

# Changes in salivary flow rate following *Chlorella*-derived multicomponent supplementation

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**Decreases in saliva secretion compromise food mastication and swallowing, reduce mucosal immune function, and increase the risk for oral diseases like dental caries. *Chlorella* is a green alga that contains a variety of nutrients including amino acids, vitamins, and minerals. In our previous study, *Chlorella*-derived multicomponent supplementation did not affect salivary flow rates in healthy young individuals, but *Chlorella*-derived supplementation attenuated a decrease in saliva secretion that was observed during a *kendo* training camp. Hence, we hypothesized that *Chlorella*-derived supplementation increases saliva secretion in individuals with lower rates of saliva flow. Sixty-four subjects took *Chlorella*-derived tablets for four weeks. Before and after supplementation, saliva samples were collected by chewing cotton. In the complete study group, there was no difference in saliva production before and after supplementation ( $1.91 \pm 0.11$  ml/min before vs  $2.01 \pm 0.12$  ml/min after). Analysis of subgroups based on saliva production before supplementation found an increase in saliva secretion in the lower saliva flow group ( $1.18 \pm 0.06$  vs  $1.38 \pm 0.08$  ml/min), but no change in the higher saliva flow group ( $2.63 \pm 0.11$  vs  $2.64 \pm 0.15$  ml/min). These results suggest that *Chlorella*-derived multicomponent supplementation increases saliva production in individuals with lower levels of saliva secretion.**

**Key Words:** *Chlorella*, immunoglobulin A, multicomponent supplementation, saliva secretion

Saliva protects the oral mucosa and the teeth. Decreases in saliva secretion can compromise the mastication and swallowing of food, reduce mucosal immune function, and increase the risk for oral diseases such as dental caries. Lifestyle modifications that prevent reductions in saliva secretion would therefore be of clinical significance. A number of factors can influence salivation, including dehydration,<sup>(1)</sup> age,<sup>(2)</sup> the number of remaining teeth,<sup>(3)</sup> and bite force.<sup>(4)</sup> In addition, nutritional status is associated with saliva secretion. Dormenval *et al.*<sup>(5)</sup> reported that salivary flow rate was correlated with serum albumin concentrations and that the relative frequency of malnutrition was greater in subjects with lower salivary flow rates, as compared to those with higher levels of saliva secretion. Saito and coworkers reported that ingestion of astaxanthin,<sup>(6)</sup> coenzyme Q10,<sup>(7)</sup> isoflavones,<sup>(8)</sup> and quercetin<sup>(9)</sup> corrected impaired salivary secretion. Therefore, dietary modification or the use of a dietary supplement may prevent hyposalivation.

*Chlorella* is a unicellular green alga that grows in fresh water and contains high levels of proteins, chlorophylls, vitamins, minerals, and dietary fibers. In our previous study, *Chlorella*-derived multicomponent supplementation did not affect salivary flow rates in healthy young individuals, although it increased the salivary secretory immunoglobulin A (SIgA) secretion rate.<sup>(10)</sup> However, *Chlorella*-derived supplementation attenuated the

decrease in salivary flow rate observed in university *kendo* athletes during a training camp.<sup>(11)</sup> Hence, we hypothesized that *Chlorella*-derived multicomponent supplementation may increase saliva production in individuals with lower salivary flow rates. To test this hypothesis, we compared the effects of a *Chlorella*-derived supplement on saliva production in subjects with lower and higher baseline salivary flow rates. In addition, we re-examined the effects of *Chlorella*-derived supplementation on the salivary SIgA secretion rate, because the sample size of the previous study ( $n = 15$ )<sup>(10)</sup> was small.

## Materials and Methods

**Subjects and experimental design.** First, pre-supplementation saliva samples were obtained from 43 male and 21 female non-smokers. The mean  $\pm$  SE values for the age, height, weight, and body mass index (BMI) of all subjects were  $32.2 \pm 2.5$  years,  $1.66 \pm 0.01$  m,  $64.0 \pm 1.3$  kg, and  $23.3 \pm 0.4$  kg/m<sup>2</sup>, respectively. Subjects refrained from alcohol consumption from the day before testing, and from caffeine consumption and intense physical activity on the day of testing. They then took 30 *Chlorella*-derived tablets per day (15 tablets twice daily, after breakfast and dinner) for four weeks, as in the previous studies.<sup>(10-14)</sup> This was consistent with the recommended dosage for Japanese consumers. The participants were asked not to modify their lifestyle during the trial period. A post-supplementation saliva sample was obtained one day after the final tablet intake.

This study was approved by the Ethics Committee of Ryutsu Keizai University and the Ethical Committee of the Institute of Health and Sport Sciences of the University of Tsukuba. The protocol of this study conformed to the principles of the Helsinki Declaration. All participants gave their written informed consent prior to study initiation.

***Chlorella* tablets.** The *Chlorella* tablet used in this study (SunChlorella A; Sun Chlorella Corp., Kyoto, Japan) was the same as that used in our previous studies.<sup>(10-14)</sup> The mass of each tablet was 200 mg and dried *Chlorella pyrenoidosa* powder was the main ingredient. The nutritional values of these tablets (per 100 g) were: energy, 399 kcal; moisture, 5.3 g; protein, 60.8 g; lipid, 9.2 g; saccharide, 6.3 g; dietary fiber, 11.9 g; and ash, 6.5 g.

**Saliva samples.** Saliva samples were obtained as described previously.<sup>(15-17)</sup> The subjects rinsed their mouths with distilled water (three times for 30 s) and then rested for 5 min. Saliva production was stimulated by chewing sterilized cotton (Salivette; Sarstedt, Nümbrecht, Germany) at a frequency of 1 chew/s for 60 s. The amount of saliva (g) was converted to milliliters, assuming a saliva density of 1 g/ml. The subjects were divided

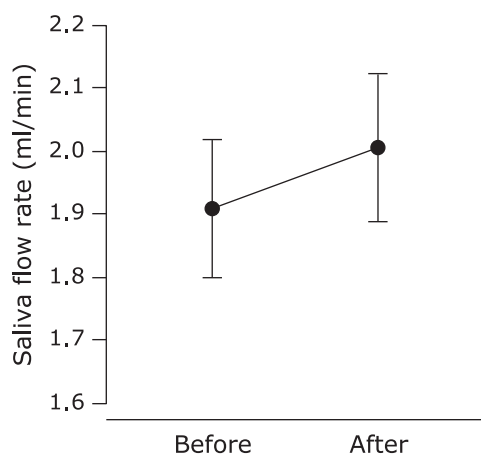
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into lower and higher saliva secretion groups based on the median value before supplementation (1.72 ml/min). After the saliva sample was separated from the cotton by centrifugation at 1,460 g for 15 min, the samples were frozen at  $-60^{\circ}\text{C}$ . We measured salivary SIgA concentrations in duplicate using an enzyme-linked immunosorbent assay, as described previously.<sup>(15-17)</sup> The inter-assay coefficient of variation for this method was reported to be 6.2%.<sup>(16)</sup> The SIgA secretion rate was obtained from the product of the absolute SIgA concentration and the saliva flow rate.

**Statistical analysis.** The results are presented as means  $\pm$  SEs. The paired  $t$  test was used to assess the effects of supplementation. Inter-group comparisons of patient compliance and variables prior to the supplementation period were made by unpaired  $t$  tests. The group male:female ratios were compared by chi-squared test. The analysis of covariance was used to detect group differences in the change in saliva flow rate. If a significant  $F$  value was found, a post-hoc Bonferroni/Dunn test was performed.  $P$  values  $<0.05$  were considered statistically significant.

## Results

In all subjects, compliance with respect to regular tablet intake was  $94.4 \pm 1.1\%$ . There was no difference between the saliva flow rate of all subjects before and after the supplementation period



**Fig. 1.** Saliva flow rate before and after *Chlorella*-derived multi-component supplementation in all subjects. Values are means  $\pm$  SE.

( $p = 0.11$ , Fig. 1). Subjects were assigned to subgroups, depending on their salivary flow rates before supplementation. There were no significant differences in the physical characteristics of these subgroups (Table 1) or their compliance with tablet intake (lower vs higher saliva flow subgroups;  $95.1 \pm 1.3$  vs  $93.6 \pm 1.8\%$ ,  $p = 0.50$ ), although the differences in male:female ratio, age, and height were close to statistical significance. Saliva flow rate increased after supplementation in the lower saliva flow group, whereas there was no change in the higher group (Fig. 2). Changes in saliva flow rate before and after supplementation were greater in the lower saliva flow group than in the higher group, independent of sex (male = 0, female = 1), age, or height (Fig. 3). The salivary SIgA secretion rate increased in all subjects after supplementation, relative to the baseline (Fig. 4).

## Discussion

We investigated the effects of a 4-week *Chlorella*-derived multicomponent supplementation on saliva flow rate. The supplement increased the saliva flow rate in the lower saliva flow group, as compared to their baseline, but not in the higher saliva flow group. These results suggest that this *Chlorella*-derived multicomponent supplement selectively increased saliva production in individuals with lower saliva flow rates.

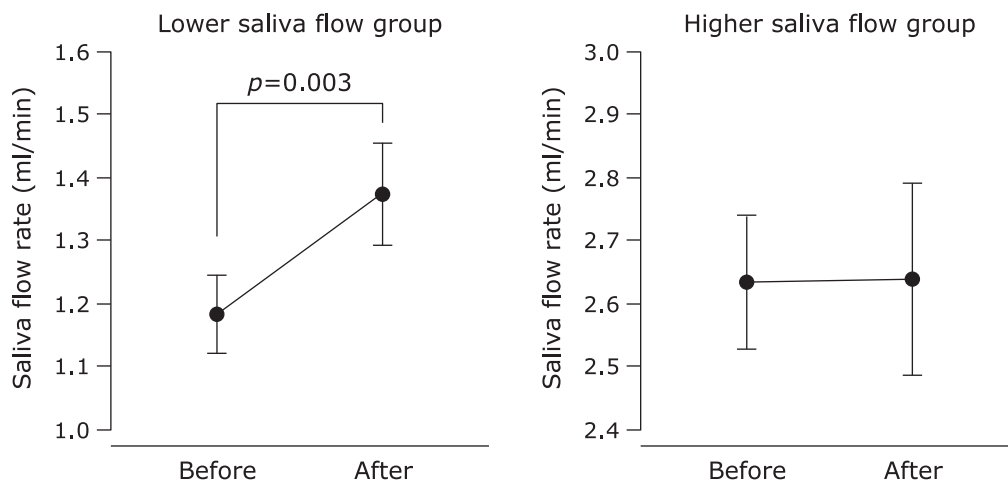
There are two components of saliva secretion: the stimulated and unstimulated saliva flow rates. The stimulated flow rate reflects the ability to produce saliva during eating and speaking, whereas the unstimulated flow rate reflects the normal level of saliva in the mouth. The stimulated saliva flow rate is correlated with the unstimulated saliva flow rate.<sup>(18,19)</sup> However, it is possible that *Chlorella*-derived multicomponent supplementation has different effects on the stimulated and unstimulated saliva levels. For example, age is related to the unstimulated saliva flow rate,<sup>(3)</sup> but there are conflicting reports regarding the relationship between age and the stimulated saliva flow rate.<sup>(2,3,20)</sup> This study could not investigate unstimulated saliva secretion and the present findings therefore suggest that *Chlorella*-derived multicomponent supplementation may provide effective improvement of stimulated saliva secretion during chewing.

*Chlorella*-derived multicomponent supplementation increased the saliva flow rate of the lower saliva flow group. Unfortunately, this study could not identify the individual nutrients responsible for this increase, or the mechanisms underlying this effect. The effect of tablet caloric intake can be excluded, because subject BMI was within the normal range and did not change during the supplementation period. The National Health and Nutrition

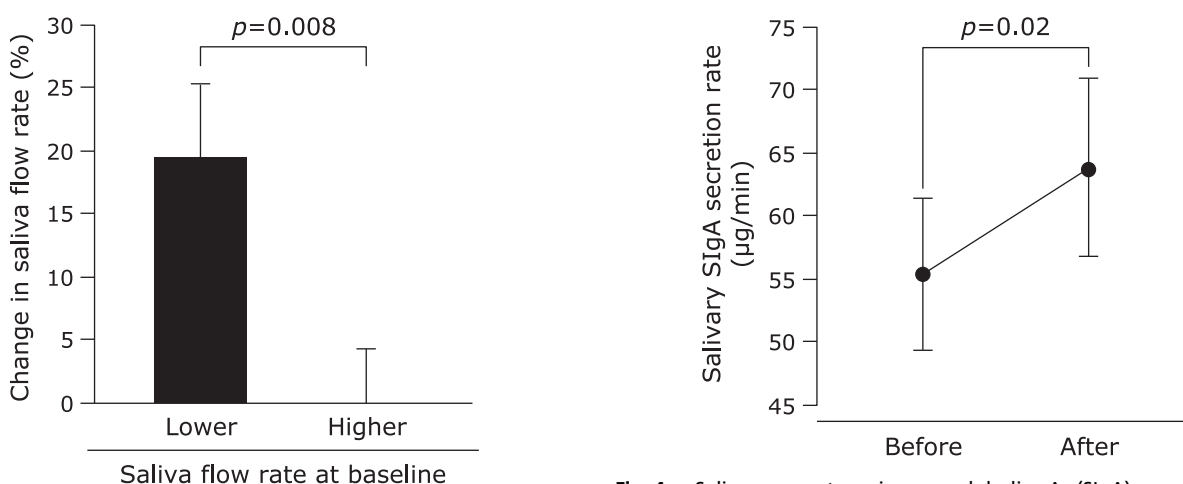
**Table 1.** Characteristics of subjects

	Saliva flow	Before	After	Before vs After
Sex (male/female)	Lower	18/14	—	—
	Higher	25/7	—	—
	Lower vs Higher	$p = 0.06$	—	—
Age (years)	Lower	$36.7 \pm 3.7$	—	—
	Higher	$27.3 \pm 3.1$	—	—
	Lower vs Higher	$p = 0.06$	—	—
Height (m)	Lower	$1.64 \pm 0.02$	—	—
	Higher	$1.68 \pm 0.01$	—	—
	Lower vs Higher	$p = 0.06$	—	—
Body weight (kg)	Lower	$62.3 \pm 1.4$	$62.4 \pm 1.4$	$p = 0.62$
	Higher	$65.7 \pm 2.2$	$65.8 \pm 2.1$	$p = 0.69$
	Lower vs Higher	$p = 0.18$	$p = 0.18$	—
Body mass index ( $\text{kg}/\text{m}^2$ )	Lower	$23.3 \pm 0.6$	$23.4 \pm 0.5$	$p = 0.52$
	Higher	$23.2 \pm 0.4$	$23.3 \pm 0.6$	$p = 0.67$
	Lower vs Higher	$p = 0.94$	$p = 0.91$	—

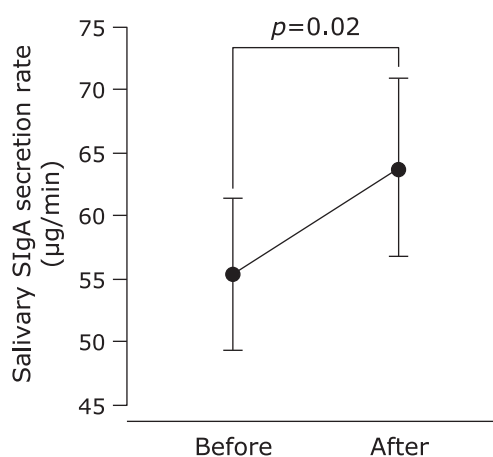
Values are means  $\pm$  SE.



**Fig. 2.** Saliva flow rate before and after *Chlorella*-derived multicomponent supplementation in the lower and higher saliva flow groups. Values are means  $\pm$  SE.



**Fig. 3.** Change in saliva flow rate before and after *Chlorella*-derived multicomponent supplementation in the lower and higher saliva flow groups. Values are means  $\pm$  SE.



**Fig. 4.** Salivary secretory immunoglobulin A (SIgA) secretion rate before and after *Chlorella*-derived multicomponent supplementation in all subjects. Values are means  $\pm$  SE.

Examination Survey by the Ministry of Health, Labour, and Welfare of Japan has pointed out that the intake of vegetables by Japanese people is below the target level. An epidemiological study by Iwasaki *et al.*<sup>(21)</sup> reported that consumption of vegetables, fish, and shellfish, and intake of *n-3* polyunsaturated fatty acid, potassium, vitamin B6, vitamin D, vitamin E, and folate were lower in individuals with hyposalivation, relative to those without hyposalivation. A previous multicomponent analysis demonstrated that the tablet used in the present study contained *n-3* polyunsaturated fatty acid and many vitamins and minerals, including those mentioned above, although no vitamin D data was available.<sup>(11,12)</sup> These nutrients may be implicated in the observed increase in the saliva flow rate of individuals with lower baseline saliva production. On the other hand, the saliva flow rate in the higher saliva group did not change during the supplementation period. In this group, the baseline saliva secretion might have been close to the individual's upper production limit. Another possibility is that sufficient nutrients had already been ingested prior to supplementation in this group.

Salivary SIgA secretion rate was previously shown to increase after *Chlorella*-derived multicomponent supplementation.<sup>(10)</sup> How-

ever, the sample size of this previous study was only 15. The present study investigated 64 participants and verified these previous results. In addition, this study demonstrated that saliva production increased after supplementation in the lower saliva flow group. Saliva provides a physical barrier for the mucosa and washes away viruses and bacteria. These findings indicated that *Chlorella*-derived supplementation may have dual benefits for mucosal immune function in individuals with low saliva secretion.

This study has several limitations. First, no placebo group was included and the results might therefore include a placebo effect. Second, we did not investigate dietary habits or collect blood samples. Further studies are needed to elucidate the mechanisms underlying the observed effect of this *Chlorella*-derived multicomponent supplement on saliva production. Additionally, a combination of dietary and exercise habits can affect SIgA secretion.<sup>(22)</sup> This study could not adjust for not only dietary but also exercise habits of subjects, even though the participants were asked not to modify their lifestyle during the trial period.

In conclusion, *Chlorella*-derived multicomponent supplementation increased the saliva flow rate of individuals with lower baseline saliva secretion.

## Abbreviations

BMI	body mass index
SIgA	secretory immunoglobulin A

## Conflict of Interest

Sun Chlorella Corp. provided funding for the study and supplied the test supplements used in this study. TO has received speaker's honorarium from Sun Chlorella Corp. KS, AZ, and SM have no competing interests.

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