$ in both groups). Saliva osmolality, IgG concentration, and IgG secretion rate were unaffected by the dietary intake with or without WBM ($P > 0.05$). There was, however, a significant postdietary WBM intake increase in the IgA:osmolality ratio ($P < 0.0015$). Furthermore, the sIgA:osmolality ratios were significantly higher in the WBM group than in the control group at weeks 1 and 2 ($P < 0.0001$).

Discussion

This study has shown for the first time that a dietary intake of WBM resulted in higher sIgA secretion in the saliva of healthy

Table 1
Salivary IgA concentrations, salivary IgA secretion rate, and saliva flow rate in dietary intake with and without the WBM*

	Week 0	Week 1	Week 2	Week 3
Dietary intake without WBM				
Saliva samples	12	8	8	8
sIgA concentration (μg/mL)	26.5 ± 5.8	26.4 ± 4.8	24.3 ± 4.5	23.5 ± 6.7
sIgA secretion rate (μg/2 min)†	36 ± 24.6	38.4 ± 26.6	34.4 ± 21.8	43 ± 26.4
Saliva flow rate (mL/2 min)	1.33 ± 0.67	1.4 ± 0.67	1.35 ± 0.76	1.27 ± 0.69
Osmolality (osmol/kg)	80.5 ± 13.3	80.5 ± 13.3	75.7 ± 10.5	74.3 ± 15.6
sIgA:osmolality‡	0.33 ± 0.10	0.33 ± 0.08	0.34 ± 0.06	0.33 ± 0.07
IgG concentration (μg/mL)	18.7 ± 10.3	14.3 ± 10.4	15 ± 7.5	19 ± 6.9
IgG secretion rate (μg/2 min)†	25 ± 15.9	30.7 ± 22.7	31 ± 17.1	24.8 ± 18.2
Dietary intake with WBM§				
Saliva samples	12	12	12	12
sIgA concentration (μg/mL)‖	37 ± 3.8	53.3 ± 5.5	56 ± 0.57	32 ± 7.9
sIgA secretion rate (μg/2 min)†,¶	50.6 ± 22	78.2 ± 37	79.8 ± 39	43.8 ± 26.4
Saliva flow rate (mL/2 min)	1.26 ± 0.57	1.46 ± 0.67	1.41 ± 0.73	1.36 ± 0.63
Osmolality (osmol/kg)	83 ± 25	82 ± 15	88 ± 19.6	86.4 ± 21
sIgA:osmolality‡,#	0.47 ± 0.13	0.68 ± 0.16	0.67 ± 0.19	0.38 ± 0.13
IgG concentration (μg/mL)	12.6 ± 0.57	14.6 ± 0.67	14.1 ± 0.73	23 ± 6.7
IgG secretion rate (μg/2 min)†	26.6 ± 27.1	22 ± 30.6	24.1 ± 25.5	30.5 ± 17.7

IgA, immunoglobulin A; sIgA, salivary immunoglobulin A; WBM, white button mushroom

* Values are presented as mean ± SD. Differences between the control group (without WBM) and the WBM group for each variable were analyzed by analysis of variance.

† Secretion rates were calculated by multiplying the sIgA and IgG concentrations by the saliva flow rate.

‡ This ratio was calculated by dividing the sIgA concentration by osmolality.

§ One hundred grams of blanched *Agaricus bisporus* WBM taken with the normal diet per day for 1 wk.

¶ $P < 0.0005$.

‖ $P < 0.0005$.

\# $P < 0.0001$.

adult subjects. The elevated sIgA secretion rate in the WBM group remained stable at week 2. The change in the sIgA concentration and secretion rate was not due to the change in saliva osmolality, which remained relatively constant in both groups over time. In addition, elevated sIgA secretion occurred in the absence of an increase in sIgG secretion over the same period. Taken together, the data show that sIgA responds to the dietary intake of WBM in a manner consistent with an enhanced mucosal immunity.

How did dietary intake of WBM lead to an accelerated sIgA secretion in saliva? The IgA response is promoted by the regulatory activities of T-cell helper type 2 cytokines, integrins, and chemokines secreted within the tissues of the mucosal immune system linking the gut and other mucosal sites such as the salivary and mammary glands through the traffic of effector cells [3,6,7]. Thus, a mucosal immune response initiated in an inductive site (e.g., Peyer's patch in the intestine) would result in the generation of activated mucosal immunocytes, which then leave the inductive site through the lymph and enter the circulation to populate distant mucosal sites such as the lacrimal gland, where they differentiate into memory or effector IgA-producing plasma cells [7–9]. The existence of such a common mucosal immune system appears to be supported by experimental and clinical studies demonstrating that oral intake of viable [10,28,29] or killed bacteria [7,30] or bacterial antigen [6,8] can lead to the induction of strain-specific and non-specific SIgA in saliva. For example, Czerkinsky et al. [7] administered enteric-coated capsules of killed *Streptococcus mutans* to healthy volunteers and found an increase in specific IgA antibody-secreting cells in the peripheral blood within 7 d, followed by an increase in SIgA antibodies in the parotid saliva and tears of human adults. In addition, healthy volunteers ingesting a vaccine containing *S. mutans* produced and secreted specific anti–*S. mutan* IgA antibodies in colostrum, milk, tears, and saliva after immunization [6,30]. In recent studies, an oral intake of live or killed probiotic bacteria has been found to induce a strain-specific IgA response and a non-specific total IgA in the saliva of healthy subjects [28–31]. High levels of salivary SIgA have been found in infants fed fermented milk containing *Lactobacillus casei* [29]. Taken together, these studies support our hypothesis that the dietary intake of WBM stimulates the gut mucosal immune system to enhance the secretion of SIgA in human saliva.

In the present study, we used the WBM because it is widely consumed and it exhibits immunomodulatory [25,26], hypoglycemic and hypocholesterolemic [32], and antitumor [26,27] activities. In recent studies, we and other investigators have found that the WBM contains a wide range of soluble polysaccharides that have the ability to activate macrophages [26,33], a key event for effective innate and adaptive immunity. These polysaccharides have been identified as mainly water-soluble β-D-glucans with a backbone formed by linear or branch β-(1 → 4), β-(1 → 3), or β-(1 → 6) linked glucose, xylose, mannose, galactose, ribose, and fucose molecules [34]. It has been reported that oral administration of a yeast-derived soluble branched β-1, 3-D-glucan in enteric-coated capsules can lead to increased salivary concentrations of IgA in healthy subjects, whereas an increase in sIgG concentrations does not occur [31]. Furthermore, a high carbohydrate diet taken with a self-served meal for 6 d has been shown to result in higher concentrations of sIgA after exercise than before exercise in triathletes, whereas cortisol concentrations alone have been found to be increased in the self-served dietary group [35]. Our findings are consistent with these observations and suggest that the increase in the sIgA secretion rate by a dietary intake of the WBM may be due to the gut immunostimulatory effect of glucans in the mushroom.

Saliva is the most accessible of mucosal secretions for the assessment of the status of the mucosal immune system. The parotid, submandibular, and sublingual glands are the most important source of SIgA in the upper respiratory tract [13]. This study adopted the non-invasive passive dribble method for the collection of saliva samples to monitor the resting state of sIgA secretion and minimize the potential errors associated with saliva collection using mechanical suction or swab method. In our experience, the latter methods produce less stable results

owing to stimulation of the oral mucosa by the suction device or by the action of swabbing, thereby potentially increasing the saliva flow rate, which could affect the study results. There were two outcome measures in our study, namely sIgA concentration and secretion rate. We focused on salivary IgA secretion rate because it provides an indication of the total amount SIgA secreted per unit time. More importantly, it represents the actual amount of SIgA for protecting the mucosal surfaces against potential pathogens by the formation of an exclusion barrier and forms the first line of innate immune defense [2–4]. Thus, it is commonly used as a measurement of mucosal immune competence in athletes after prolonged and strenuous exercise [21,23], children with nutritional deficiencies [19,20], in individuals with psychological or occupational stress [15–18], and in the elderly [14,16]. In these populations, a decrease in sIgA secretion has been associated with the incidence of upper respiratory illness symptoms [19,20,24,36,37]. The present study showed that a dietary intake of the WBM for 1 wk produced 53% to 56% increases in sIgA secretion compared with the baseline in a group of healthy human participants, thereby indicating its potential clinical relevance. However, the elevated sIgA secretion rate in the WBM group seemed to reach a plateau at week 2 and then returned to a baseline level. This suggests that, in arresting or slowing the decrease of sIgA in individuals such as the elderly or those with immune compromise, a continuous daily intake of the WBM may be necessary to maintain an increased sIgA secretion. Although its relevance to human nutrition remains to be established, the ability to manipulate mucosal immunity through dietary intervention may have the potential for tipping the immunologic balance in favor of the host.

However, this study had some limitations. Four subjects who met the predetermined exclusion criteria were excluded from the saliva collection for data analysis at follow-up owing to poor compliance. This resulted in different sample sizes in the control and the WBM groups. However, the missing data did not occur in the WBM group. Nonetheless, using all subjects at entry, the data analysis by an intention-to-treat approach produced similar results (data not shown). Blood chemistry profiles of subjects before and after the study were not taken. However, a review of the individual records from the control and WBM groups showed no accumulated incidences of illness or symptoms that could have affected the results during the study. Moreover, this study did not distinguish the effect of the WBM from that of the rest of the participants' diet. The latter may contain factors such as vitamins, antioxidants and dietary fiber, which may contribute to mucosal immunity. However, the fact that salivary SIgA did not increase in the control group suggests that the effect observed was caused by the WBM intake.

Conclusions

This study has shown that a dietary intake of the WBM increases SIgA secretion in the saliva of healthy subjects. The increase of salivary SIgA may have the potential for improving mucosal immunity. It is important to determine the significance of such changes induced by a dietary intake of the WBM on the overall defense capacity of saliva and how this could lead to increased protection against the risk of infections such as upper respiratory illness in susceptible populations.

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