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Effect of Processed Soybeans (Tofu and Tempeh) Consumption, and Exercise on Upper Respiratory Tract Immunity

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Abstract

Background: IgA is widely used as Upper Respiratory Tract Infection (URTI) risk marker, as a lower concentration in sIgA indicates a higher incidence of URTI. This study aimed to investigate the effect of different types of exercise; combined with Tempeh consumption in increasing sIgA concentration in saliva sample.

Methods: 19 sedentary male subjects aged 20-23 were recruited and assigned into 2 groups based on the exercise type, endurance (n=9), and resistance (n=10). These subjects underwent 2 weeks of Tofu and Tempeh consumption, then were assigned to do exercises based on their groups.

Results: This study showed an increased mean value of sIgA concentrations in the endurance group; the baseline value, after food treatment, and after food and exercise treatment were 71.726 ng/mL, 73.266 ng/mL, and 73.921 ng/mL, respectively for Tofu treatment; and 71.726 ng/mL, 73.723 ng/mL, and 75.075 ng/mL, respectively for Tempeh treatment. While in the resistance group, there was also an increase in the mean value of sIgA concentrations; baseline, after food treatment, and after food and exercise treatments were 70.123 ng/mL, 71.801 ng/mL, and 74.430 ng/mL, respectively for Tofu treatment; and 70.123 ng/mL, 72.397 ng/mL, and 77.216 ng/mL, respectively for Tempeh treatment. These results indicated that combining both Tempeh consumption and moderate intensity resistance exercise was more effective to increase sIgA concentration.

Conclusions: This study showed that combining moderate intensity resistance exercise with consumption of 200 gr Tempeh for 2 weeks was more effective in increasing sIgA concentration; compared to endurance exercise and Tofu consumption.

Keywords: Exercise, IgA, Paraprobiotics, Tempeh, Upper Respiratory Tract Infection (URTI).

Introduction

Upper respiratory tract infection (URTI) is an infectious disease caused by various types of bacteria and viruses that infect the upper respiratory tract, including nose, sinuses, pharynx, larynx, and large airways (1). These are the most common infectious illness in general population, having the ability to spread amongst people easily. It usually involves direct invasion of the upper airway mucosa by the organism that acquired by inhalation of infected droplets. Common symptoms of URTIs are coughing, sore throat, runny nose, headache, facial pressure, sneezing, etc. (2).

One of the immunity proteins that plays a role in preventing URTI is Immunoglobulin A (IgA). IgA is the main class of antibodies present in the body secreted fluids such as saliva, tears, nasal fluids, and mucus from the intestines, usually known as secreted IgA (sIgA) (1). sIgA in saliva takes a part in URTI prevention system because it primarily interacts and defends the various secretory surfaces from

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invasion and has the highest concentration than other antibodies in saliva (3,4). sIgA is the first line of defense of the host against pathogens which colonize or invades the mucosal surface. sIgA main function is to limit microbial adherence as well as penetration of foreign antigens into the mucosal tissue (5). Research by Tiernan et al. (6), found out that low level of salivary sIgA is correlated with higher incidence of URTI. This finding indicated that salivary sIgA level might serve as an effective, non-invasive, and rapid biomarker for URTI infection risk.

Corona virus disease, well known as COVID-19 is a disease that caused by SARS-CoV-2 virus and can be included as URTI. Many studies have explored ways to improve levels of salivary sIgA to prevent URTI, especially during the pandemic situation.

Exercising is one of many activities that people believed have many positive effects on the body, such as improving brain function, enhancing immunity, modulate neurogenesis (7), and also said to be one of the factors that affect salivary sIgA level (8). According to Trochimiak & Hübner-Woźniak (8), acute sIgA level may depend on the intensity, duration, and the type of physical activity. The research also stated that moderate intensity exercise increased sIgA concentration most efficiently, where prolonged high intensity could lead to immunosuppression, decreasing sIgA concentration. Generally, there are two types of exercise, resistance and endurance exercise. Both type of exercises had been proven to increase IgA levels acutely (9); however, no study has ever investigated which type of exercise is more effective to improve salivary sIgA levels.

Diet also plays an important role in influencing sIgA concentration. One of functional food that has been said to be effective to increase sIgA concentration is Tempeh, a traditional Indonesian food made from fermented soybeans. Research by Subandi et al. (4), stated that Tempeh could increase sIgA concentrations and activity in human intestinal, due to the para-probiotics properties, consisted of *Rhizopus oligosporus* and lactic acid bacteria that are used during the fermenting process. However, Tempeh's potential to increase sIgA concentration in the upper respiratory tract, specifically in the salivahas not been reported. The objectives of this research were to analyze and measure the effect of Tempeh consumption on the salivary IgA level, compared to unfermented soybean; and to analyze the influence of different exercise patterns combined with Tempeh consumption on sIgA concentration level in saliva.

Materials and Methods

There were several main steps, consisted of subjects recruitment, preliminary exercise, Tempeh and Tofu consumption, exercise treatment, saliva samples collection, salivary IgA quantification, and statistical analysis. Twenthy healthy adult men (age 21 ± 0.78 years; BMI 23.02 ± 3.67 kg/m^2) participated in the study, and there was one drop out due to not fulfilling the minimum Tempeh consumption requirement. Based on means difference and SD on similar previous study by Klentrou *et al.* (10), minimal of 14 participants were required for the study. In the beginning of the research, the subjects were required to fill a form that consist of personal identity, height, weight, past injuries, allergies, International Physical Activity Questionnaire (IPAQ), and Physical Activity Readiness Questionnaire (PARQ). Subjects were divided into two groups based on the exercise types. The first group (n=9) was Endurance Exercise group, and the second group (n=10) was Resistance Exercise group. There were five major steps for both groups (endurance and resistance): body composition measurement, preliminary exercise, Tofu and Tempeh consumption, exercise, and sample collection.

Subjects Recruitment

Subjects' recruitment was carried out at the Atma Jaya Catholic University of Indonesia. Criteria for subjects in this study were male subject (17-25 years old), normal BMI (18.0-22.9 kg/m^2), sedentary physical activity, not smoking, not allergic to soy, and did not have any injury or congenital disease. The subjects' body composition was measured by Omron

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HBF375 to collect physical data including Body Mass Index (BMI), Basal Metabolic Rate (BMR), weight, height, body fat, and skeletal muscle in order to balance body composition of both group's participant. The subjects were also given diet and activity guidelines to be followed during the whole research.

Prelminary Exercise

Preliminary exercise was performed to collect their physical abilities data and to familiarize the subjects with the tool that would be used during the exercise treatment. For resistance group, subjects did a strength test to determine their one repetition maximum (1-RM) weight for each exercise, consisted of bench press, dumbbell row, dumbbell squat, and overhead press. The 1-RM data was used to be the baseline for each subject's exercise. For endurance group, subjects were assigned to run on the tracks and reach the target heart ranges for moderate exercise which was 60-70% from their maximum heart rate based on ages (11); heart rate was measured using Fitbit Inspire HR. The 1-RM data were calculated using Epley's formula: 1 RM = weight * [1 + (Reps/30)] (11).

Tempeh and Tofu Consumption

Tempeh and Tofu consumption dose was adapted from Subandi et al. (4); with some modification. Subjects were assigned to consume 200 gr of steamed Tofu every day for two weeks, followed by 200 gr of steamed Tempeh every day for the next two weeks. Between the Tofu and Tempeh consumption period, there was a two week of washout period. In the washout period, subjects were not given any food treatment. The washout period was required to prevent the carryover effect between both food treatments that might otherwise confound the estimates of treatment effects. During this whole 6-week period, subjects were prohibited to consume any foods that contain probiotics and para probiotics properties from other sources.

Exercise Treatment

Each subject was assigned to two days of exercise in moderate intensity based on normal BMI calculation. The first exercise day was scheduled after the 2-week Tofu consumption period, and the second exercise was scheduled after the 2-week Tempeh consumption period. For the endurance group, the exercise method was adapted from McDowell et al. (13); with some modification. The subject was assigned to an exercise session consisting of 3 bouts of 10 mins jogging with 1 minute rest on each bout, while maintaining their heart rate at 60-70% of their age maximum heart rate as in Table 1. For resistance group the exercise method was adapted from Neves Jr et al. (14); with some modification. Subjects were assigned to do four exercises movement that focused on major muscles that consist of bench press, dumbbell row, dumbbell squat, and overhead press with 60% of their 1-RM weight. Each movement was done for 2 sets with 10 reps each, 60 seconds rest between each set, and 120 seconds rest between each movement.

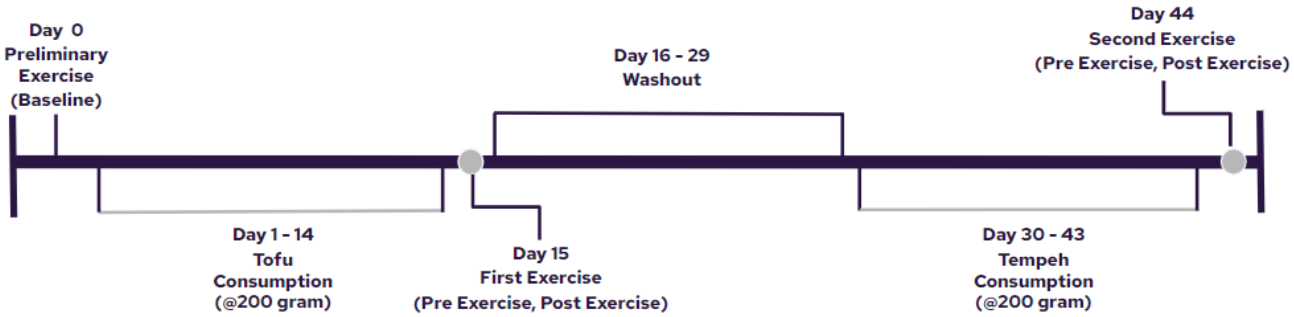

Fig. 1. Research timeline.

Saliva Samples Collection

Figure 1 shows the research timeline, and sample collection timestamps. Each subject's saliva was collected five times. The first saliva sampling was done before the preliminary

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exercise, and act as a baseline (no treatment). The second and third saliva sampling was done in the first exercise day after the Tofu consumption period. The second sample was taken before the exercise (Tofu treatment, no exercise), and the third sample was taken after the exercise (Tofu and exercise treatment). The fourth and fifth samples were taken at the same day, in the second exercise day after the Tempeh consumption period. The fourth sample was taken before the exercise (Tempeh treatment, no exercise), and the fifth sample was taken after the exercise (Tempeh and exercise treatment).

Saliva was collected using the passively drooling method adapted from Miletic et al. (15). Prior to collection, subjects should avoid eating or drinking for at least 30 minutes before start salivating. 10 minutes before start salivating, subjects were asked to rinse their mouth with clean water. To stimulate saliva, subjects were told to swab their inner cheeks with their tongue for 30-60 seconds. Then, subjects passively drooled the saliva into the tube until reaching 1 mL. Then, the samples were stored at -20 °C until sample was ready to be analyzed. Sample was thawed by heating it at 56°C for 30 minutes, then cooled at room temperature, centrifuged at 12.500 rpm for 20 minutes. Then, the supernatant was taken to be analyzed.

Salivary sIgA Quantification

Salivary sIgA quantification method was adapted from Miletic et al. (15); with some modification. sIgA concentration is quantified by using Human IgA ELISA kit from Elabscience (E-EL-H6071). First, 100 µL each dilution of standard, blank and sample was added into 96 well ELISA microplate, then incubated for 90 min at 37 °C. The liquid was decanted from each well, then immediately 100 µL of Biotinylated Detection Ab working solution was added to each well and then incubated for 1 hour at 37 °C. The solution was decanted from each well, then 350µL of wash buffer was added to each well. Soak for 1-2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. This wash step was repeated 3 times. 100µL of HRP Conjugate working solution was added to each well then incubated for 30 min at 37 °C. The solution was decanted from each well, then the wash process was repeated for 5 times. 90 µL of substrate reagent was added to each well then incubated for about 15 min at 37 °C. Then, 50µL of Stop Solution was added to each well, while keeping the plate protected from light. The optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 nm.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 25. Statistical data was considered significant if $P< 0.05$. All data were tested for normal distribution using Saphiro-Wilk Test, and some of the data were tested homogeneity of variance among groups using Levene's test. After passing the normality test, result was tested by independent t-test, or repeated measure ANOVA test based on the data type, while the data that failed the normality test will be analyzed by non-parametric using Mann-Whitney.

Results

The subject's physical data consisted of mean value of age, weight, body fat, skeletal muscle, and BMI is shown in Table 1.

Table 1. Physical data of the subjects.

Parameters	Resistance (Mean ± SD)	Endurance (Mean ± SD)
Biodata	N = 10	N = 9
Age (Years)	21.2 ± 0.78	20.89 ± 0.78
Weight (kg)	71.11 ± 11.50	68.71 ± 11.29
Body Fat (%)	17.74 ± 6.41	17.33 ± 5.82
Skeletal Muscle (%)	35.32 ± 2.84	35.18 ± 2.37
BMI (kg/m^2)	23.66 ± 3.66	22.31 ± 3.68

[a]Independent t-test, significant difference at α=5%.

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Food and Exercise Influence in Endurance Group

Data between groups were compared using independent t-test and tested for homogeneity using Levene's test. The result showed no significant difference between each data among the groups, and the data were homogeneous.

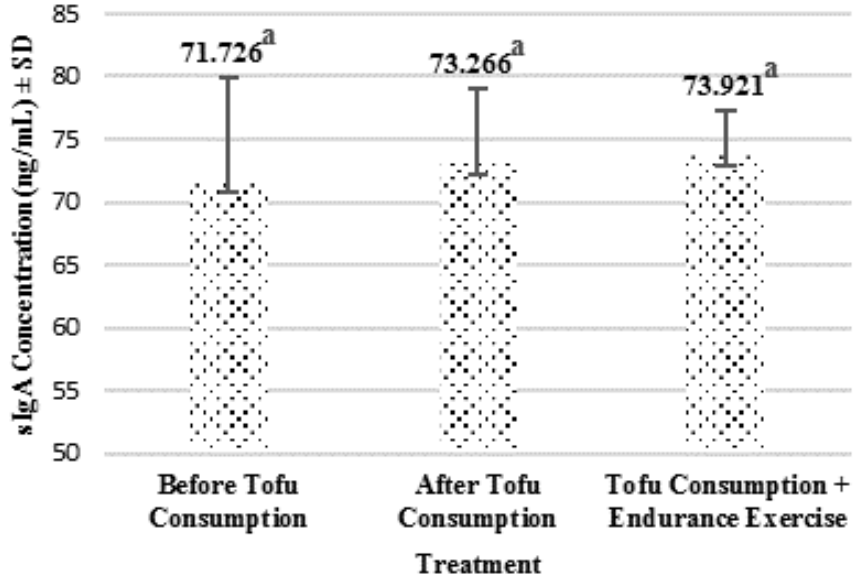

Fig. 2. Salivary sIgA concentration on endurance group before & after Tofu consumption [a]Repeated Measure ANOVA, significant difference at α=5%.

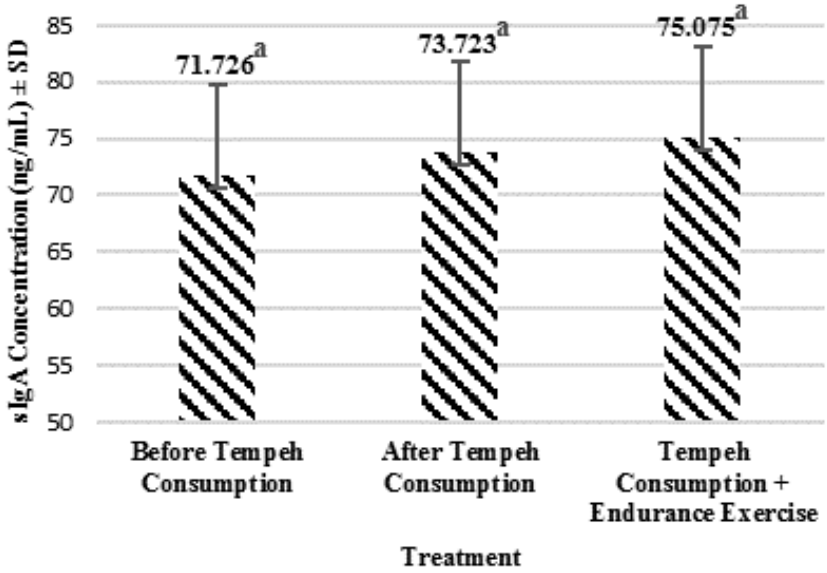

Fig. 3. Salivary sIgA concentration on endurance group before & after Tempeh consumption [a]Repeated Measure ANOVA, significant difference at α=5%.

The mean values for the baseline, after Tofu treatments, and after exercise and Tofu treatments were 71.726 ng/mL, 73.266 ng/mL, and 73.921 ng/mL, respectively. The data were analyzed using repeated measure ANOVA. Result showed there was an increase in sIgA concentration after each treatment, but the value was not statistically significant (Fig. 2).

The mean values for the baseline, after Tempeh consumption, and after exercise and 2 weeks Tempeh consumption were 71.726 ng/mL, 73.723 ng/mL, and 75.075 ng/mL, respectively. Result showed that there was an increase in sIgA concentration after each treatment, although the value was not statistically significant (Fig. 3).

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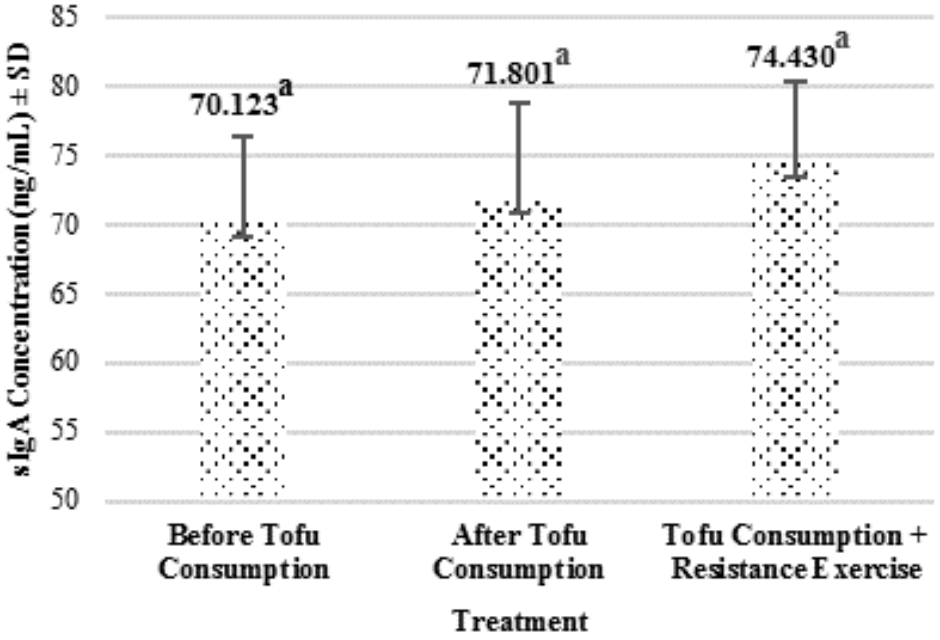

Fig. 4. Salivary sIgA concentration on resistance group before & after Tofu consumption [a]Repeated Measure ANOVA, significant difference at α=5%.

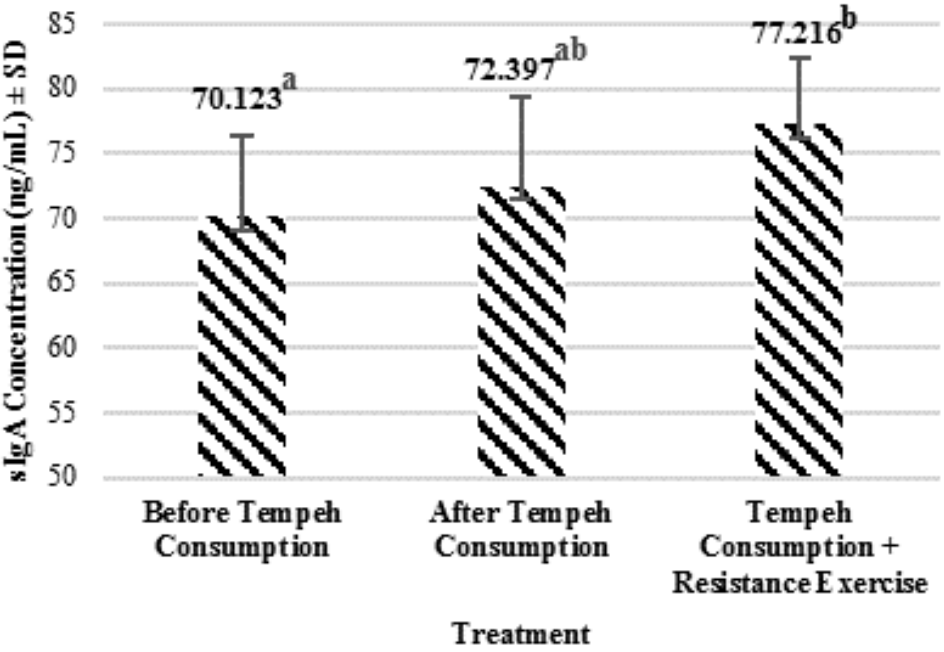

Fig. 5. Salivary sIgA concentration on resistance group before & after Tempeh consumption [a]Repeated Measure ANOVA, significant difference at α=5%.

Food and Exercise Influence in Resistance Group

The mean values for the baseline, after Tofu consumption, and after exercise and Tofu consumption were 70.123 ng/mL, 71.801 ng/mL, and 74.430 ng/mL, respectively. The data were analyzed using repeated measure ANOVA and there was an increase in sIgA concentration, although the value was not statistically significant (Fig. 4).

The mean values for the baseline, after Tempeh consumption, and after exercise and Tempeh consumption were 70.123 ng/mL, 72.397 ng/mL, and 77.216 ng/mL respectively. The data were analyzed using repeated measure ANOVA, and the result showed significant increase between the baseline and after Tempeh and resistance exercise treatment (Fig. 5).

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Discussion

Tempeh Effect on sIgA Concentration

In endurance group, the data showed that both Tofu and Tempeh treatment could increase the sIgA concentration from the baseline, but Tempeh treatment showed higher increase than Tofu treatment. However, both values were not statistically significant. The same pattern also applied to the resistance group, where both of Tofu and Tempeh treatment increased sIgA concentration. The increase in Tofu treatment was not significant; yet, Tempeh gave a significant increase on sIgA concentration. This result indicated that combination of resistance exercise and Tempeh treatment was more potent in increasing sIgA concentration.

Tofu is used as alternative food in this study because Tofu's main ingredients is similar to Tempeh, which is soybean. However, Tofu did not go through fermentation process. Meanwhile Tempeh also contains of soybean as its main ingredients, but it went through a fermentation process with the help of white mold called *Rhizopus oligosporus* and lactic acid bacteria. After the cooking process, these microbes will die because of the heat, making Tempeh a paraprobiotic food (16).

Based on the previous study by Sun et al. (17), Tofu could increase the sIgA concentration because of the protein property contained in soybean called Glycinin. Glycinin was proven to increase production of T helper 2 cell cytokines, which are IL-4 and IL-6. IL-4 main function is to stimulate IgE production and induction of naïve CD4+ T cells to differentiate into Th2 cells rather than Th1 cells. IL-4 also can help B lymphocytes to proliferate and differentiate into plasma cells, which are the main source of IgA in mucosa. IL-6 main function is to induce proliferation and differentiation of B lymphocytes to secrete IgA antibodies, increasing sIgA concentration in the secreted liquid body, such as saliva (18). Systemic immune responses to soybean glycinin with increased lymphocytes CD4+ T cells differentiation into Th2 pathways by increasing production of IL-4 and IL-6, which finally increase sIgA concentration in saliva.

Meanwhile, Tempeh also has glycinin protein because it has soybean as its main ingredients (16). Fermentation process can help to break down antinutritional factors like glycinin into simpler amino acids to facilitate the digestion and absorption. According to study by Yan et al. (19), it is said that fermented soybean contained 60% less glycinin protein, and respectively contained more indispensable amino acids (Isoleucine, Leucine, Phenylalanine, Threonine, Valine), and dispensable amino acids (Serine, Glycine). Those amino acids are said to be positively correlated with increased IgA production, thus secreting more sIgA in the saliva (19).

During fermentation process, not only molds (*R. oligosporus*) are present, but there are other microorganisms such as lactic acid bacteria, yeasts, and different gram-negative bacteria. In previous study, Barus et al. (20) showed that the process of cooking in Tempeh or soybean can reduce microbial populations in large numbers. In this research, Tempeh was steamed before given to the subjects to be consumed. Therefore, most of the microorganism that were used during the fermentation process were inactivated. However, our immune systems still can recognize the dead cells as antigens, responded by increasing Th2 cytokines (IL-4, IL-6, and IL-10), and the inhibition of IL-12 led to increased sIgA production. This concept is called paraprobiotics effect (4, 21).

The concept was also supported in research by Soka et al. (22) that showed both raw and cooked Tempeh consumption could stimulate similar increases in IgA secretion, despite the fact most of the microorganism in cooked Tempeh had been inactivated. Tofu does not have paraprobiotics effect because Tofu did not undergo a fermentation process during the production process, therefore, Tofu does not have much microorganism antigens to stimulate the immune systems. According to Moradi-kalbolandi et al. (23), delivering the antigens into a region simultaneously can provoke the regional immune systems

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response, in this case is salivary sIgA, and stimulating the production of sIgA in that area.

The data show that, in both type of food treatment, resistance group had higher increase in sIgA concentration compared to the subjects in the endurance group. This indicated that resistance type exercise is better in stimulating sIgA production and secretion than endurance type exercise. "Tissue Injury" hypothesis could be used as a model to explain this exercise induced immune response. This model proposes that repetitive mechanical trauma resulting from exercise can cause tissue injury. This injury can induce a pro-inflammatory response involving cytokines produced by Th1 cells, followed by Th2 cytokines response. Th2 is the host response to restore homeostasis and prevent further tissue damage by dampening the differentiation process from T lymphocytes to Th1. As mentioned before, differentiation into Th2 means that it will boost the production of IL-4 and IL-6, therefore increasing sIgA production and secretion from mucose tissue, and affecting sIgA concentration on saliva.

The difference between both type exercises is on the muscle usage. The resistance exercise mostly focused on the muscle strength, for example weight training and free-weight training (24). The endurance exercise mostly focused on heart rate and breathing capacity, which goals is to increase body endurance (25). Both type of exercises caused muscle injury, however resistance exercise involves more muscle in the process than endurance exercise, meaning that resistance exercise will give more tissue injury, activating more Th2 cells, and increasing sIgA production and secretion. Refering to Kushkestani et al. (26); resistance exercise generally reduced systemic inflammation. However, it should be noted that higher intensity of resistance exercise (>75% 1RM, and lasting more than 1.5 hours) could lead to immunosuppression, where sIgA concentration will decrease acutely. This is due to the repetitive mechanical trauma, injuring more muscle tissue, dampening more Th1 cells, resulting to an imbalanced inflammatory response. Factors that could lead to sIgA concentration decrease are the combination of sub-optimal recovery, high training load, and acute increase in training load or frequency (27). Whereas for endurance exercise, refering to Klentrou et al. (10); three sessions a week of endurance exercise training also resulted in a significant increase in sIgA post-exercise compared to the none exercising group. Difference in the result can be caused by the amount of exercise session during the study, and device that were used at that study such as bicycles, treadmills, and stair climbers.

Limitation of this study are the gender, age, and BMI of the subjects participated in this study were all homogenized, which might show different phenomenon if the same methods used in different categories. Combination of resistance exercise and Tempeh consumption dose were also a limitation in this study, which made it unknown which factor has more impact to increase the sIgA concentration. Thus, these factors also need to be investigated in further studies.

To conclude, our data showed that resistance exercise in moderate intensity or around 60% 1 RM weight capacity and Tempeh consumption for 2 weeks period were an effective combination to improve salivary sIgA concentrations. Thus, Tempeh can be considered as a sport food alternative, where people start to boost their immune system in the pandemic situation to prevent incidence of URTI.

Due to the pandemic situation in the past 2 years, people has focused on ways to improve their immunal system in order to lower the risk of URTI, including COVID-19. Salivary sIgA concentration is often used as immunity marker for URTI risk, as lower sIgA concentration is correlated with higher incidence in URTI. Many studies had showed ways to improve sIgA concentration such as exercising or consuming certain types of food. This study aimed to investigate the effect of different type of exercise and consumption of Tempeh in increasing sIgA concentration in saliva. This study showed that Tempeh consumption per se did not increase sIgA

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significantly. However, when being combined with resistance exercise, Tempeh increased sIgA concentration significantly. In the same group of resistance exercise, Tempeh gave a higher increase of sIgA concentration than non-fermented soybean (Tofu). The proposed mechanism was due to the fermentation process during Tempeh production, resulting in Tempeh's paraprobiotic property, and soy's antinutritional protein breakdown into more digestible amino acids. Both paraprobiotics and the disgestible amino acids could provoke the production and secretion of sIgA as the regional immune systems. Endurance and resistance type exercises were said to increase sIgA concentration acutely. Yet, this study showed that resistance exercise gave higher increase of sIgA concentration, compared to endurance exercise. To conclude, this research indicated that combination of moderate intensity resistance training and Tempeh consumption was effective in increasing sIgA concentration. However, there were limitations of this study such as are the gender, age, and BMI of the subjects participated in this study were all homogenized, that might show different result if the methods are used in different categories. Combination of resistance exercise and Tempeh consumption dose were also a limitation which made in unknown which factor has higher impacts on increasing sIgA concentration; and thus, these factors need to be considered in the future studies.

Ethical Approval

Ethical approval was obtained from The Institution of Research and Community Services Atma Jaya Catholic University of Indonesia (approval No. 00031Z/III/LPPM-PM.10.05/12/2021). All participants provided written informed consent, and were informed that they could terminate their participation any time.

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Conflicts of Interest

The authors certify that there is no conflict of interest.

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