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Original Article

The Unique Correlation between Anti-Mutagenicity of Human Saliva and Change in Body Weight

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Abstract

The purpose of this study was to investigate the effect of weight reduction on the anti-mutagenicity of human saliva. Subjects were 16 male college judo players. The anti-mutagenicity of the saliva was measured using the umu test. There was an inhibiting effect of the saliva on the mutagenicity of AF-2. However, a modifying effect of the saliva on Trp-P-1 was not observed. On the day before a competition and 7 days after the competition, the inhibiting capacity of the saliva for the mutagenicity of AF-2 decreased and increased in two non-weight reduction and two weight reduction groups, respectively.

However, on the day before the competition, the changed body weights ($r=-0.77$, $p<0.01$) and BMI ($r=-0.77$, $p<0.01$) were significantly correlated with that of the inhibiting capacity of the saliva for the mutagenicity of AF-2. In addition, the BMI at 20 days before the competition was not significantly but markedly correlated with it ($r=0.50$, $p=0.057$). At 7 days after the competition, however, these correlations were not found.

These findings suggest a unique correlation between the anti-mutagenicity of human saliva and body weight or BMI.

Key words: anti-mutagenicity, human saliva, umu test, fasting, judo players

Introduction

There are a large number of mutagens in the environment or in food, and some of them are markedly related to carcinogenesis; i.e. humans ingest daily various mutagens or carcinogens[1]. However, previous studies have also found many anti-mutagens[2,3] and the modifying effect of components of the human body on mutagens[3–8]. Above all, it may be important to investigate the effect of human saliva, because it reacts first with mutagens in food. The anti-mutagenicity of human saliva has been found in some studies[3–6].

The purpose of the present study was to clarify the effect of weight reduction on the anti-mutagenicity of human saliva for judo players. In general, many judo players and boxers select a lower weight class to compete with an advantage. Although the effect of the change of the body weight has been studied widely in various situations[9–13], there are no known studies that have investigated the relationship with the anti-mutagenicity of human saliva. However, such a study appears to be important to advance the epidemiologic research of the cancer.

At present, the Ames test[14] and umu test[15–19] are widely used as the method of the mutagenicity assay, and this study was carried out using the umu test. This is the method that utilizes the SOS responses observed when the DNA of Escherichia coli is damaged unlike the usual one detecting the expression of the mutation directly. It is a practical and usable method because of many advantages, when compared with the Ames test; i) the sensitivities are similar, ii) only one bacterial tester strain is required for various types of mutation, iii) the assay is carried out in a shorter time and iv) it is not influenced by histidine. Thus, it is suggested to be more suitable for large groups or samples that could include histidine such as components of the human body.

We found, and report here, an interesting correlation between the anti-mutagenicity of human saliva and a change in body weight.

Materials and Methods

Subjects and protocol

Subjects were 16 male judo players who belonged to the Nippon Sport Science University Judo Club. Their mean age was

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19.5 years (range 18–21 years). All individuals were non-smokers. Seven of them reduced their weight prior to a competition. The competition was for choosing some representative players of the club, and, therefore, it was very important for them. One subject was excluded from the study, because the quantity of his saliva sample was insufficient. In this study, therefore, the assay was carried out for 15 individuals.

All measurements were carried out on three different days: 20 days before the competition (before weight reduction), the day before the competition (the peak of weight reduction) and 7 days after the competition. On the each collection day, the physical characteristics (height, body weight, fat percentage), lifestyle (Health Practice Index[20,21]) and the mental health status (GHQ-28[22], Zung-SDS[23,24]) were measured, and the saliva was collected.

The saliva was collected using Salivette® (Sarstedt Co. Ltd., Nümbrecht) before breakfast (between 07:20 and 07:50). This is an instrument to obtain saliva by centrifuging (at 3,000 rpm for 15 min) from cotton that subjects have bitten (for 2 min). The saliva was stored at −50°C until all samples were collected.

Anti-mutagenicity assay

The anti-mutagenicity of the saliva was measured using the umu test. Furylfuramide (AF-2, Wako Pure Chemical Ind., Osaka) and 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1, Wako Pure Chemical Ind., Osaka) of 0.1 ml were used as a mutagen. Their mutagenicity has previously been confirmed by the umu test[2,6,7].

O-nitrophenyl-β-D-galactopyranoside (ONPG) was obtained from Wako Pure Chemical Ind., Osaka. The S9 mix derived from the livers of rats pretreated with the polychlorobiphenyl (PCB) for enzyme induction was obtained from Kyowa Hakko Ind., Osaka. Other chemicals were of the purest grade available.

Bacteria were grown in either Luria's broth or TGA medium (1% bacto tryptone, 0.5% NaCl and 0.2% glucose) supplemented with ampicillin (20 mg/ml). Z-buffer was prepared as described by Miller[25].

The umu-test was essentially carried out as described previously[15–17] using a tester strain, *Salmonella typhimurium* TA1535/pSK1002. The saliva was added as described by Okada et al[6]. In the pretest, there was no effect of the saliva on the proliferation of the tester strain. The SOS responses were measured as the β-galactosidase activity by the method of Miller[25].

The SOS responses-inhibiting capacity of the saliva (%) was obtained using the following equation[8]:

$$\text{The SOS responses-inhibiting capacity} = [1-(A-C)/(B-D)]\times 100$$

Here, A is the β-galactosidase activity induced by the mutagen mixed with the saliva, B is that by the mutagen, C is that by the saliva, and D is that by no addition (baseline).

Statistical analysis

Measurements on the day before the competition (the peak weight reduction) and 7 days after the competition were compared with that of 20 days before the competition (before weight reduction). Student's paired *t* test was used for data analysis. In addition, Pearson's correlation coefficients were used to examine the relationship between the variables. Values were considered to be significantly different if $p<0.05$. All data were expressed as the mean±SD.

Results

Anti-mutagenicity of saliva (Table 1)

In this study, no modifying effect of the saliva on Trp-P-1 was observed. However, the mutagenicity of AF-2 was modified by the addition of the saliva. Therefore, the anti-mutagenicity of the saliva with respect to AF-2 is discussed here.

Grouping of subjects

The inhibiting capacity of the saliva for the mutagenicity of AF-2 ranged from −5.71 to 66.58% at 20 days before the competi-

Table 1 Modifying effect of human saliva on SOS responses induced by mutagens

	Chemicals (μg/ml)	20 days before the competition	The day before the competition	7 days after the competition
		β-galactosidase activity (units)		
S9 (−)	AF-2 (0.024)			
	−saliva	481.0±40.1	481.0±40.1	481.0±40.1
	+saliva	395.6±114.9	418.5±46.5	439.3±66.6
	Control (DMSO)			
	−saliva	138.7±28.8	138.7±28.8	138.7±28.8
	+saliva	117.1±17.3	115.3±11.2	121.8±19.7
		Inhibiting capacity (%)		
		18.6	11.4	7.2
		β-galactosidase activity (units)		
S9 (+)	Trp-P-1 (0.4)			
	−saliva	251.2±9.1	251.2±9.1	251.2±9.1
	+saliva	238.1±18.3	241.6±8.4	238.6±13.8
	Control (DMSO)			
	−saliva	122.3±7.0	122.3±7.0	122.3±7.0
	+saliva	108.0±4.7	113.5±9.2	110.7±7.2
		Inhibiting capacity (%)		
		−0.9	0.6	0.8

Values are expressed as the mean±SD.

Table 2 Characteristics of subjects on 20 days before the competition (before weight reduction)

	Low BMI group		High BMI group	
	non-weight reduction (n=5)	weight reduction (n=4)	non-weight reduction (n=3)	weight reduction (n=3)
BMI (kg/m^2)	25.5±2.7	25.7±1.9	31.7±1.0	32.3±1.3
Height (cm)	171.4±7.7	168.1±6.7	175.0±1.0	171.3±3.5
Body weight (kg)	75.1±10.2	72.6±8.6	97.0±3.9	94.9±6.7
Fat percentage (%)	11.0±2.9	10.0±4.9	16.4±1.4	21.4±2.9
Inhibiting capacity of saliva for mutagenicity of AF-2(%)	40.4±19.2	10.0±9.6	15.5±10.6	3.7±8.5
Weight reduction rate (%)	0.2±1.2	6.1±2.7	0.9±1.1	6.8±2.2

Values are expressed as the mean±SD.
Weight reduction rate=[(a–b)/a]×100: a is the body weight at 20 days before the competition, and b is that on the day before the competition.

Table 3 Changes in the inhibiting capacity of saliva for mutagenicity of AF-2 and physical characteristics during weight reduction

		20 days before the competition	The day before the competition	7 days after the competition
Low BMI group				
non-weight reduction(n=5)	Inhibiting capacity (%)	40.4±19.2	–1.3±19.0*	–5.2±13.3*
	Body weight (kg)	75.1±10.2	75.0±10.1	75.3±9.8
	BMI (kg/m^2)	25.5±2.7	25.5±2.8	25.6±2.7
	Fat percentage (%)	11.0±2.9	10.8±3.1	9.8±3.6
weight reduction (n=4)	Inhibiting capacity (%)	10.0±9.6	13.7±12.5	15.4±36.8
	Body weight (kg)	72.6±8.6	68.2±9.1*	72.7±8.8
	BMI (kg/m^2)	25.7±1.9	24.1±1.6*	25.7±2.0
	Fat percentage (%)	10.0±4.9	9.1±4.6	9.5±4.8
High BMI group				
non-weight reduction (n=3)	Inhibiting capacity (%)	15.5±10.6	4.8±18.4	–6.2±26.0
	Body weight (kg)	97.0±3.9	96.1±5.0	97.8±4.6
	BMI (kg/m^2)	31.7±1.0	31.4±1.4	31.9±1.3
	Fat percentage (%)	16.4±1.4	17.6±1.3**	16.1±1.2
weight reduction (n=3)	Inhibiting capacity (%)	3.7±8.5	23.2±1.5*	15.1±3.0
	Body weight (kg)	94.9±6.7	88.4±4.8*	92.5±5.6
	BMI (kg/m^2)	32.3±1.3	30.1±1.1*	31.5±1.1
	Fat percentage (%)	21.4±2.9	20.2±3.9	22.6±4.5

Values are expressed as the mean±SD.
Significantly different from 20 days before the competition, * $p<0.05$, ** $p<0.01$ (Student's paired *t* test).

tion. This individual variation was significantly correlated with the BMI ($r=-0.61$, $p<0.05$). Therefore, subjects were divided into a high BMI group (BMI≥30) and a low BMI group (BMI<30). The classification system suggested by Garrow[26] was applied to this grouping. In addition, these groups were divided into a weight reduction group and a non-weight reduction group. The findings of each group are shown in Table 2.

According to Garrow's classification system, two high BMI groups were within the obesity range and two low BMI groups were within the overweight range.

The weight reduction rate (%) was obtained using the following equation[9]:

The weight reduction rate (WRR)=[(a–b)/a]×100

Here, a is the body weight at 20 days before the competition, and b is that on the day before the competition. According to Kurakake et al.[9], two weight reduction groups were within the marked weight reduction range (WRR≥6%) and two non-weight reduction groups were within the slight weight reduction range (WRR<3%).

There were no significant differences in the lifestyle or the mental health status among the four groups.

Effect of weight reduction (Table 3)

The change in the inhibiting capacity of the saliva during the weight reduction is shown in Fig. 1. In the low BMI/non-weight reduction group, the inhibiting capacity of the saliva decreased significantly on the day before the competition ($p<0.05$) and 7 days after the competition ($p<0.05$). However, that of the high BMI/weight reduction group increased significantly on the day before the competition ($p<0.05$). In addition, although there were no significant differences, that of the low BMI/weight reduction and the high BMI/non-weight reduction group showed a tendency to increase and decrease, respectively.

In two weight reduction groups, the body weight decreased significantly on the day before the competition ($p<0.05$). This was accompanied by a significant decrease in the BMI ($p<0.05$). However, in the high BMI/non-weight reduction group, the fat percentage increased significantly on the day before the competition ($p<0.01$).

There were no significant changes in lifestyle or the mental health status during the weight reduction.

Next, the change in the levels of each parameter were obtained by subtracting the values at 20 days before the competition from those on the day before the competition or 7 days after

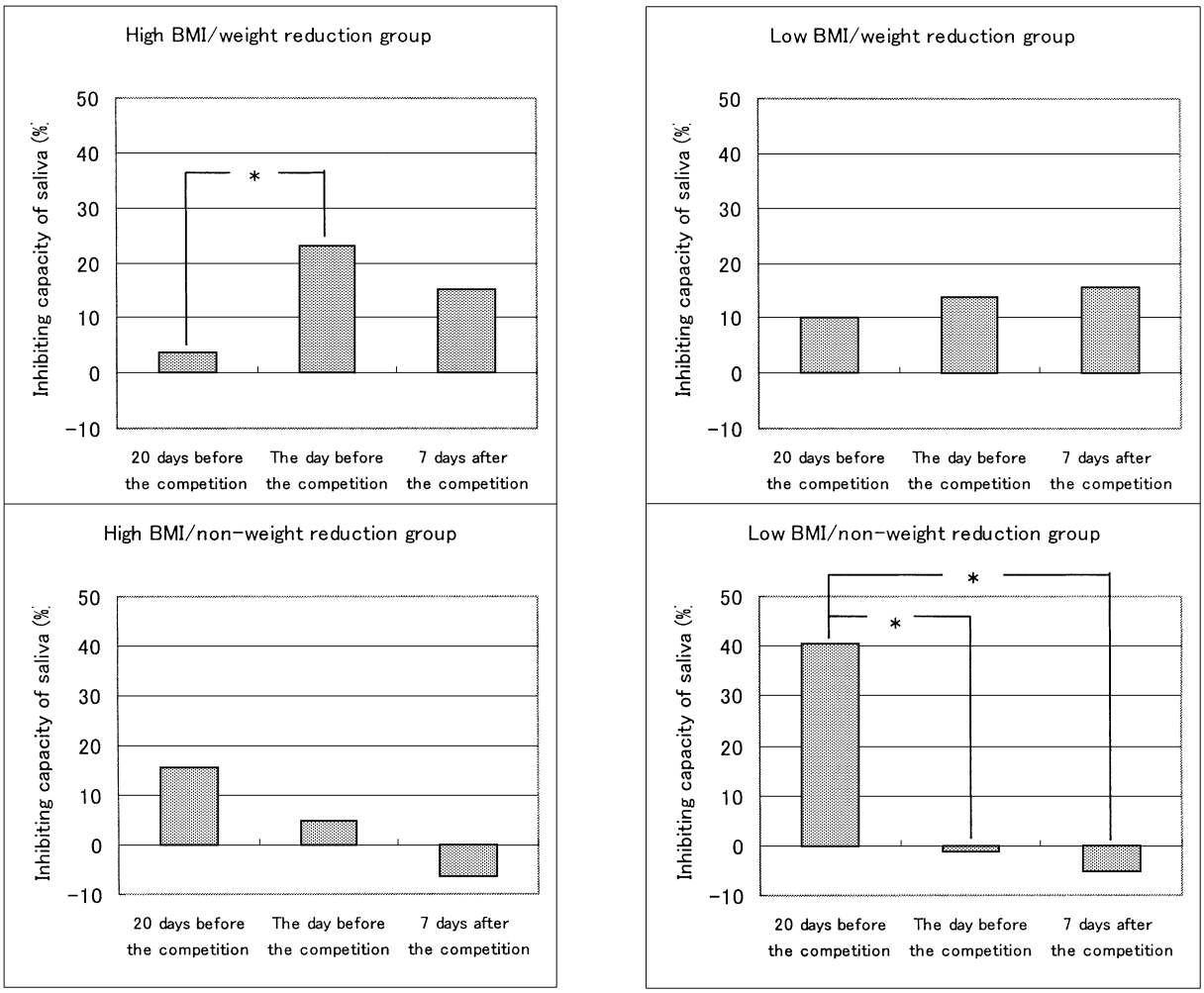

Fig. 1 Changes in the inhibiting capacity of saliva for mutagenicity of AF-2 during weight reduction.
***Significantly different from 20 days before the competition, $p<0.05$ (Student's paired *t* test).**

Table 4 Changes in the levels of each parameter on the day before the competition and at 7 days after the competition

		The day before the competition	7 days after the competition
Low BMI group			
non-weight reduction (n=5)	Change levels of		
	Inhibiting capacity	−41.7±27.0	−45.6±24.3
	Body weight (kg)	−0.1±0.8	0.2±0.6
	BMI	0.0±0.3	0.1±0.2
	Fat percentage	−0.2±0.9	−1.3±1.2
weight reduction (n=4)	Change levels of		
	Inhibiting capacity	3.7±21.1	5.4±36.3
	Body weight (kg)	−4.4±2.0	0.1±0.5
	BMI	−1.6±0.8	0.0±0.2
	Fat percentage	−0.9±1.2	−0.6±0.6
High BMI group			
non-weight reduction (n=3)	Change levels of		
	Inhibiting capacity	−10.8±28.9	−21.8±28.5
	Body weight (kg)	−0.8±1.1	0.8±0.9
	BMI	−0.3±0.4	0.3±0.3
	Fat percentage	1.2±0.2	−0.3±0.6
weight reduction (n=3)	Cange levels of		
	Inhibiting capacity	19.5±7.5	11.3±8.7
	Body weight (kg)	−6.5±2.5	−2.5±1.3
	BMI	−2.2±0.8	−0.8±0.4
	Fat percentage	−1.2±1.1	1.2±1.6

Values are expressed as the mean±SD.
These findings were obtained by subtracting the values at 20 days before the competition from those on the day before the competition or at 7 days after the competition.

the competition (Table 4). On each day, each factor that contributed to the change in the inhibiting capacity of the saliva was examined. As a result, on the day before the competition, the change in body weight ($r=-0.77$, $p<0.01$) and BMI ($r=-0.77$, $p<0.01$) were significantly correlated with that of the inhibiting capacity of the saliva. In addition, the BMI at 20 days before the competition was not significantly but markedly correlated with it ($r=0.50$, $p=0.057$). At 7 days after the competition, however, these correlations were not found.

Thus, the following equation was obtained by the multiple regression analysis:

$$Z=2.469X-7.246Y-100.025 \quad (R^2=0.67,\ p<0.01) \qquad [1]$$

Here, X is the BMI at 20 days before the competition, Y is the change in body weight on the day before the competition, and Z is the change in the inhibiting capacity of the saliva on the day before the competition.

Discussion

Anti-mutagenicity of saliva

As described above, no modifying effect of the saliva on the mutagenicity of Trp-P-1 was observed in this study. This finding is different from those of Nishioka et al.[4,5] who observed the marked inhibiting effect of saliva on the mutagenicity of Trp-P-1. However, these previous studies were carried out using the Ames test. With regard to the modifying effect of the serum on the mutagens, similar difference between findings have also been found[5,7]. Therefore, these findings appear to be due, to some extent, from the differences in the test systems.

However, there was an inhibiting effect of the saliva on the mutagenicity of AF-2. The present findings were lower compared with the observations of Okada et al.[6] who reported an inhibiting capacity of about 66% using the umu test. Although the reason for this difference is not clear, the method of collecting the saliva may be associated with it. In contrast to the method of Okada et al.[6] who collected saliva in test tubes directly, saliva was collected using cotton swabs in the present study. Therefore, it appears that the higher molecular weight substances adhered to it. Although the mechanism of the anti-mutagenicity of the saliva is not yet known, previous studies have suggested that both higher and lower molecular weight substances are associated with it[4,6]. With regard to the method of collection of the saliva, further studies are required.

Lifestyle and mental health status

There appeared to be 2 types of psychological distress, that related to weight reduction and that for the competition, in this study. With regard to the mental health status, however, there were no significant changes during the weight reduction and no significant differences among the four groups. Subjects were first-rate judo players, even though they were students. Therefore, they should have known how to relieve such psychological distress.

In contrast to our expectations, the lifestyle was not associated with the anti-mutagenicity of the saliva. This was probably because the present subjects were first-rate athletes. In general, they were always careful about their health and had good lifestyles. In fact, all subjects were non-smokers. Thus, there was no significant individual variation or change in lifestyle. In the saliva of individuals who used some luxury goods, however, previous studies have found that there are some mutagens[27,28]. Further studies are necessary using more various groups.

Correlation between anti-mutagenicity of saliva and BMI

At 20 days before the competition, the inhibiting capacity of the saliva for the mutagenicity of AF-2 was significantly correlated with the BMI ($r=-0.61$, $p<0.05$). As described above, the mechanism of the anti-mutagenicity of the saliva has not been clarified. However, Nishioka et al.[5] suggested that the inhibiting capacity of the saliva or serum is related to the activity of enzymes such as peroxidase, catalase and superoxide dismutase. In addition, a previous study on serum[29] suggested a relationship between a low level of lipids and a high activity of oxidative enzymes. The present findings may support this hypothesis.

Correlation between the change in the level of anti-mutagenicity of saliva and that of body weight

On the day before the competition, the change in body weight ($r=-0.77$, $p<0.01$) and BMI ($r=-0.77$, $p<0.01$) were significantly correlated with that of the inhibiting capacity of the saliva. In addition, the BMI at 20 days before the competition was not significantly but markedly correlated with it ($r=0.50$, $p=0.057$). At 7 days after the competition, however, these correlations were not found.

From the above results, equation [1] was obtained. This equation leads to the following hypothesis: When the body weight decreases, the inhibiting capacity of the saliva increases and this tendency is greater in those individuals with a higher BMI. In contrast, when the body weight increases, the inhibiting capacity of the saliva decreases and this tendency is greater in those individuals with a lower BMI.

Although this hypothesis explains the present findings well, some points should be considered.

Firstly, despite the similar BMI levels between the two low BMI groups at 20 days before the competition, the inhibiting capacity of the saliva was higher in the low BMI/non-weight reduction group. The reason for this was not clear. However, there was a similar tendency between the two high BMI groups. Therefore, the individuals without the necessity to reduce their weight may have already been in good physical condition. This should be confirmed in a larger group study because the number of the present subjects was very small.

Secondly, in this study, there was no significant change in the body weight in the two non-weight reduction groups. In the low BMI/non-weight reduction group, however, the inhibiting capacity of the saliva decreased significantly on the day before the competition ($p<0.05$) and at 7 days after the competition ($p<0.05$). In addition, in the high BMI/non-weight reduction group, it showed a tendency to decrease. These findings were probably because the present subjects were athletes preparing for a competition. Since the intensity of the training was higher than usual, they needed to intake a considerable amount of energy to maintain their body weights. This must have been more serious in those individuals with lower body weights. In fact, the tendency to take unlimited amounts of various foods was widely observed among them, and it probably led to the confusion of the energy intake. Thus, the unusual situation appears to have contributed to the present findings.

Finally, the BMI of the present subjects was generally high: the BMI at 20 days before the competition was 25.6±2.3 (mean±SD, range 22.0–28.2) in the low BMI group, 32.0±1.1

(range 30.7–33.4) in the high BMI group, and 28.1±3.7 in the whole study group. Therefore, our hypothesis may be applied only to overweight or obese individuals. Further studies should be carried out for lean individuals.

Conclusions

In this study, we found an interesting correlation between the modifying effect of human saliva on the mutagenicity of AF-2 and body weight or BMI. A similar study should be carried out for other various mutagens. In addition, to elucidate other factors that affect the anti-mutagenicity of the saliva, further studies should be carried out using more various and larger groups.

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