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Title: Effects of *Lactiplantibacillus pentosus* ONRICb0240-containing food on Reducing Immunosuppression in Judo Athletes During Rapid Weight Loss

Brief running head: Lactiplantibacillus and immune function in judo

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ABSTRACT

Despite the known health impacts of rapid weight loss (RWL) in athletes, effective mitigation strategies, especially nutritional ones, remain underexplored. This study aimed to investigate the effects of *Lactiplantibacillus pentosus* ONRICb0240-containing food (b240) intake on salivary secretory immunoglobulin A (SIgA) secretion in judo athletes undergoing RWL. Therefore, 17 male competitive college judo athletes were categorized into two groups: the b240 intake (active group; n = 9) and no b240 intake (control group; n = 8). Both groups engaged in 4 weeks of regular training followed by 1 week of RWL. The active group consumed b240 daily, every evening before dinner, throughout the 5-week experiment. Participants documented upper-respiratory symptoms (URS) and abdominal conditions during the study. Saliva samples were collected, and physical fitness tests were performed on each participant at 0 weeks (before intervention), 4 weeks (before weight loss), and 5 weeks (after weight loss). Results showed that salivary SIgA secretion was significantly decreased in the control group after weight loss compared to before the intervention (0 weeks, $p < 0.05$), while no significant changes were observed in the active group. Compared to the control

group, URS decreased, and the percentage of days participants reported their abdominal condition as “good” increased in the active group ($p < 0.05$). These findings suggest that regular intake of b240 may be beneficial for reducing the frequency of URS among judo athletes, potentially due to better maintenance of salivary SIgA secretion during training and RWL.

Key words: *Lactiplantibacillus pentosus*, judo, salivary SIgA, mucosal immunity, upper-respiratory symptoms

63 タイトル：

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65 ONRICb0240 の効果

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要約

アスリートにおける急速減量（RWL）の健康への影響が知られているにも関わらず、効果的な軽減策、とくに栄養による解決策は不明である。本研究は、RWL を行う柔道選手の唾液分泌型免疫グロブリン A（SIgA）に対する乳酸菌（*Lactiplantibacillus pentosus* ONRICb0240）含有食品（b240）の効果を検討することを目的とした。男子大学柔道選手 17 名を対象とし、乳酸菌摂取群（active 群; n = 9）と摂取なし群（control 群; n = 8）の 2 群に分けた。両群とも 4 週間の通常トレーニングの後に 1 週間の RWL を行った。active 群は 5 週間の実験期間中、毎日夕食前に乳酸菌を摂取した。参加者は試験期間中、上気道症状（URS）と腹部の状態を記録した。0 週（介入前）、4 週（減量前）、5 週（減量後）に唾液採取と体力テストを行った。その結果、減量後の control 群では、介入前（0 週、$p < 0.05$）と比較して唾液中の SIgA 分泌量が有意に減少したが、active 群では有意な変化は認められなかった。また、Control 群に比べて active 群では URS が少なく、腹部の状態について「良好」と回答した日数の割合が多かった

（$p < 0.05$）。これらの結果より、乳酸菌 b240 の継続的な摂取は減量期の柔道選手の免疫低下抑制や URS の予防に働き、良好なコンディション維持に役立つ可能性が示唆された。

INTRODUCTION

Judo, an Olympic sport played worldwide, employs a weight class system to ensure fairness by accounting for individual differences in physical characteristics [1]. Many athletes strategically use weight loss to gain a physical advantage over their opponents in competitions [2]. Rapid weight loss (RWL) [3] has been shown to impose significant physiological and psychological burdens, as detailed in a systematic review [4]. Specifically, methods of RWL, such as dietary restriction and dehydration, can cause various physiological issues, including upper-respiratory symptoms (URS) [5] and abdominal problems [6].

The frequent occurrence of URS is attributed to impaired immune function. Indeed, athletes undergoing RWL have been observed to exhibit URS alongside reductions in secretory immunoglobulin A (SIgA) in saliva [7] and blood lymphocytes [8]. Furthermore, intense exercise training has been shown to significantly reduce salivary SIgA secretion in combat sports [9]. Therefore, preventing immunosuppression is inferred to be the most

effective intervention for inhibiting URS during RWL. Therefore, it is speculated that preventing immunosuppression is the most effective intervention for suppressing URS in the RWL.

Lactiplantibacillus pentosus ONRICb0240, an anaerobic/non-spore-forming gram-positive bacterium isolated from fermented tea leaves [10], secretes SIgA in Peyer's patch cells in a strain-specific manner [11]. We hypothesized that its action might contribute to maintaining immune function in humans. Indeed, it has been demonstrated that heat-killed ONRICb0240promotes salivary SIgA secretion in elderly adults with low physical fitness [12]. Therefore, intake of heat-killed ONRICb0240 is expected to preserve immune functions and suppress URS.

Athletes who routinely perform intense exercise training often experience abdominal issues, such as nausea, abdominal cramping, and diarrhea. These problems are believed to result from a decrease in intestinal membrane permeability, inducing a pro-inflammatory cascade [6,13]. This effect is speculated to be more severe in athletes undergoing RWL because the combination of intense exercise, dehydration (via water restriction and sweating), and caloric restriction amplifies the elevation of inflammatory cytokine levels [14]. Conversely, several clinical trials have indicated that heat-killed bacteria, such as *Lactiplantibacillus plantarum* L-137 [15] and *Enterococcus faecalis* EC-

12 [16] can regulate the intestinal environment by producing regenerating family member 3 (Reg3), a major antimicrobial peptide. Similarly, heat-killed ONRICb0240 might promote intestinal regulation through antimicrobial actions, as the bacteria activate toll-like receptor 2 (TLR-2) [17], a receptor upstream of increased Reg3 gene expression [18]. Therefore, ONRICb0240 may help inhibit gastrointestinal distress in athletes during RWL.

This study aimed to investigate the effects of ONRICb0240 intake on salivary SIgA secretion, URS, and abdominal condition in judo athletes undergoing RWL. It is thought that the ingested b240 is recognized by dendritic cells in the intestinal tract, which produce cytokines such as interleukin 6 (IL-6) to induce IgA production by B cells. B cells then contribute to SIgA secretion in the intestinal tract, and some B cells migrate to the salivary glands and contribute to salivary SIgA secretion [17,19]. Therefore, we hypothesized that continuous intake of b240 may reduce the risk of infection and the occurrence of URS in athletes during RWL by stimulating salivary SIgA secretion and that its antibacterial effect in the intestinal tract may suppress the deterioration of abdominal conditions.

MATERIALS AND METHODS

151 **Experimental Approach to the Problem**

152 In this study, we investigated the effects of continuous b240 intake on immune function
153 by requesting judo athletes perform RWL and using salivary SIgA secretion, URS
154 appearance, and abdominal condition as evaluation indices. This study was conducted in
155 an open-label randomized controlled trial.

156

157 **Participants**

158 This study adhered to the principles of the Declaration of Helsinki and was approved
159 by the Ethics Committees of Japan Institute of Sports Sciences [approval #033 (2017)].
160 Participants were provided with detailed information about the study's risks, stressors,
161 and potential benefits before signing an informed consent form. A total of 26 competitive
162 male college judo athletes participated in this study (Fig. 1). According to a self-reported
163 questionnaire, none of the participants had been treated with medications known to affect
164 immune function, experienced acute infectious illnesses in the preceding 3 months, or
165 regularly smoked tobacco. Participants were randomly assigned to either the b240 intake
166 (active group; n = 13) or no b240 intake (control group; n = 13) based on age, height,
167 body weight, susceptibility to URS, and salivary SIgA secretion rate. Finally, 17
168 participants (active group: n = 9; control group: n = 8) were included in the analysis (Fig.

169 1). Participants had an average judo practice duration of 13.1 ± 0.6 years. All participants
170 trained regularly for 16.5 h per week and held technical levels between 1st and 2nd Dan
171 black belts, competing in weight categories ranging from 66 to 100 kg.
172 [*Insert Fig. 1*]
173
174 **Procedures**
175 The experimental design of this study is illustrated in Fig. 2. Over a 5-week
176 intervention period, participants were instructed to continue their normal training routines.
177 In the last week, participants engaged in self-determined weight loss programs, including
178 dietary energy restriction, fluid restriction, wearing sauna suits during intense exercise
179 training, and sauna use, in addition to their regular judo training. No food other than that,
180 including b240 was provided during the experimental periods, and each participant used
181 his own selection for the intake of each nutrient, etc. The consumption of supplements
182 (e.g., vitamins, minerals) and additional probiotics (e.g., yogurt) was prohibited during
183 the study. The active group consumed heat-killed b240 (2×10^9 cells containing) every
184 evening before dinner for 5 weeks. A trial tablet containing heat-killed ONRICb0240 was
185 prepared as follows: after cultivating the bacteria and performing repeated washes, the
186 cells were sterilized in an autoclave and freeze-dried. The non-viable b240 was counted

187 using a flow cytometer and adjusted to produce the test sample with 2×10^9 cells. The
188 nutritional content per 100 g of the food included: energy, 95 kcal; protein, 10 g (L-valine,
189 500 mg; L-leucine, 1,000 mg; L-isoleucine, 500 mg; L-arginine, 500 mg; whey protein,
190 7.5 g); carbohydrate, 14 g; fat, 0 g; sodium chloride equivalent, 0.16 g; vitamin B_6, 5.0
191 mg; vitamin D, 10.0 μg; citric acid, 1,250 mg. Assessments, including anthropometric
192 measurements, saliva sampling, subjective physical condition reporting, and physical
193 fitness tests, were conducted at 7:00 AM at their normal weight at three time points: 0
194 weeks (before intervention), 4 weeks (before weight loss), and 5 weeks (after weight loss).
195 During the initial 4 weeks, participants performed their regular judo, interval, and
196 resistance training regimens for 2.5–3.0 h daily. Judo training sessions included stretching,
197 judo-specific skills and drills, and high-intensity randori (fighting practice), targeting
198 approximately 60–80% of the maximal oxygen uptake and 80–85% of the maximum heart
199 rate [20].
200
201 [*Insert Fig. 2*]
202
203 **Subjective physical assessments**

Participants were asked to evaluate their physical condition over the past week at the 0-, 4-, and 5-week marks using a questionnaire. This questionnaire inquired about the appearance of URS and the rating of abdominal condition, as described in a previous report [21]. The assessment of URS was based on prior studies [22], in which complaints of URS on any day, assuming the presence of at least one URS (sore throat, runny nose, coughing, headache, lassitude, chillness, and fever, were noted. The percentage of days with complaints was calculated for each period (before intervention (1 week before the experiment), normal training (4 weeks), and weight loss (1 week) and compared between groups. Responses to abdominal conditions ("good", "usual", "bad") were also compared between groups by calculating the percentage of each response for each period. The data are presented as the percentage of days with reported conditions for each period.

Anthropometric measurements

Body weight, percentage of fat, fat mass, fat-free mass, and body water content were recorded using a multi-frequency bioelectrical impedance device (InBody® 770; InBody Japan Inc., Tokyo, Japan). Measurements were taken with each participant in light clothing and without footwear.

222 **Saliva analyses**

223 Participants were instructed to abstain from alcohol on the day before the test and fast
224 from 10:00 PM the previous day. Saliva samples were collected between 6:00 AM and
225 7:00 AM after overnight fasting. Participants rinsed their oral cavity three times with
226 mineral water and then rested for at least 5 min. Saliva production was stimulated by
227 chewing paraffin wax at a rate of 180 times/180 s. The stimulated saliva was collected by
228 passive dribbling into a sterile collection tube. Following the measurement of sample
229 volume, saliva samples were frozen at -40°C. SIgA concentration was determined using
230 an enzyme-linked immunosorbent assay [12]. The SIgA concentration (μg/mL) was
231 multiplied by the saliva flow rate over 3 min (mL/3 min) to calculate the SIgA secretion
232 rate (μg/3 min).

233

234 **Physical fitness tests**

235 The participants underwent physical fitness tests at 0, 4, and 5 weeks, encompassing
236 five tests: isometric grip strength measured with a handgrip dynamometer, muscle
237 endurance assessed through a 30-s sit-up test, muscular power evaluated using vertical
238 jump and standing broad jump tests, and whole-body reaction time. The isometric grip
239 strength, sit-up, and standing broad jump tests followed protocols described in the

240 “Physical Fitness Test” by the Japan Ministry of Education, Culture, Sports, Science, and
241 Technology. The vertical jump test was performed on a mat switch (660 × 1000 mm)
242 connected to a PC via an A/D converter (Multi Jump Tester, DKH Co., Ltd., Tokyo,
243 Japan). Whole-body reaction time was measured by instructing participants to jump
244 vertically as quickly as possible upon the activation of a red light placed 2 m ahead, with
245 the time from light stimulus to both feet leaving the ground recorded (T.K.K.1264p, Takei
246 Scientific Instruments Co., Ltd., Nigata, Japan).
247
248 **Assessment of nutritional intake**
249 Nutritional intake data were collected using a detailed 3-day food record immediately
250 before the intervention and during the weight loss period. Participants were encouraged
251 to accurately record all foods and fluids consumed, ensuring precise reporting of amounts
252 and types. Daily nutritional intake values were calculated using dietary assessment
253 software (Excel Nutrition ver. 2.3; Kenpakusha, Tokyo, Japan). Participants were also
254 asked to adhere to their normal dietary patterns throughout the study.
255
256 **Statistical analysis**

Data are presented as means ± standard error (SE). A p-value of < 0.05 was considered statistically significant in all analyses. Variables obtained from questionnaires (including physical assessments of URS and abdominal conditions) were analyzed using the chi-squared test and residual analysis. Other variables were analyzed using two-way repeated measures analysis of variance (ANOVA). A Tukey-Kramer post-hoc test was applied to identify specific differences when ANOVA revealed significant effects.

RESULTS

Table 1 presents the physical characteristics of the active and control groups during the study periods. No significant group-time interaction was found for body weight, fat mass, fat-free mass, and body water. A significant decrease in body weight (3.6% average loss), fat mass, fat-free mass, and body water was observed in both groups after weight loss (at 5 weeks) compared to before the intervention (at 0 weeks, $p < 0.05$). The percentage of body fat in the control group at 5 weeks was significantly decreased compared to that at 0 weeks ($p < 0.05$). Similarly, body weight, fat-free mass, and body water in both groups at 5 weeks were significantly decreased compared to those before weight loss (at 4 weeks, $p < 0.05$). Fat mass in the control group at 5 weeks was significantly decreased compared to that at 4 weeks ($p < 0.05$).

[*Insert Table 1*]

Table 2 and Fig. 3 detail salivary SIgA-related measurements. No significant group-time interaction was found for saliva flow, SIgA concentration, and SIgA secretion rate. The saliva flow rate at 5 weeks in both groups was significantly decreased compared to that at 0 weeks ($p < 0.05$), and in the active group, it was significantly decreased compared to that at 4 weeks ($p < 0.05$). The salivary SIgA secretion rate in the control group at 5 weeks was significantly decreased compared to that at 0 weeks ($p < 0.05$), whereas the active group did not show a significant change.

[*Insert Table 2*]

[*Insert Fig. 3*]

Fig. 4 illustrates subjective physical assessments, including URS and abdominal condition. The percentage of days with complaints of URS during the intervention periods tended to be lower in the active group than in the control group, particularly during the normal training period, where it was significantly lower in the active group ($p < 0.05$).

293 The percentage of respondents reporting their abdominal condition as “good” in the active
294 group was significantly increased during the intervention periods compared to that in the
295 control group ($p < 0.05$).
296
297 [*Insert Fig. 4*]
298
299 Table 3 shows the results of physical fitness tests during the experimental periods. No
300 significant group-time interaction was found for any fitness test items. Sit-up
301 performance in both groups at 5 weeks was significantly increased compared to that at 0
302 weeks ($p < 0.05$). The vertical jump in the control group at 4 weeks was significantly
303 decreased compared to that at 0 weeks ($p < 0.05$).
304
305 [*Insert Table 3*]
306
307 Table 4 presents nutritional intake during the experimental periods. No significant
308 group-time interaction was found for any nutritional intake. Energy, carbohydrate, and
309 water intake in both groups at 5 weeks were significantly decreased compared to those at

0 weeks ($p < 0.05$). Protein and fat intake in the active group at 5 weeks were significantly decreased compared to those at 0 weeks ($p < 0.05$).

[*Insert Table 4*]

DISCUSSION

Athletes competing in weight classifications often suffer from URS and various abdominal problems due to the stress of RWL. Therefore, strategies are needed to maintain physical condition during competitive events. In this study, we investigated the effects of b240 on immune function, URS, and abdominal condition in judo athletes during RWL. Our results showed that approximately 3.6% body weight loss induced changes in salivary SIgA secretion, although physical fitness levels remained unchanged. Importantly, b240 intake mitigated the significant decrease in salivary SIgA secretion and reduced the percentage of URS-affected days during RWL. Additionally, the percentage of days participants reported having a "good" abdominal condition increased. These findings indicate that regular ingestion of b240 is beneficial for maintaining a favorable physical condition in athletes during RWL through the inhibition of immune dysfunction and the prevention of URS and gastrointestinal distress.

328 Salivary SIgA plays a crucial role as an initial defense mechanism in the human
329 organism, specifically capturing various pathogens (bacteria and viruses) in the oral
330 cavity and either inactivating or aggregating them. It has been revealed that salivary SIgA
331 levels dramatically decrease in athletes under physiological stressors such as long-
332 distance running [23], amenorrhea [24], and RWL [5]. A review examining changes in salivary
333 SIgA secretion over time in patients with URS found a significant decrease in salivary
334 SIgA secretion beginning 2-3 weeks before the onset of symptoms [25]. Therefore,
335 maintaining salivary SIgA levels several weeks prior to competition is crucial for
336 ensuring optimal physical condition during the event. In this study, we observed that
337 RWL reduced salivary SIgA secretion in the control group over the 5-week experimental
338 period, whereas the active group, who regularly took b240, maintained salivary SIgA
339 secretion levels. Moreover, URS incidents in the active group were also lower than in the
340 control group. Therefore, these results suggest that regular ingestion of b240 may inhibit
341 immunodeficiency and reduce URS over several weeks. Ingested b240 is thought to
342 activate dendritic cells in the intestinal tract to produce cytokines and induce IgA
343 production in B cells, which then migrate to the salivary glands and contribute to salivary
344 SIgA secretion, potentially reducing the risk of infection [17,19].

345 The abdominal discomfort in athletes is attributed to an inflammatory cascade, which
346 increases intestinal membrane permeability. This increase allows for the invasion of
347 bacteria and toxins due to intense exercise [6,13]. Conversely, the intake of b240 resulted in
348 a higher percentage of "good" responses regarding abdominal condition compared to
349 cases without intake. We propose that b240 contributes to this favorable abdominal
350 condition through the secretion of antimicrobial peptides, such as SIgA, in the intestinal
351 tract. Certain heat-killed bacteria are known to regulate intestinal function via the
352 secretion of antimicrobial peptides [15,16]. Moreover, b240 might also improve abdominal
353 conditions because these heat-killed bacteria stimulate receptors in the intestinal cells,
354 promoting SIgA secretion [17]. The consumption of *Lactiplantibacillus* has been reported
355 to positively affect the intestinal environment of athletes [26], and the results of our study
356 align with these findings. Notably, the percentage of "good" responses increased not only
357 during the weight loss phase but also during the normal training phase. Therefore, we
358 infer that b240 attenuates the excessive increase in intestinal membrane permeability,
359 contributing to better abdominal conditions in athletes.

360 RWL may exert a greater strain on immune function in a shorter period than exercises
361 that induce a reduction in SIgA, as previously reported. For instance, peak training over
362 4 weeks did not affect salivary SIgA concentration and secretion rate in triathletes [27], and

363 intense training for 21 days also had no effect on cyclists [28]. Moreover, in a study on
364 triathletes, it took 20 days for a decrease in salivary SIgA secretion to occur if they did
365 not maintain sufficient energy intake from their diet [21]. Therefore, RWL may pose a
366 higher risk of inducing immune dysfunction than the training conditions previously
367 reported, at least in terms of salivary SIgA.

368 While we expect that b240 is primarily responsible for the observed improvements in
369 abdominal condition and immune function, we cannot exclude the possibility that other
370 components of the intake might have played a role in the study's outcomes. For instance,
371 it has been suggested that protein intake can inhibit exercise-induced intestinal protein
372 catabolism [29] and bacterial/viral infections [30]. However, the participants in this study
373 consumed a lower amount of protein than that previously identified as effective in
374 suppressing these symptoms [31]. The protein intake of our study participants (active
375 group: 61.2 g/day, control group: 71.6 g/day, Table 4) was lower than the high protein
376 intake (220 g/day) reported to be effective [31]. This discrepancy suggests that abdominal
377 conditions may not improve without sufficient total protein intake, even with protein
378 supplementation. Therefore, it appears that b240, rather than the protein content in the
379 food, notably contributed to the better abdominal condition observed in the active group
380 compared to the control group, despite the active group's total protein intake being

significantly lower during the weight loss period in our study. In addition, while a relationship between vitamin D and immune function has been established [32,33], the vitamin D content in the food (10 μg, approximately 400 IU) in this study was likely insufficient to impact immune function and URS. Effective vitamin D intake for increasing salivary SIgA secretion and inhibiting URS is estimated to be approximately 1000–5000 IU [34,35]. This is consistent with previous research indicating that 10 μg of vitamin D intake in recreational athletes did not affect URS or salivary SIgA secretion [36]. These findings suggest that the improvements in abdominal condition and reduction in URS observed in the present study are attributable to the effects of b240.

This study had some limitations. First, we prioritized the reduction of salivary SIgA secretion by approximately 4% during 1 week, based on a previous study [7], and allowed each subject to select an RWL method that was easily implementable for them. Therefore, this study lacked a standardized method for RWL due to variations in b240 intake, training volume, and dehydration methods during the experimental periods. To achieve the targeted 4% weight loss, participants chose methods that were most feasible for them. Therefore, this study cannot dismiss the potential influence of individual RWL methods on the results. Second, the design of this study did not include a placebo group. Future studies need to demonstrate the effects of ONRICb0240 on immune function, URS, and abdominal condition in athletes through a double-blind, placebo-controlled design. Third, the small sample size limits the degree to which the study results can be generalized. We examined the effects of b240 intake on SIgA secretion in

healthy male athletes during the RWL, which presented the difficulty of finding athletes who played the same sport, had similar years of experience, and conducted RWL. Additional studies must involve an appropriate number of participants. Fourth, in this study, the percentage of days with URS complaints over the 4 weeks was significantly lower in the active group than in the control group; however, no significant changes were observed in salivary SIgA in either group at 4 weeks. This result may be due to the intake of b240 affecting not only salivary SigA but also other immunological factors. It is thought that ingested b240 is recognized by dendritic cells, which then produce IL-6 to induce IgA production by B cells [17, 19]. IL-6 induces the activation of T cells, monocytes, neutrophils, and other cells, in addition to B cells. Therefore, it is possible that b240 activated these immune cells. In addition, when mice were administered b240 for 2 weeks and subsequently infected with pneumococcus, the production of inflammatory cytokines in the lungs was reduced compared to that in mice administered saline [37]. Furthermore, it has been reported that 5 weeks of b240 intake increased the expression of the antiviral protein radical S-adenosyl methionine domain containing 2 in the lungs of mice [38]. These factors, in addition to SIgA, may contribute to the changes in URS. However, there is a lack of evidence in humans, and these factors were not measured in this study; therefore, this remains speculative. Future studies are required to clarify the effects of b240 intake on these immunological factors in response to intensive exercise and RWL.

In conclusion, we found that RWL could rapidly decrease salivary SIgA secretion over approximately one week. However, continuous intake of b240 for 5 weeks was

found to inhibit reductions in salivary SIgA secretion, the appearance of URS, and improve abdominal conditions. These results suggest that b240 plays a crucial role in maintaining physical condition by suppressing the occurrence of URS through immunosuppression inhibition and enhancing abdominal conditions in athletes during RWL.

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Author Contributions

K.S. and S.H conceived the study, the main conceptual ideas, and the proof outline. M.M. and H.H. support designed in the experimental protocol and setup of the experimental environment. K.N., Y.T., Y.H., T.O., and F.K. collected the data. K.W. and K.H. aided in interpreting the results. T.K. supervised the project and advised on the research concept. All authors discussed the results and commented on the manuscript.

Conflict of interest

Satoshi Hattori and Koichiro Hamada are employees of Otsuka Pharmaceutical Co., Ltd.

The other authors have no conflicts of interest to declare.

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583

584 **Figure Legends**

585 **Fig.. 1.** Flow diagram for study participants

586

587 **Fig.. 2.** Experimental design

588 Subjects are divided into the control group and active group. The study consists of a
589 normal training period (4 weeks) and a weight loss period (1 week), totaling 5 weeks of
590 experimental periods. Subjective physical assessments, anthropometric measurements,
591 physical fitness tests, and saliva sampling are conducted at the time points of 0, 4, and 5
592 weeks.

593

594 **Fig. 3.** Change in Salivary SIgA secretion rate during the study periods

595 Values are presented as means ± standard error (SE). *, values that differ significantly
596 from pre-intervention (Pre) within the group ($p < 0.05$).

597

598 **Fig. 4.** Subjective physical assessments of upper-respiratory symptoms (URS) and
599 abdominal condition. The percentage of days with URS (A) and ratings of abdominal
600 conditions (“good”, “usual”, “bad”) (B) before intervention (0 weeks), after 4 weeks of
601 intervention (4 weeks), and after 1 week of weight loss (5 weeks).

602 Table 1. Physiological characteristics of the active and control groups during the
603 study periods

		0 week	4 week	5 week
Age(yr)	Active	20.2 ± 0.4		
	Control	19.5 ± 0.4		
Height(cm)	Active	172.1 ± 1.6		
	Control	173.9 ± 1.7		
Body mass(kg)	Active	79.8 ± 5.8	79.8 ± 5.7	76.5 ± 5.3 *, †
	Control	83.2 ± 4.1	83.7 ± 4.0	79.9 ± 3.9 *, †
Fat(%)	ACT	14.4 ± 1.9	13.9 ± 2.2	14.0 ± 2.1
	CON	16.2 ± 1.8	16.1 ± 1.7	15.4 ± 1.8 *
Fat(kg)	Active	12.3 ± 2.9	12.0 ± 3.1	11.5 ± 2.8 *
	Control	13.9 ± 2.2	13.9 ± 2.2	12.7 ± 2.1 *, †
Fat free mass(kg)	Active	67.5 ± 3.1	67.8 ± 2.9	65.0 ± 2.8 *, †
	Control	69.3 ± 2.4	69.9 ± 2.4	67.2 ± 2.3 *, †
Body water(kg)	Active	49.6 ± 2.3	49.8 ± 2.2	47.6 ± 2.1 *, †
	Control	50.8 ± 1.7	51.2 ± 1.7	49.2 ± 1.7 *, †

604

605 Values are presented as means ± SE. *Values that significantly differ from those
606 at 0 week within the same group ($p < 0.05$). †Values that significantly differ from those
607 at 4 week within the same group ($p < 0.05$).
608

609 Table 2. Saliva flow rate and salivary secretory immunoglobulin A concentration
610 for the active and control groups during the study periods
611

		0 week	4 week	5 week
Saliva flow rate (ml/3min)	Active	4.30 ± 0.65	4.61 ± 0.58	2.79 ± 0.41 *, †
	Control	4.45 ± 0.60	4.03 ± 0.64	3.31 ± 0.68 *
SIgA concentration(μg/ml)	Active	73.3 ± 8.1	74.2 ± 10.3	92.6 ± 20.0
	Control	65.9 ± 6.0	62.0 ± 5.4	58.7 ± 7.9

612
613 Values are presented as means ± SE. *Values that significantly differ from those
614 at 0 week within the same group ($p < 0.05$). †Values that significantly differ from those
615 at 4 week within the same group ($p < 0.05$).
616
617

618 Table 3. Physical fitness test results for the active and control groups during the
619 study periods

		0 week	4 week	5 week
Grip strength (kg)	Active	46.3 ± 2.8	46.8 ± 2.8	46.5 ± 2.8
	Control	45.4 ± 2.1	44.7 ± 2.1	45.1 ± 2.0
Sit-up (times/30sec)	Active	34.5 ± 1.2	35.9 ± 1.1	37.3 ± 1.3 *
	Control	36.8 ± 3.3	37.8 ± 3.2	40.3 ± 2.8 *
Vertical jump (cm)	Active	43.4 ± 2.9	42.4 ± 2.7	43.6 ± 2.7
	Control	42.1 ± 1.8	39.3 ± 2.1 *	41.8 ± 2.0
Standing broad jump (cm)	Active	211 ± 10	209 ± 6	212 ± 7
	Control	210 ± 10	220 ± 7	220 ± 8
Whole body reaction time (msec)	Active	0.32 ± 0.01	0.32 ± 0.02	0.32 ± 0.01
	Control	0.30 ± 0.01	0.30 ± 0.01	0.30 ± 0.01

620
621 Values are presented as means ± SE. *Values that significantly differ from those
622 at 0 week within the same group ($p < 0.05$).
623
624

Table 4. Nutritional intake of the active (ACT) and control (CON) groups before and after 5 weeks of intervention

		0 week	5 week
Energy (kcal/day)	ACT	2,299 ± 135	1,605 ± 123 *
	CON	2,592 ± 75	1,857 ± 123 *
Protein (g/day)	ACT	76.0 ± 3.8	61.2 ± 3.8 *
	CON	87.6 ± 6.2	71.6 ± 9.3
Fat (g/day)	ACT	60.8 ± 2.0	44.7 ± 3.7 *
	CON	70.8 ± 2.4	58.1 ± 5.5
Carbohydrate (g/day)	ACT	345 ± 28	230 ± 24 *
	CON	381 ± 15	250 ± 17 *
Water (g/day)	ACT	883 ± 65	630 ± 78 *
	CON	1,082 ± 56	720 ± 37 *

Values are presented as means ± SE. *Values that significantly differ from those at 0 week within the same group ($p < 0.05$).