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Banana Intake Reduces Oral Cavity-Derived Bacteria in the Gut Microbiota

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Abstract Bananas, rich in prebiotics, dietary fibre, oligosaccharides, vitamins and minerals, have positive effects on gut health. However, their impact on oral cavity-derived gut microbiota and the intestinal environment remains unclear. This study investigated changes of the gut microenvironment and oral cavity-derived gut microbiota populations induced by banana intake. Twenty-six healthy women were instructed to consume two bananas per day for 2 weeks. We measured urinary indoxyl sulfate levels (a general gut microenvironment index) and the proportion of oral microbiota species within the gut microbiota before and after the 2-week banana consumption period. Banana intake significantly reduced urinary indoxyl sulfate levels. Additionally, participants aged < 40 years showed decreased indole levels, while no significant change occurred in those aged ≥ 40 years. The total number of bacterial species decreased due to banana intake. However, oral microbiota and *Porphyromonas* spp. populations remained unchanged in all participants. Nevertheless, participants with a high urinary indoxyl sulfate levels before banana intake showed slightly reduced oral microbiota and *Porphyromonas* spp. prevalence after banana intake, along with significantly lower urinary indoxyl sulfate levels. Therefore, the decreases in urinary indoxyl sulfate and the prevalence of oral cavity-derived bacteria and *Porphyromonas* spp. indicates the regulatory activity of bananas on gut microbiota.

Keywords: *oral bacteria, gut microbiota, Porphyromonas spp, indoxyl sulfate, banana intake*

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1. Introduction

The mouth is the starting point of the digestive tract where food is mechanically broken down before being digested and absorbed through the intestines and the waste excreted as faeces. The human gut microbiota is composed of approximately 700-1,000 bacterial species, with an estimated total of about 40 trillion bacteria [1]. In contrast, the oral cavity contains about 500-700 species of bacteria [2]. Among the oral bacteria, *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* are the most important periodontal pathogens [3]. Recent studies have associated periodontal disease with various diseases, such as coronary artery disease, myocardial infarction, stroke, diabetes and adverse pregnancy outcomes [4,5,6,7,8]. Therefore, the abnormal proliferation of periodontal pathogens can not only induce oral diseases but also cause dysbiosis of the gut microbiota, potentially increasing the risk of developing diseases [9,10]. In the older people, periodontal disease may lead to symptoms such as dementia and pneumonia [11,12]. The effects of gut microbiota have been extensively reported, indicating that gut microbiota may be involved in various diseases, such as obesity, diabetes, myocardial infarction, dementia, allergic diseases, rheumatoid arthritis and cancer. Furthermore, both oral and gut bacteria can affect the whole body [13,14,15]. Although oral and gut bacteria have different characteristics due to their different habitats, their inter-relationships suggest potential mutual influences [16,17]. Simply put, people with periodontal disease may inadvertently ingest a considerable number of periodontal bacteria [18,19]. Therefore, the proportion of oral cavity-associated bacteria to the gut microbiota needs to be evaluated. Recent studies have clarified the relationship between oral and gut bacteria [16,20]. Studies on mice have demonstrated that the ingestion of oral bacteria associated with periodontal disease changes the gut microbiota [21,22]. Notably, these changes were accompanied by a decrease in intestinal barrier function and inflammation. Therefore, oral bacteria can disrupt the balance of the gut microenvironment. Pre-, pro- and symbiotics are well-known regulators of the balance of gut microbiota [23,24,25,26,27]. Bananas are among the well-known prebiotics. We previously found that banana intake improves the gut microenvironment and reduces the levels of stress markers cortisol and chromogranin A [28]. However, no studies have examined the effect of banana intake on oral bacteria populations in the gut microbiota. Therefore, in this study, we investigated the effect of banana intake on the prevalence

of oral cavity-associated bacteria in the intestinal microbiota of Japanese adult women.

2. Materials and Methods

2.1. Participants and Ethics Statement

This study enrolled 26 healthy Japanese women aged 20–60 years, all of whom have provided their informed consent in writing. Excluded from participation were individuals who were taking medications that potentially influenced the study outcomes, those who were at risk of developing allergies related to the study and those who were deemed unsuitable for participation due to other reasons. These criteria were established by a physician. All research procedures adhered to the Declaration of Helsinki and the ethical guidelines for medical and health research involving human subjects and were approved by the Ethics Committee of Medical Corp. Koyokai (KEC-2021-10).

2.2. Banana Variety Used

We selected the Cavendish Dole Sweetio banana variety that is cultivated in highland regions (Dole Japan, Inc., Tokyo, Japan). Each banana had an edible portion of approximately 60 g. The nutritional values per 100 g of the edible portion, as outlined in the Standard Tables of Food Composition in Japan-2020 (8th revised edition) [29]. The nutritional values per 100 g of the edible portion are outlined in the Standard Tables of Food Composition in Japan-2020 (8^{th} revised edition) [29].

2.3. Experimental Design

The participants in the study were tasked with assessing the effects of eating two bananas daily for a span of 2 weeks, without any specific intake times. Urine and faecal samples were collected at regular intervals before and after the 2-week period to assess daily fluctuations. The influence of banana intake was gauged by measuring urinary indole to assess changes in the intestinal environment and observing variations in the proportion of oral cavity-associated bacteria to understand their impact on the gut microbiota.

2.4. Measurement of Urinary Indoxyl Sulfate Levels

Urine samples (approximately 5 ml) collected from all subjects were stored at 4°C and sent to Healthcare Systems Co., Ltd. (Nagoya, Japan), the third-party research institute contracted to perform measurements of indoxyl sulfate levels. The urine samples were centrifuged at 1,500 ×*g* and 4°C for 15 min before determining the level of indoxyl sulfate using a QuantiChrom Indole Assay Kit (BioAssay Systems, Hayward, CA, USA), as previously described [28].

2.5. Evaluation of Gut Microbiota in Faecal Samples

A SheepMedical next-generation sequencer (Tokyo, Japan) was used to determine the genetic sequence of the gut microbiota isolated from faecal samples. The sequencer can detect approximately 50–150 bacteria out of more than 3,000 different types of intestinal bacteria. Data from all participants before and after banana consumption were analysed to determine the total number of bacteria, types of bacteria and percentage of orally derived bacteria. The orally derived bacteria that could be measured in this study were *Streptococcus*, *Porphyromonas* spp. and *Fusobacterium* spp. After physically disrupting the stool sample using zirconia beads, DNA was extracted and purified using a Maxwell automated extraction device (Promega, Madison, WI, USA). Using this DNA as a sample, approximately 300 base pairs of the 16S rRNA hypervariable regions V1 and V2 were sequenced using the Ion S5 next-generation sequencer (Thermo Fisher Scientific). Sequence data were analysed using Ion Reporter software (Thermo Fisher Scientific) to identify the bacterial species obtained and calculate proportions. We analysed 30,000–100,000 sequences per participant and no one had more than 150 bacterial species. For a more detailed examination, sub-analysis was conducted on the above items for subjects with a high proportion of oral bacteria (more than 5%).

2.6. Statistical Analysis

All values are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using the Wilcoxon matched-pairs signed rank test in GraphPad Prism 9 (San Diego, CA, USA). P values <0.05 were considered statistically significant. A reanalysis was conducted on subjects aged <40 years with high urinary indoxyl sulfate levels (40 μg/mg Creatinine) prior to banana intake.

3. Results

3.1. Effect of Banana Consumption on Urinary Indole

The effect of banana intake on urinary indoxyl sulfate levels is demonstrated in Figure 1. The overall urinary indoxyl sulfate levels significantly decreased from 42.5 ± 4.7 μg/mg Creatinine(Cr.) to 35.5 ± 4.5 μg/mg(Cr.) following banana intake (Figure 1-A). Little change in urinary indoxyl sulfate levels was observed in individuals in their 20s and 30s; however, changes were observed in individuals aged ≥40 years. Therefore, a reanalysis was conducted by dividing the subjects into two groups: those aged <40 years and those aged ≥40 years. Age-stratified analysis (Figure 1-B) revealed a significant decrease in indole in participants aged <40 years (n = 12), from 40.0 ± 11.5 μg/mg to 30.0 ± 8.0 μg/mg. In contrast, no significant change in urinary indole was observed in those aged ≥40 years (n = 14).

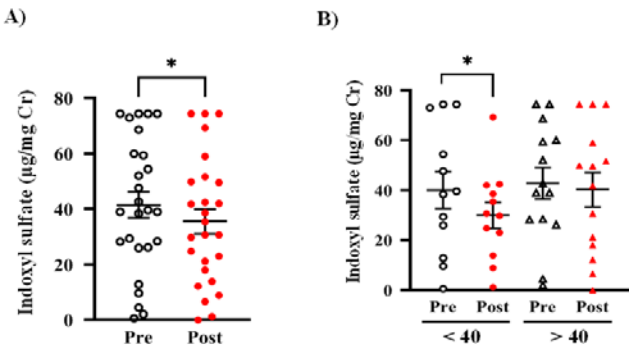

Figure 1. The effects of banana intake on urinary indoxyl sulfate

A). Amount of urinary indoxyl sulfate in all participants before (Pre) and after (Post) banana intake. Data are expressed as mean ± SEM (n = 26), *P < 0.05.

B). Indole levels in individuals aged <40 years and ≥40 years. Each value represents the mean (aged <40 years, n = 12; aged ≥40 years, n = 14), *P < 0.05.

3.2. Influence of Banana Consumption on Gut Microbiota

Banana intake caused a significant reduction in the number of gut microbiota species (Figure 2-A). Age-stratified analysis of the number of gut microbiota species could not assess the difference between individuals aged <40 years and ≥40 years (data not shown). Although the number of bacterial species decreased significantly, no change in diversity was observed (Figure 2-B). The proportion of oral cavity-associated bacterial species found in the intestinal flora is shown in Figure 3-A. Further analysis of participants aged <40 years and ≥40 years revealed that the proportion of oral bacteria decreased in those aged <40 years after banana consumption (Figure 3-B). Focusing particularly on the genus *Porphyromonas*, a periodontopathogenic genus of oral bacteria (Figure 4-A), the proportion of this genus in participants aged <40 years is shown in Figure 4-B. A slight decrease in the value of the genus *Porphyromonas* was observed, but the difference is not significant. Furthermore, a decrease in the number of genera in the gut microbiota (Figure 2-A) did not affect diversity (Figure 2-B).

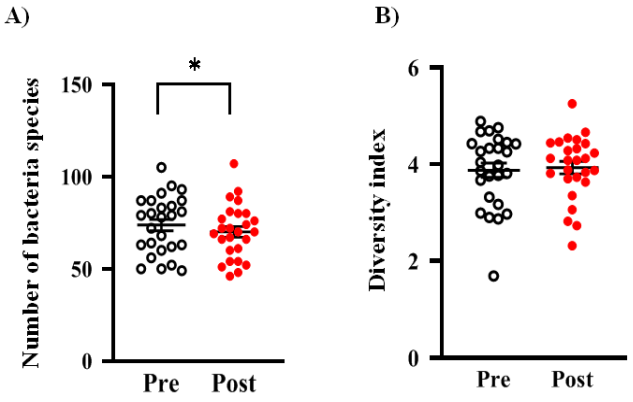

Figure 2. Changes in gut microbiota due to banana intake

A). Number of bacterial species in all participants before (Pre) and after (Post) banana intake. Data are expressed as mean ± SEM (n = 26), *P < 0.05.

B). Diversity of bacterial species in all participants before (Pre) and after (Post) banana intake. Data are expressed as mean ± SEM (n = 26).

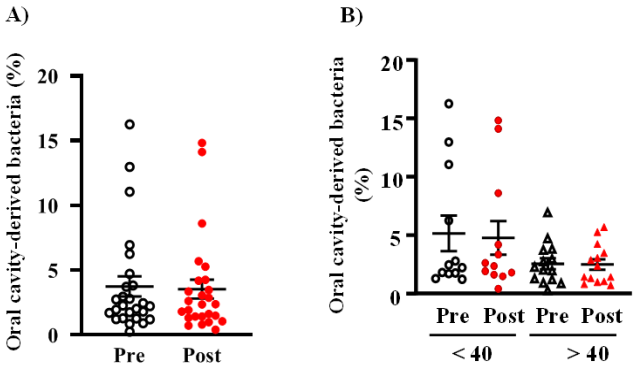

Figure 3. Percentage of oral cavity-derived bacteria

A).Percentage of oral cavity-derived bacterial species in all participants before (Pre) and after (Post) banana intake. Data are expressed as mean ± SEM(n = 26).

B).Percentage of oral cavity-derived bacterial species in individuals aged <40 years and ≥40 years. Each value indicates the mean ± SEM (aged <40, n = 12; aged ≥40 years, n = 14).

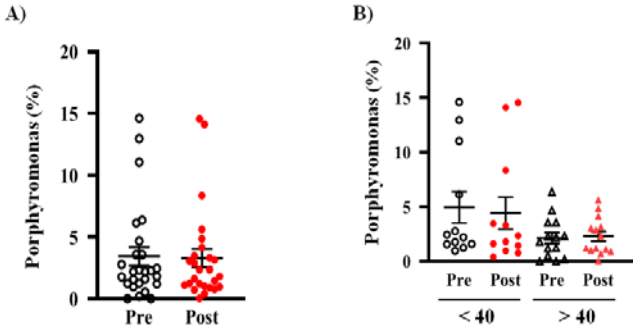

Figure 4. Percentage of *Porphyromonas* spp

A). Percentage of *Porphyromonas* spp. in all participants before (Pre) and after (Post) banana intake. Data are expressed as mean ± SEM (n = 26).

B). Percentage of *Porphyromonas* spp. in individuals aged <40 years and ≥40 years. Each value indicates the mean ± SEM (aged <40 years, n = 12; aged ≥40 years, n = 14)

3.3. Correlation Between Urinary Indoxyl Levels and Dominant Oral Bacteria

The correlation between urinary indole levels and oral cavity-derived bacteria was investigated. Participants aged <40 years with high urinary indoxyl sulfate levels (40 μg/mg Cr.) before banana consumption (n = 5) were re-analysed. The five participants with a high proportion of oral-derived bacteria had high urinary indoxyl sulfate levels (40 μg/mg(Cr.)) (Figure 5-A). Urinary indoxyl sulfate levels decreased slightly with banana intake, but not significantly. Figure 5-B demonstrates that the proportion of oral cavity-derived bacteria was slightly reduced by banana consumption. Furthermore, the prevalence of *Porphyromonas* spp. was slightly reduced by banana consumption (Figure 5-C). Subjects with a greater proportion of oral cavity-derived bacteria before

banana consumption were also found to have higher indoxyl sulfate levels.

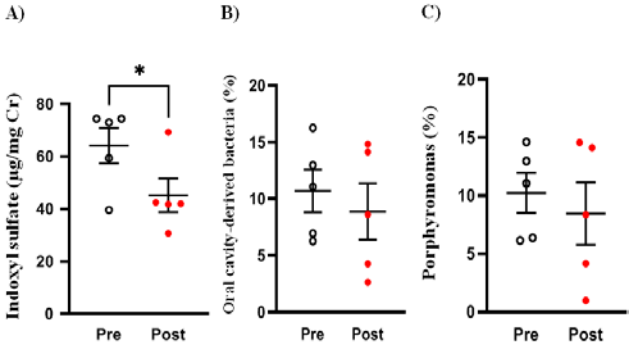

Figure 5. Reanalysis of participants with high urinary indoxyl sulfate levels before banana intake

A). Changes in urinary indoxyl sulfate before and after banana intake in participants with high basal levels of urinary indoxyl sulfate (>40 μg/mg(Cr.)). Each data represents the mean ± SEM(n = 5), *P < 0.05.

B). Changes in oral cavity-associated bacterial occupancy in the gut microbiota before and after banana intake in subjects with high basal levels of urinary indoxyl sulfate. Each data represents the mean ± SEM (n = 5)

C). Changes in *Porphyromonas* genus occupancy in the gut microbiota before and after banana intake in subjects with high basal levels of urinary indoxyl sulfate. Each data represents the mean ± SEM (n = 5)

4. Discussion

In this study, we demonstrated that banana intake as a prebiotic intervention modulates the intestinal microenvironment while reducing indole levels. In addition, a reduction in oral bacteria associated with periodontal and cariogenic pathogens was observed with banana consumption.

Approximately 10% of the intestinal flora in healthy humans consists of harmful bacteria, including *Escherichia coli*, which produces the putrefactive substance indole in the intestine [1,14]. The study measured urinary indoxyl sulfate levels as a marker for assessing the intestinal microenvironment and observed a decrease in indole levels with banana consumption. These results concur with those we reported previously [28]. The indigestible carbohydrates in bananas serve as a food source for probiotic bacteria, promoting their growth. Banana-derived oligosaccharides increase the faecal viable counts of probiotic bacteria and decrease Enterobacteriaceae [23,24]. Furthermore, banana improves the human gut microbiota, promoting the growth of beneficial bacteria and promoting the production of short-chain fatty acids [25,26,27]. Furthermore, banana intake decreased urinary indoxyl sulfate levels, indicating its mitigating activity on bacterial imbalance in the gut microbiota.

Banana consumption also improved the intestinal microenvironment and reduced oral bacteria populations in the gut microbiota (Figure 3). In the <40 years age group, a decrease in indole levels was observed with banana consumption, as well as a decrease in oral cavity-derived bacteria, including *Porphyromonas*. However, no significant changes were observed in the older age group.

In addition, higher indoxyl sulfate levels were observed in participants with a higher proportion of oral cavity-derived bacteria prior to banana consumption. Furthermore, a trend towards a decrease in urinary indoxyl sulfate was observed with banana intake. These findings indicate that reducing oral bacteria, including *Porphyromonas* spp., in the intestinal flora mitigates intestinal floral imbalance caused by periodontopathic bacteria.

The cause of the intestinal floral change in the younger age group is more efficient metabolisation, which means a greater efficiency in harnessing the prebiotic effects of bananas. Generally, the metabolic rate decreases after the age of 40 due to a decrease in muscle mass, activity level, hormonal changes and ageing of organs [30]. Furthermore, the number of patients with periodontal disease increases after the age of 40 [31]. Thus compared with people aged <40 years, those aged ≥40 years had a lower proportion of oral bacteria prior to banana intake.

To further elucidate the prebiotic effect of bananas and their effects on intestinal flora, including on oral bacteria, oral cavity-derived bacteria need to be ingested in advance and a comparison of the oral and intestinal bacteria need to be performed. The effects of urinary indoxyl sulfate should also be investigated. In future research, men and women should be included, along with a comparison with a control group. Herein, the gut environment can be relatively easily measured by assessing indoxyl sulfate and the gut microbiota. While previous research revealed that oral bacteria are part of the gut microbiota [32], there has been limited investigation into the impact of banana consumption on variations in oral cavity-derived bacteria within the gut microbiota. Because this study only focuses on banana intake without restricting other foods, dietary restrictions should be considered to accurately assess the effects of banana consumption. Additionally, it is important to evaluate whether banana intake improves the gut environment in participants with a history of digestive disorders or symptoms such as abdominal bloating, constipation or diarrhoea.

5. Conclusions

This study showed that consuming bananas may reduce the number of oral bacteria in the intestinal flora and improve the intestinal microenvironment by reducing indoxyl sulfate levels.

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