Annals of Clinical and Medical Case Reports

Research Paper ISSN 2639-8109 | Volume 13

Examination of Changes in Various Biomarkers by Intake of Enterococcus Faecalis Kawai

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Received: 12 Jun 2024
Accepted: 15 July 2024
Published: 22 July 2024
J Short Name: ACMCR

Citation:

Kawakami S, Examination of Changes in Various Biomarkers by Intake of Enterococcus Faecalis Kawai. Ann Clin Med Case Rep. 2024; V13(22): 1-16

1. Introduction

Starting in 2022, the WHO disease classification system ICD-11 (International Classification of Diseases, 11th Revision) will have a code called “aging-related.” [1] In other words, while aging is irreversible, aging is reversible and is a target for treatment to promote healthy longevity and prevent other diseases, and it is considered important to take measures to prevent it. [2] It is known that many lifestyle-related diseases such as cancer, Alzheimer’s, and cardiovascular events such as myocardial infarction and stroke are caused by aging. [3] In other words, it is considered important to take measures against the causes of aging in order to prevent lifestyle-related diseases.

It is known that aging is not simply an effect of aging, but progresses as tissues and cells are damaged by inflammation. [4] It is believed that the intestinal flora is related to one of the factors that cause inflammation. [5] In other words, aging can also be seen as a problem of the intestinal environment. [6] There are about 1,000 types of intestinal bacteria, about 100 trillion of them, that constantly reside in the intestines, forming the intestinal flora. [7] Disturbance in the balance of the intestinal flora is called dysbiosis, and it causes inflammation and abnormal insulin resistance through cell metabolism regulators such as AMPK and NF-κB [8]. It is thought that these abnormalities lead to a vicious cycle of aging [6]. Enterococcus faecalis Kawai strain lactobacillus was discovered among lactobacillus living in healthy humans by Dr. Yasuo Kawai while he was searching for lactobacillus that are considered to be most effective in preventing the three major lifestyle-related diseases of cancer, heart disease, and cerebrovascular disease, and is a lactobacillus that has been proven to be particularly effective in treating arteriosclerosis [9]. Lactobacillus faecalis Kawai strain lactobacillus has the effect of suppressing inflammatory cytokines that cause arteriosclerosis through the intestinal environment, and it is suggested that it may also be highly effective in preventing aging. In recent years, it has become possible to quantitatively measure the degree of aging that correlates with disease risk through the “epigenetic clock test,” which examines the sequence of all genes and calculates biological age from their methylation levels [10]. The best-known epigenetic clock test is the “Horvath’s Clock,” introduced by Steve Horvath in 2013 [11]. Senescent cells may increase in the body because it becomes difficult to remove them due to the decline in immune system function caused by the aging phenomenon in the body [12]. Therefore, aging can also be seen as a problem of immune function [13]. Various immune cells act to remove senescent cells, but it is possible that their action may be weakened by the decline in immune function associated with aging [14]. Therefore, this time, we planned this study with the aim of elucidating the disease prevention effect of Lactobacillus faecalis Kawai strain in healthy individuals using this epigenetic clock test. We believe that by conducting this study, we can contribute to the construction of a new preventive medical treatment strategy from the perspective of suppressing the progression of aging by improving the intestinal environment.

In addition, the concept of fasting has become popular in Japan in recent years. Fasting means fasting, but it means restricting the

intake of solid foods. There are types of regular fasting that are applied around the world based on traditional, cultural, or religious backgrounds. In ancient medicine, fasting is an established treatment method since Hippocrates. Since then, it has been recommended by most old European medical schools for the treatment of acute and chronic diseases [15]. The food intake frequency of modern people tends to be longer and the fasting period shorter, and high-calorie diets and sedentary lifestyles affect the body's metabolism and increase the incidence of obesity, diabetes, cardiovascular disease, stroke, and dementia year by year [16]. It is also said that fasting at the same time changes the balance of the intestinal flora, so it may be useful for preventing lifestyle-related diseases such as strengthening the immune system [17].

The purpose of this study was to statistically analyze the changes in various biomarkers using Enterococcus faecalis Kawai strain lactobacillus. We also further classified the subjects into two groups: a group that fasted while taking Enterococcus faecalis Kawai strain lactobacillus, and a group that did not fast.

The items measured this time were as follows:

1. Biological age
2. Adiponectin
3. Urinary creatinine
4. HbA1c
5. Blood glucose level
6. Insulin amount
7. CRP

The above seven items were measured and any changes were examined. The flowchart is as follows.

2. Material and Methods

2.1. Type of Studies

Single-blind functional evaluation

2.2. Intervention Methods

Ethics approval was obtained for this study through the International Society for Geriatrics and Gerontology Ethics Committee (ISGN_NI10022023). Before the study began, 12 subjects were divided into two groups, and one group orally ingested the test substance before meals or on an empty stomach, 2 sachets in the morning, 2 sachets in the afternoon, and 2 sachets in the evening (total of 1770 mg of bacterial components) for 12 weeks. The other group also orally ingested the test substance for 12 weeks, while additionally undergoing the lactic acid bacteria festing program described below. The results of each group were evaluated by measuring the following test items and observation items before and after the start of the study, and the results of the two groups were compared to verify the effects of the test substance itself and the program using the test substance. Based on the results obtained, a statistical analysis software, IBM SPSS Statistics version 25, was used to perform a significant difference test at a significance level of 5%. Since the n number for both the fasting group and the non-fasting group was 5 to 6, a non-parametric test was used as the test method. Furthermore, since there was a correspondence in the non-parametric tests, the Wilcoxon signed-rank test was used. At the start of the study, there were 12 subjects, but one error was recognized, so in the end there were a total of 11 subjects, with 6 in the fasting group and 5 in the non-fasting group.

2.3. Evaluation Items

1. Biological age
2. Adiponectin
3. Urinary creatinine
4. HbA1c
5. Blood glucose level
6. Insulin amount
7. CRP

The above seven items were examined in the treatment group, the treatment group with fasting, and the treatment group without fasting. The biological significance of each item will be described in the results and discussion.

3. Results

The n numbers are listed below.

Treatment Group: n=11, Fasting Group: n=6, No Fasting Group: n=5

3.1. Biological Age

3.1.1. Biological Age: Treatment Group (Figure 1, Table 1)

Regarding biological age, no change was observed, with a total $P>0.05$. Since long-term administration may result in changes, it is necessary to continue with a long-term perspective in the future.

3.1.2. Biological Age: Fasting Group (Figure 2, Table 2)

In the fasting group, biological age showed no change ($P>0.05$). It is considered necessary to further examine fasting methods in the future.

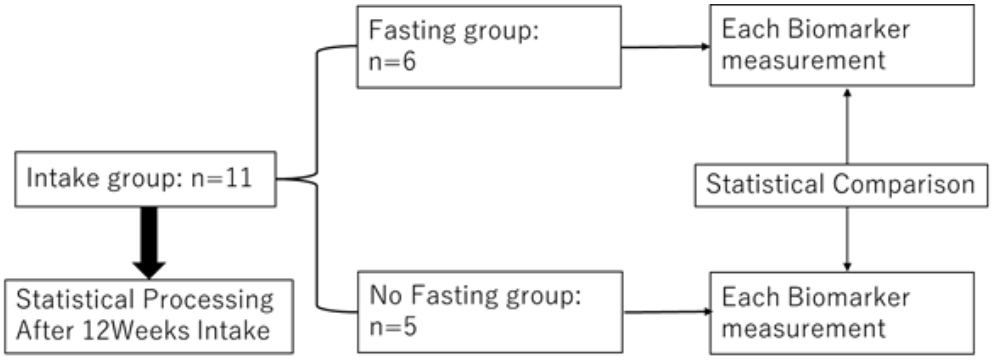
3.1.3. Biological Age: No Fasting Group (Figure 3, Table 3)

When the biological age of the non-fasting group was measured, the result was $P>0.05$, and no change was observed. Although a slight upward trend was observed, this is considered to be an error, and therefore it is considered that there was no increase in biological age.

3.2. Adiponectin

3.2.1. Adiponectin: Treatment Group (Figure 4, Table 4)

Overall, adiponectin was measured, with a significant difference of $P<0.05$. An increase in adiponectin was confirmed by using this test substance. As will be discussed in the discussion, the fact that adiponectin increased significantly is important, and therefore this test substance is considered to be useful.



Flow Chart

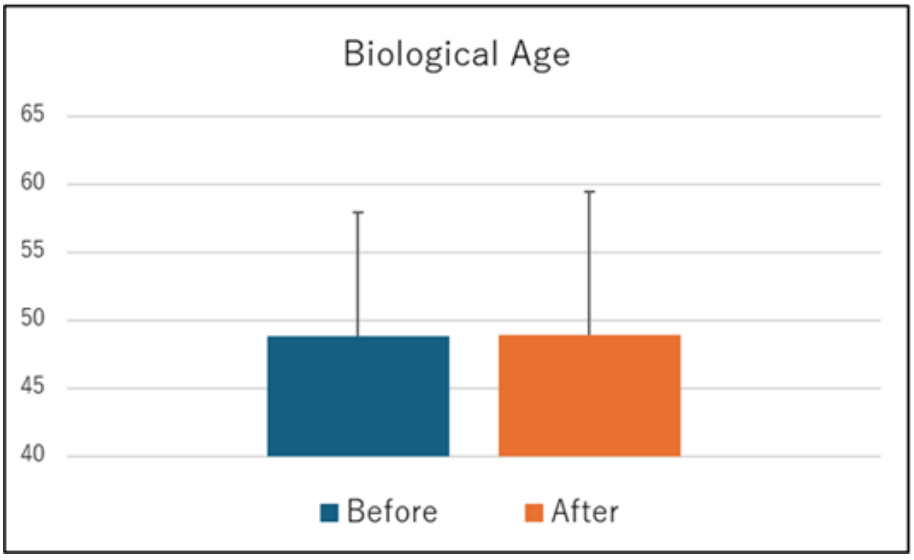


Figure 1: Biological Age Average

Table 1: Biological age Average

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Biological Age	48.8±9.10	48.9 ± 10.57	0.864

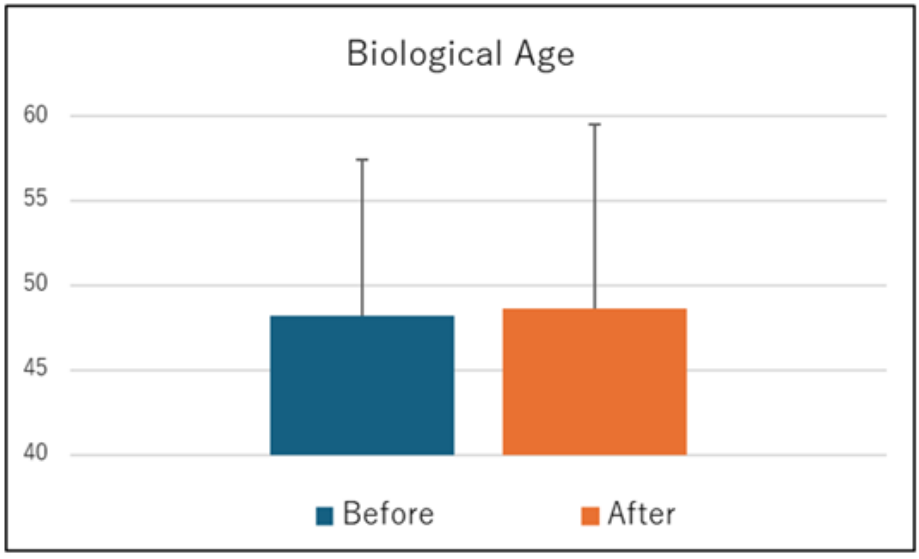


Figure 2: Biological Age Average: Fasting

Table 2: Biological age Average: Fasting

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Biological Age	48.2 ± 9.23	48.6±10.92	0.581

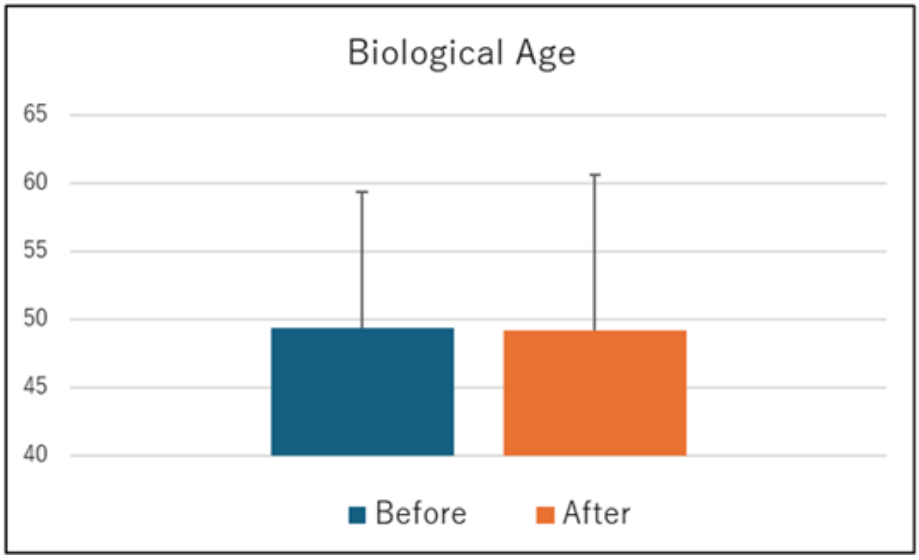


Figure 3: Biological Age Average: No Fasting

Table 3: Biological age Average: No Fasting

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Biological Age	49.4±10.01	49.2±11.48	0.109

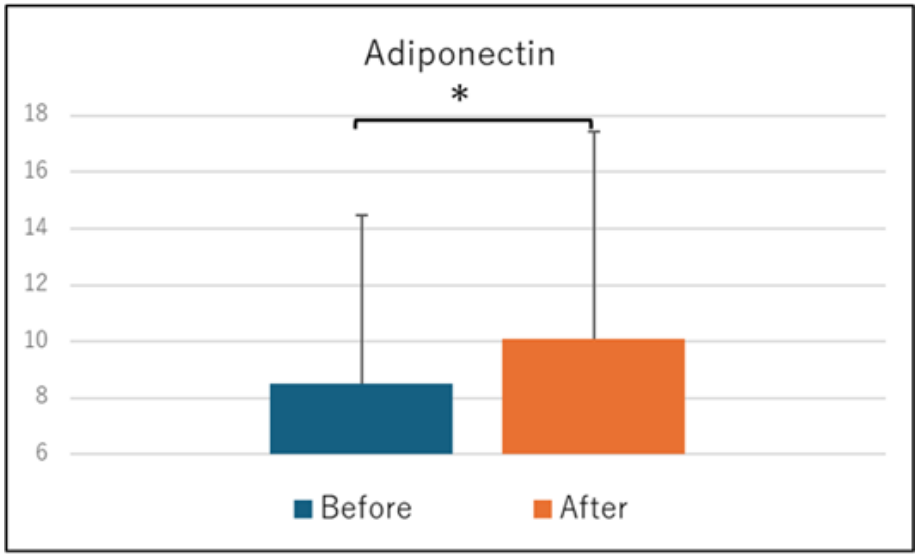


Figure 4: Adiponectin Average

Table 4: Adiponectin Average

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Adiponectin	8.52 ± 5.96	10.09±7.33	0.037

3.2.2. Adiponectin: Fasting Group (Figure 5, Table 5)

When adiponectin levels in the fasting group were measured for this test substance, the P value was P>0.05, and no significant difference was confirmed. However, since the P value was 0.08, it is possible that changes may be observed by increasing the number of cases in the future.

3.2.3. Adiponectin: No Fasting Group (Figure 6, Table 6)

When the test substance was used in the non-fasting group, statistical analysis revealed no significant difference (P>0.05). However, since the P value was 0.05, it is possible that a significant difference will be found by increasing the number of cases in the future.

3.3. Urine Creatinine

3.3.1. Urine creatinine: Treatment Group (Figure 7, Table 7)

When the test substance was used to measure urinary creatinine, the result was P>0.05, indicating no significant difference. It is believed that the substance has no effect on the glomeruli in the kidney.

3.3.2. Urine creatinine: Fasting Group (Figure 8, Table 8)

In the group that fasted using this test substance, urinary creatinine was measured, and no significant difference was observed (P>0.05). As with the Treatment Group, it is considered that there is no effect on the kidneys.

3.3.3. Urine creatinine: No Fasting Group (Figure 9, Table 9)

When urinary creatinine was measured for the group that used this test substance and did not fast, a significant difference was observed (P<0.05). It is possible that not fasting improved renal function.

3.4. HbA1c

3.4.1. HbA1c: Treatment Group (Figure 10, Table 10)

When HbA1c was measured using this test substance, a significant difference was confirmed with P<0.05. Since HbA1c is an indicator of diabetes, it is thought that long-term use of this test substance may lead to the prevention of diabetes.

Table 5: Adiponectin Average: Fasting

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Adiponectin	10.02±7.78	12.92±9.5	0.08

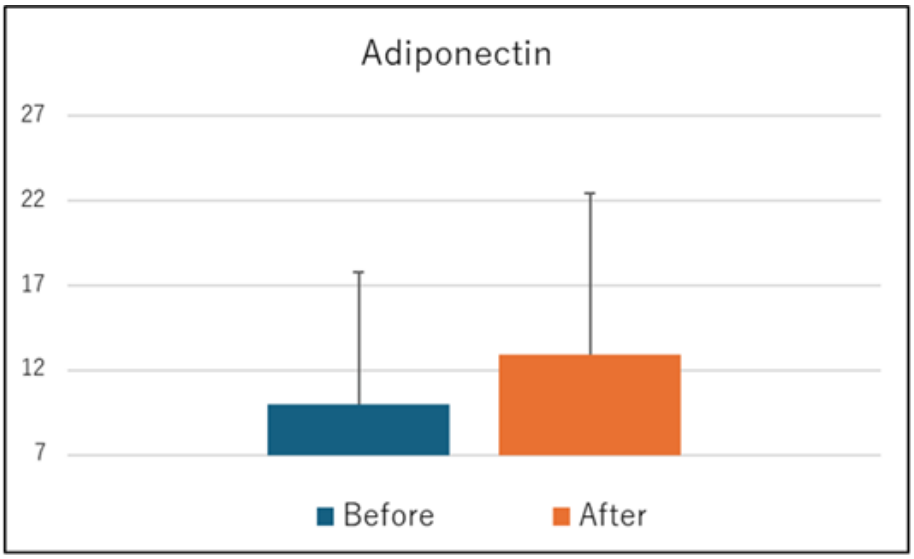


Figure 5: Adiponectin Average: Fasting

Table 6: Adiponectin Average: No Fasting

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Adiponectin	7.02±3.70	7.26±3.23	0.05

Table 7: Urine creatinine Average

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Urine creatinine	124.4±81.31	92.44±60.29	0.212

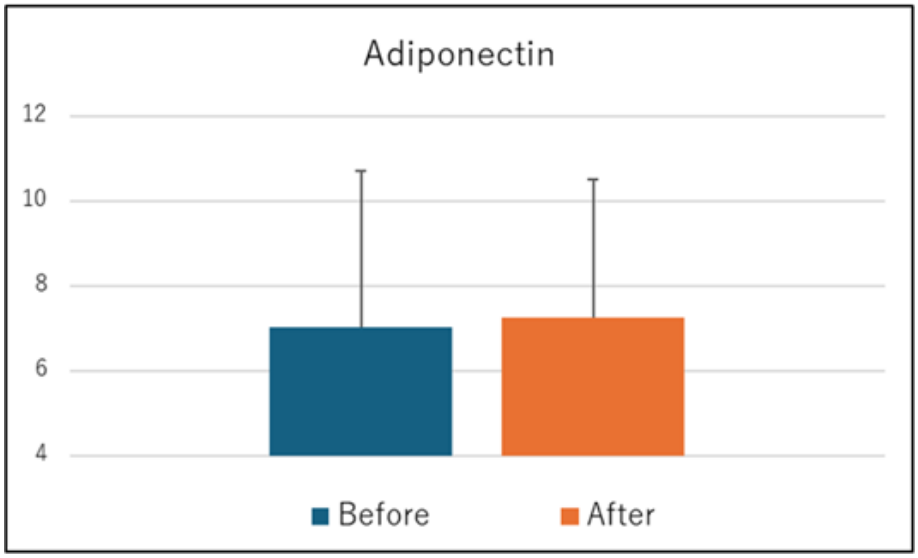


Figure 6: Adiponectin Average: No Fasting

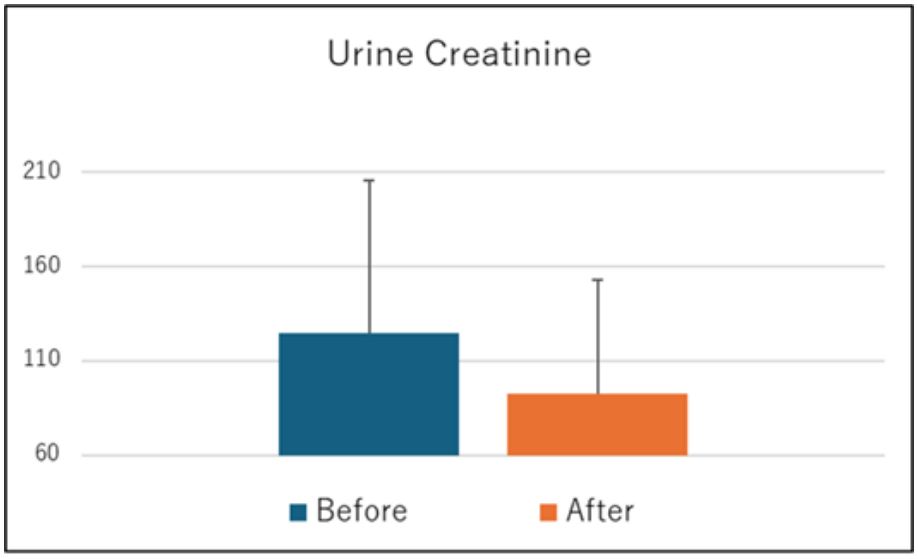


Figure 7: Urine Creatinine Average

Table 8: Urine creatinine Average: Fasting

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Urine creatinine	92 ± 30.23	116.25 ± 84.96	0.715

Table 9: Urine creatinine Average: No Fasting

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Urine creatinine	150.4± 103.16	73.4±28.90	0.042

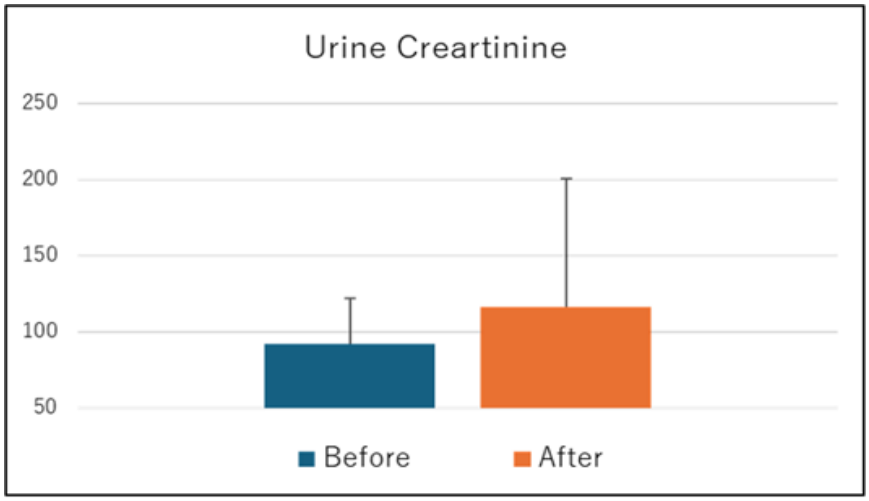


Figure 8: Urine Creatinine: Fasting

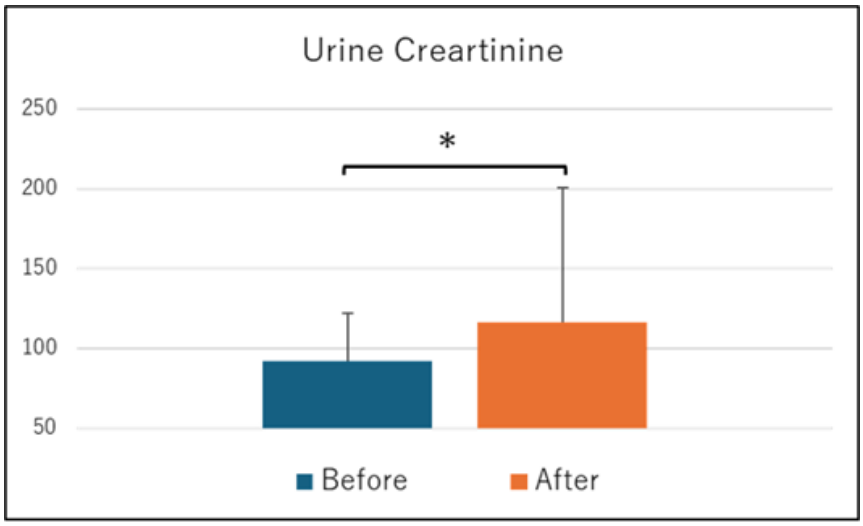


Figure 9: Urine Creatinine Average: No Fasting

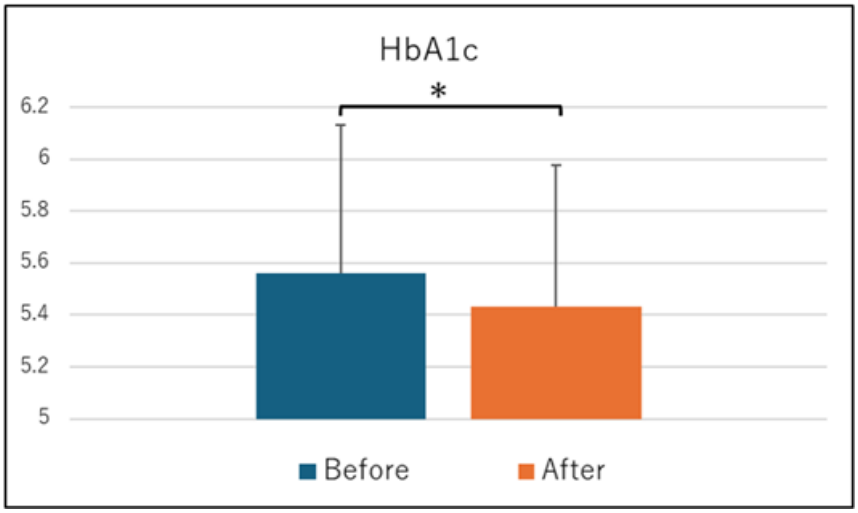


Figure 10: HbA1c Average

Table 10: HbA1c Average

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
HbA1c	5.56±0.57	5.43±0.55	0.019

3.4.2. HbA1c: Fasting Group (Figure 11, Table 11)

When HbA1c was measured for the group fasting using this test substance, the result was P>0.05, and no significant difference was observed. However, as shown in the figure below, a decreasing trend was observed, suggesting that a significant difference may be confirmed by increasing the number of cases.

3.4.3. HbA1c: No Fasting Group (Figure 12, Table 12)

When HbA1c was measured in the non-fasting group using this test substance, the result was P>0.05, and no significant difference was observed. However, as shown in the figure below, a decreasing trend was observed, suggesting that a significant difference may be observed by increasing the number of cases in the future.

3.5. Blood Glucose Level

3.5.1. Blood glucose level: Treatment Group (Figure 13, Table 13)

When blood glucose levels were measured using this test substance, the result was P>0.05, and no significant difference was observed. However, as can be seen from the figure below, a decreasing trend is observed, suggesting that a significant difference may be observed by increasing the number of cases in the future.

3.5.2. Blood glucose level: Fasting Group (Figure 14, Table 14)

When blood glucose levels were measured for the group fasting using this test substance, the result was P>0.05, and no significant difference was observed. However, as can be seen from the figure below, a trend toward a decrease in blood glucose levels was observed, suggesting that a significant difference may be observed by increasing the number of cases in the future.

3.5.3. Blood Glucose Level: No Fasting Group (Figure 15, Table 15)

When the test substance was used in a group that was not fasting, the results were P>0.05, and no significant difference was observed. However, as shown in the figure below, a tendency for blood glucose levels to decrease was observed, suggesting that a significant difference may be observed by increasing the number of cases in the future.

3.6. Insulin Level

3.6.1. Insulin Level: Treatment Group (Figure 16, Table 16)

When the amount of insulin was measured using this test substance, the result was P>0.05, and no significant difference was observed. However, the blood glucose level was on a downward trend, and the amount of insulin was increased as shown in the figure below, suggesting that it may be regulating blood glucose levels.

3.6.2. Insulin Level: Fasting Group (Figure 17, Table 17)

When the insulin levels were measured in the group fasting using this test substance, the result was P>0.05, and no significant difference was observed. However, the amount of insulin secreted showed a tendency to increase, suggesting that a significant difference may be observed by increasing the number of cases in the future.

3.6.3. Insulin Level: No Fasting Group (Figure 18, Table 18)

When the insulin amount was measured for a group not fasting using this test substance, the result was P>0.05, and no significant difference was observed. Since there was a tendency for the amount to decrease compared to the fasting group, it is thought that the measurement method needs to be reviewed.

3.7. CRP Level

3.7.1. CRP Level: Treatment Group (Figure 19, Table 19)

CRP is an index of inflammatory response. When CRP was measured using this test substance, it was significantly decreased with P<0.05, suggesting that this test substance had an anti-inflammatory response.

3.7.2. CRP Level: Fasting Group (Figure 20, Table 20)

In the group fasting using this test substance, the P value was > 0.05, and no significant difference was observed. However, as can be seen in the figure below, a decreasing trend in CRP was observed, suggesting that a significant difference may be observed by increasing the number of cases in the future.

3.7.3. CRP Level: No Fasting Group (Figure 21, Table 21)

When CRP was measured in a group that was not fasting after using this test substance, the result was a significant decrease (P<0.05), suggesting that this test substance may have suppressed inflammatory responses.

Table 11: HbA1c Average: Fasting

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
HbA1c	5.54±0.32	5.36±0.19	0.109

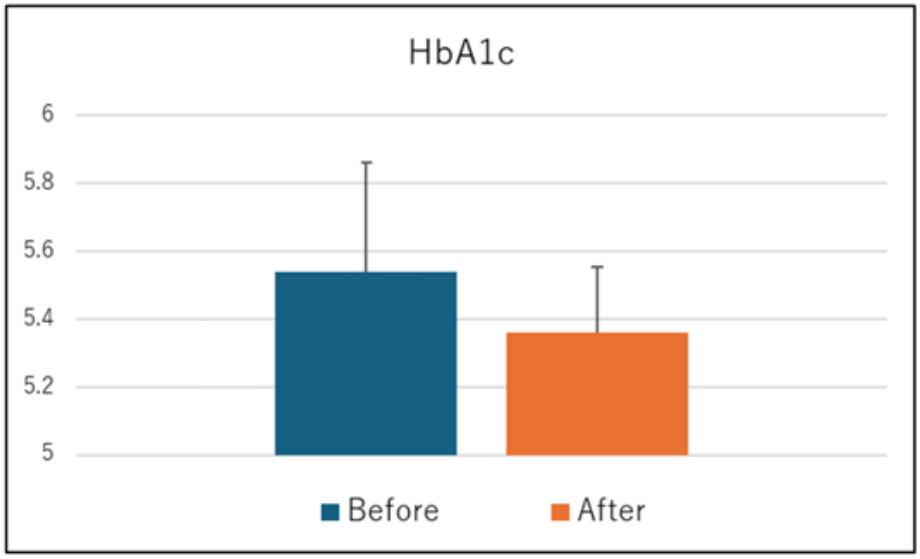


Figure 11: HbA1c Average: Fasting

Table 12: HbA1c Average: No Fasting

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
HbA1c	5.58±0.79	5.50±0.79	0.157

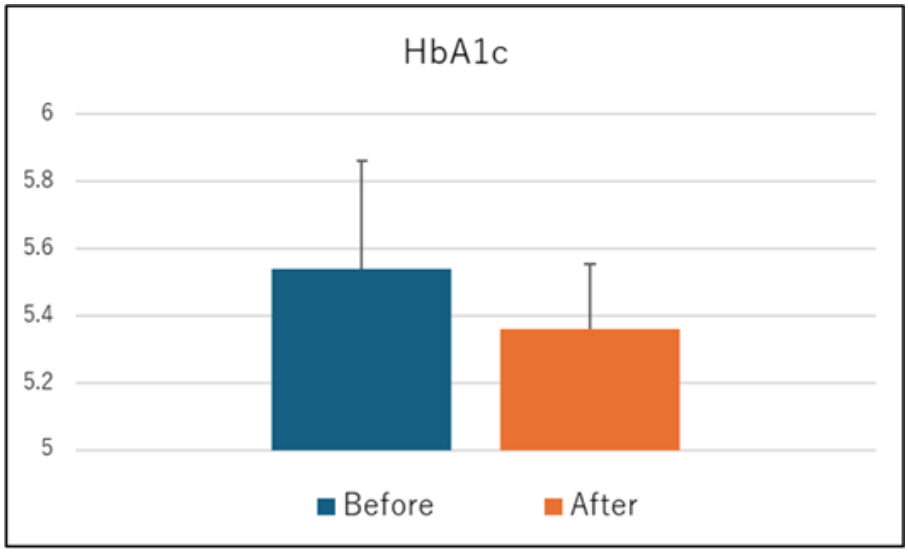


Figure 12: HbA1c Average: No Fasting

Table 13: Blood glucose level Average

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Blood glucose	96±12.75	91.5±16.08	0.155

Figure 13: Blood Glucose Level

Table 14: Blood glucose level Average: Fasting

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Blood glucose	92.8±8.76	86.6 ± 14.81	0.345

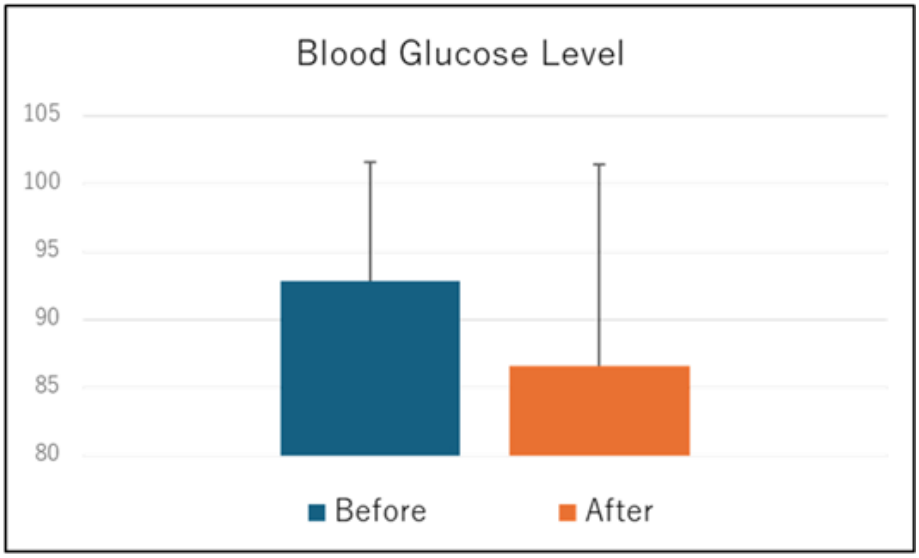


Figure 14: Blood Glucose Level Average: Fasting

Table 15: Blood glucose level Average: No Fasting

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Blood glucose	99.2± 16.24	96.4 ± 17.39	0.5

Table 16: Insulin Average

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Insulin	8.91 ± 6.20	11.32±7.24	0.328

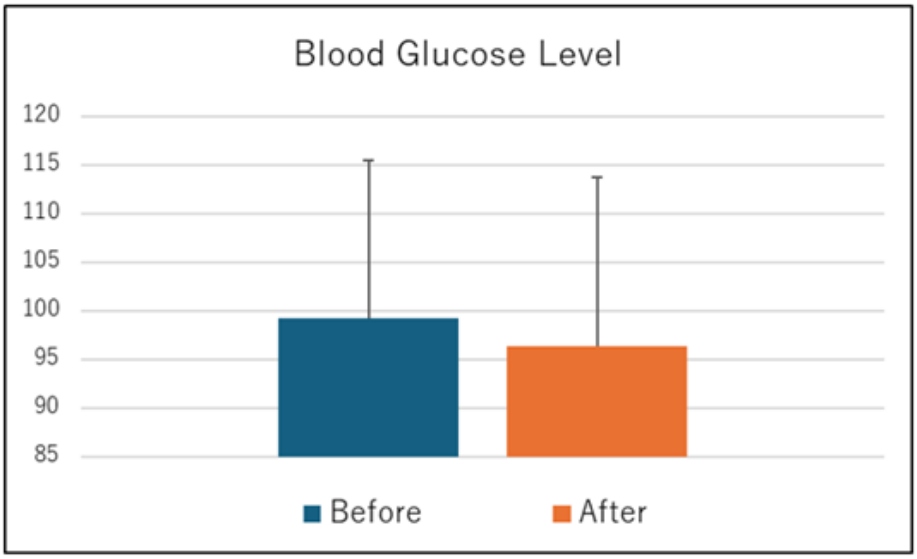


Figure 15: Blood Glucose Level Average: No Fasting

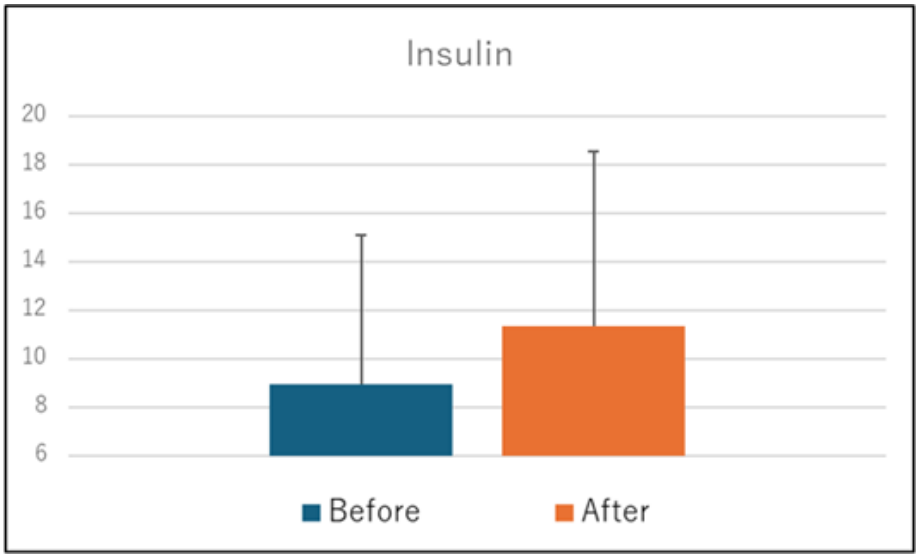


Figure 16: Insulin Average

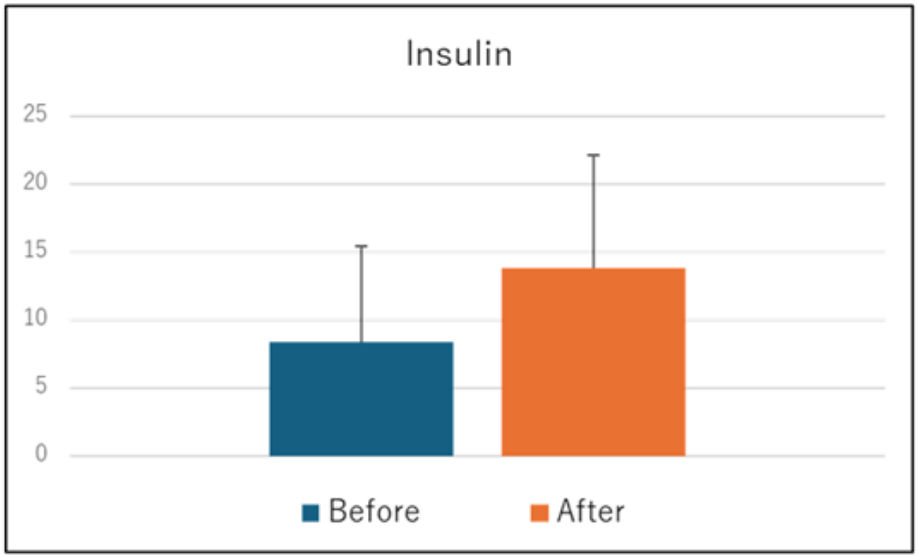


Figure 17: Insulin Average: Fasting

Table 17: Insulin Average: Fasting

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Insulin	8.36±7.04	13.83±8.26	0.225

Table 18: Insulin Average: No Fasting

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
Insulin	9.46±6.01	8.82 ± 5.83	0.686

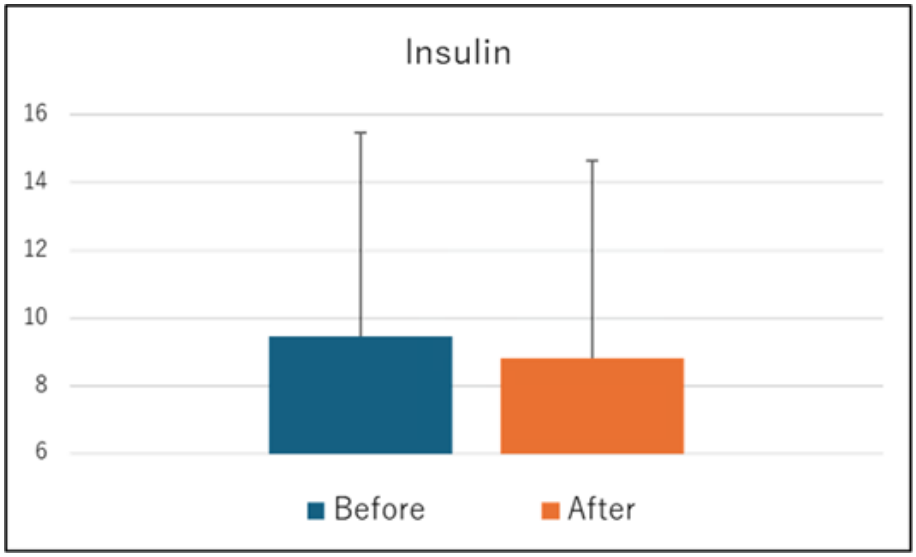


Figure 18: Insulin Average: No Fasting

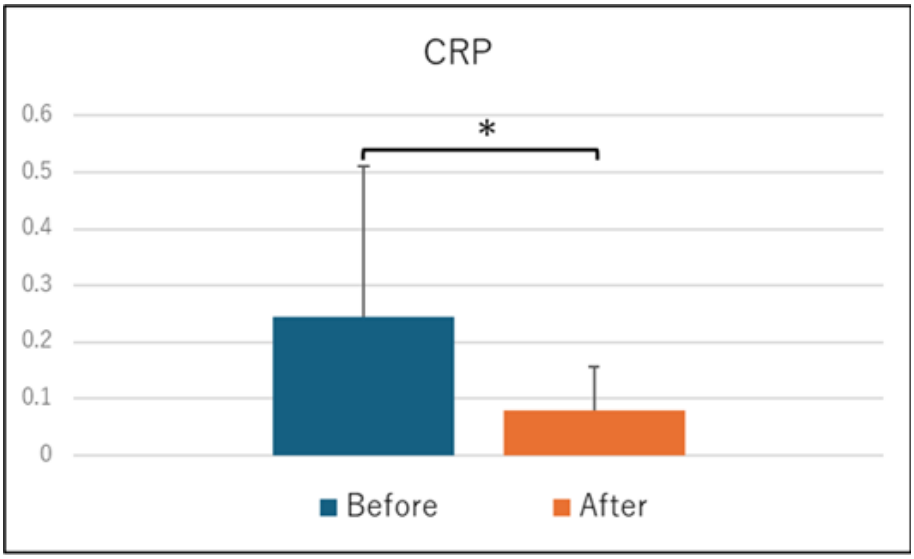


Figure 19: CRP Average

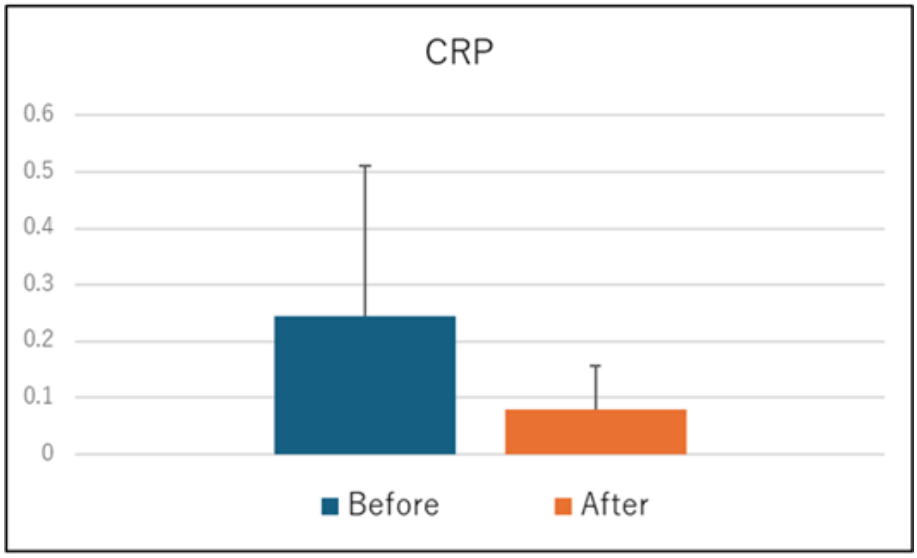


Figure 20: CRP Average

Table 19: CRP Average

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
CRP	0.24 ± 0.27	0.07±0.08	0.033

Table 20: CRP Average

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
CRP	0.19±0.21	0.08±0.09	0.273

Table 21: CRP Average

Test substance	Before	3 months later	p Value
	Mean±SD	Mean±SD	
CRP	0.29 ± 0.33	0.09±0.07	0.043

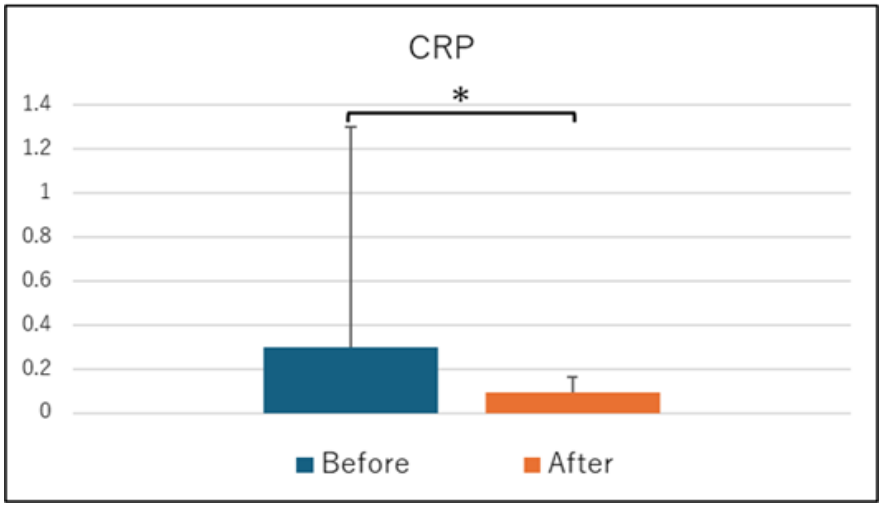


Figure 21: CRP Average

4. Discussion

As a result of this study, no significant changes were observed between fasting after taking Enterococcus faecalis Kawai strain lactobacillus and not fasting. Originally, fasting is a way of creating a state of hunger, but in the medical field, it is said that when you become hungry, motilin, which has the effect of cleaning the stomach and intestines, is secreted and promotes the excretion of waste products [18]. It is said that when the human body is in a state where there is no digestion or absorption, it naturally excretes toxins that have accumulated in the body, and has a detoxifying effect [19] but looking at the results of this study, it is thought that fasting did not have a significant meaning. In other words, it is thought that the results were due to the effect of the test substance. However, since there were some significant differences between the fasting group and the non-fasting group, how to interpret these parts is an important key, but it is thought that no statistically significant differences were observed because the n number was small in this study. Looking at the overall results, a significant increase in adiponectin was observed in the fasting group, which is noteworthy. Although no significant difference was observed, a tendency for adiponectin to increase was observed in both the fasting and non-fasting groups. Adiponectin is a type of protein secreted by fat cells and is also known as a good hormone [20], and increasing blood adiponectin levels is thought to be beneficial in preventing and treating lifestyle-related diseases and cardiovascular diseases [21]. It was suggested that taking this test substance and fasting in addition may be useful in improving lifestyle-related diseases. It is quite possible that this test substance can increase adiponectin levels even without fasting at the same time.

A decrease in CRP was observed in the non-fasting group, suggesting the possibility of an inflammation-suppressing effect. CRP stands for C-reactive protein, and is an indicator of inflammatory responses [22]. Blood CRP levels rise rapidly and show high values due to bacterial or viral infections, so they are used as a marker of acute inflammation [23]. They are also used as a marker to detect chronic microinflammation caused by factors such as obesity and lifestyle [24]. It is said that if CRP levels remain high for a long time, the risk of developing cancer increases, so monitoring the CRP level is extremely important [25]. There was no decrease in CRP in the fasting group, but this was due to the lack of statistical significance, so the trend was toward a decrease. Therefore, regardless of whether or not the subject was fasting, the test substance may have maintained a balance in the intestinal flora, thereby regulating the immune system [26], thereby reducing the inflammatory response. As it was suggested that long-term intake of the test substance may lead to the suppression of chronic inflammation, we would like to continue taking it for a long time in the future.

In addition, when blood glucose levels were examined from various angles, a significant decrease in HbA1c was observed. HbA1c is a type of glycohemoglobin, a general term for glycation products in which glucose is bound to hemoglobin [27]. It is also called glycated hemoglobin [28]. The higher the blood glucose level, the more glycohemoglobin is produced [29]. Therefore, in the case of diabetes, especially when blood sugar control is not possible, the amount of glycohemoglobin increases more than usual [30]. Even in healthy people, red blood cells, which are blood cells containing hemoglobin, have a life span of about 120 days. Old red blood cells are processed in the spleen, and glycohemoglobin is also broken down at this time [31] For this reason, the blood concentration of glycohemoglobin can be used as an indicator of the average blood glucose level over the past few months [32].

So, since a favorable trend was observed in HbA1c, we also examined blood glucose levels and insulin, which are related to blood glucose. Although no significant differences were observed, a trend toward a decrease in blood glucose levels and an increase in insulin was observed. Insulin is a hormone secreted from the β cells of the islets of Langerhans, an endocrine organ in the pancreas [33] This suggests that by improving the intestinal flora, homeostasis is achieved throughout the body [34] This suggests that the balance of blood glucose levels may also be maintained by improving the intestinal flora [35] Prolonged high blood glucose levels are of course related to lifestyle, but the possibility of developing diabetes cannot be denied [36] It is said that there are approximately 20 million people in Japan who are at risk of developing diabetes, including those at risk. Considering that the population is 120 million, about one in five to six people may have diabetes [37] When a person develops diabetes, rather than the problem itself, the three major complications are diabetic nephropathy, diabetic retinopathy, and diabetic peripheral neuropathy, and there is a possibility that artificial dialysis may also be considered.37) Artificial dialysis imposes time and lifestyle restrictions, which may lead to a decline in QOL and ADL, and measures to prevent this are urgently needed [38] Therefore, the fact that a trend toward improvement in blood glucose levels was observed using this test substance suggests that it may also be a good way to prevent diabetes. It is also said that diabetes can lead to CKD (chronic kidney disease) [39] It is known that long-term diabetes can damage blood vessels, and since the glomeruli in the kidney are also blood vessels, it is quite possible that this can damage the kidneys themselves [40] When diabetes continues for a long time, protein is confirmed in the urine, and albumin in particular is often seen [41] Albumin is the main component of protein in the blood, and when it leaks into the urine, it causes proteinuria [42]. In the early stages, the amount of albumin in the urine is small, but if it continues for a long period of time, the amount of albumin increases and is excreted as proteinuria [43]. Therefore, a decrease in the amount of albumin in the body's bodily fluids can cause hypoproteinemia, such as edema and shortness of breath [44]. When a large amount of proteinuria is produced, renal function also decreases and the

amount of creatinine can decrease [45]. This condition is called diabetic nephropathy, and although no significant differences were confirmed in the results of this study, a trend toward a decrease in blood glucose levels and an increase in creatinine levels were confirmed at the same time, indicating that blood glucose levels are being controlled. Although no improvement in biological age was observed with this test substance, the changes in the intestinal flora were observed, suggesting the possibility of maintaining homeostasis.

5. Conclusion

Although no changes were observed in biological age as a result of this test substance, when other biomarkers were looked at, including those for which no significant differences were confirmed, an overall trend towards improvement in the body was observed. In particular, a significant decrease in HbA1c was observed in blood glucose levels, suggesting the possibility of homeostasis at work. This is thought to be the result of this test substance improving the balance of the intestinal flora, maintaining homeostasis, and at the same time significantly increasing adiponectin, thereby having a positive effect on the body itself. Although fasting itself was not very meaningful in this study, it was suggested that taking this test substance could be of sufficient use in preventing lifestyle-related diseases.

6. Acknowledgement

We would like to express our gratitude to the staff and collaborators who helped us in conducting this research.

7. Conflict of Interest

There are no conflicts of interest in this study.

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