Effect of acute mental stress on bitterness perception in matcha

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Abstract

Matcha taste experiment using changes in bitterness threshold due to stress response

Aim The purpose of the present study was to investigate the effect of acute mental stress on bitterness perception in matcha. **Method** Nine healthy men and women between the ages of 20 and 39 participated. Bitterness threshold in taste disc and bitterness perception in matcha and mental stress level in visual analogue scale (VAS) were measured before and after Kraepelin test as acute mental stress loading. **Results** While mental stress significantly increased following Kraepelin test ($p < 0.05$), bitterness threshold and bitterness perception in matcha were not changed following Kraepelin test ($p > 0.05$). **Conclusion** These results suggest that changes in bitterness perception in matcha was not detected following acute mental stress.

急性的な心理的ストレスが抹茶における苦味の感じ方に及ぼす影響

目的急性的な心理的ストレスが抹茶における苦味の感じ方に及ぼす影響を調べることであった。**方法** 9 人の 20〜39 歳の健康な男女に急性的な心理的なストレスを与えるためにクレペリン検査を実施し、その前後にテーストディスクの苦味閾値の検査と VAS による抹茶の苦味知覚、加えて精神的ストレスレベルを実施した。**結果**クレペリンテストによって心理的なストレスは有意に上昇したが（$p<0.05$）、苦味閾値や抹茶における苦味の感じ方はクレペリンテストの前後で変化しなかった（$p>0.05$）。**結論**これらの結果から急性的な心理的ストレスによって検出可能なほどの抹茶における苦味の変化は生じないことが示唆された。

Matcha-Geschmacksexperiment mit Veränderungen der Bitterkeitsschwelle aufgrund von Stressreaktionen

Ziel Ziel der vorliegenden Studie war es, den Einfluss von akutem psychischem Stress auf die Bitterkeitswahrnehmung bei Matcha zu untersuchen. **Methode** Neun gesunde Männer und Frauen im Alter zwischen 20 und 39 Jahren nahmen daran teil. Die Bitterkeitsschwelle in der Geschmacksscheibe und die Bitterkeitswahrnehmung in Matcha und der mentale Stresslevel in der visuellen Analogskala (VAS) wurden vor und nach dem Kraepelin-Test als akute psychische Stressbelastung gemessen. **Ergebnisse** Während der psychische Stress nach dem Kraepelin-Test signifikant zunahm ($p < 0,05$), waren die Bitterkeitsschwelle und die Bitterkeitswahrnehmung bei Matcha nach dem Kraepelin-Test nicht verändert ($p > 0,05$). **Schlussfolgerung** Diese Ergebnisse legen nahe, dass Veränderungen der Bitterkeitswahrnehmung bei Matcha nach akutem psychischem Stress nicht festgestellt wurden.

Abbreviations:

VAS; Visual Analogue Scale

Introduction

Exports of Japanese matcha have quadrupled in 10 years due to the impact of the Japanese food boom. About changes in consumption of the four major luxury item, such as Alcohol, Tea (Green tea, Black tea), coffee or cigarette. that have no age limit. For Coffee, Global total increased by 6.2% from 2016 to 2022. (581,340kg). Tea (Green tea, Black tea), Global total increased by 10% from 2005 to 2007 (419,419t). Yokomitsu et al. (2015) suggests that luxury items are of high importance to Japanese people. 542 Japanese (278 males ＆ 264 females) were questioned about their consumption of luxury items, and the results showed that more than 80% of males and females said they consume tea, and more than 70% said they consume coffee. This shows that Japanese people consume a high percentage of luxury items, and there is no gender difference in the percentage of tea and coffee consumption (Yokomitsu et al., 2015). Yokomitsu et al. (2015) said that, the benefits of tea include relaxation, communication, enjoyment of meals, mood swings, increased motivation, made rhythm in life, improved concentration, got positive mood, and time for problem solving. (Yokomitsu et al., 2015)

Among the effects of green tea, we focused on health. Matcha green tea (Scientific name, Camellia sinensis), which originates from Japan, is commonly considered as particularly beneficial to health. A large content of polyphenols, amino acids (mainly tannins) and caffeine potentially increase the antioxidant properties of the drink (Jakubczyk et al., 2020). Studies confirming the high antioxidant

potential of tea beverages claim that it originates from the considerable content of catechins, a type of phenolic compound with beneficial effects on human health. In addition, due to its potential for preventing many diseases and supporting cognitive function, regular consumption of matcha may have a positive effect on both physical and mental health (Joanna Kochman et al., 2021). Matcha is also effective in relieving stress. Taking 3 g of matcha daily, it has been shown that matcha with more than 17 mg / g of theanine may be effective in reducing stress (Unno et al., 2018). For a typical Japanese matcha, two cups of 2 g of matcha can be consumed (ITOEN, 2009).

Depression, one of the stressful illnesses according to the WHO, is a major cause of health and disability worldwide. With more than 300 million people ill and an increase of more than 18% between 2005 and 2015, stress-induced depression has become a serious problem (WHO, 2018). Humans suffer from depression by destroying the immune system due to long-term psychological stress. There is a stress check sheet recommended by the Ministry of Health, Labor and Welfare for disease prevention (Ministry of Health, Labour and Welfare. 2021). This stress check sheet includes 52 questions about work, health, people around you, and satisfaction. In Japan, stress checks are mandatory once a year in workplaces of 50 or more people. But the current stress check is not enough. Feedback of stress survey results and interviews with doctors may not be scientifically effective (Kawakami & Tsutsumi, 2016). Improvement can be expected by incorporating additional components. Besides depression, there are other responses caused by stress. Heath (2006) revealed

that as the level of anxiety increases, so does the bitterness threshold (Heath et al. 2006). There is a paper that uses Spielberger Trait and mentions the relationship between stress and bitterness threshold. Spielberger Trait is a psychological test that can measure a patient's anxiety. In this paper, the bitterness threshold differs with and without stress (Heath et al., 2006) (Figure 1, A). It was found that the bitterness threshold was different even under high / low stress, and the bitterness threshold was higher at higher stress (Figure 1, B) (Heath et al., 2006). Other perceived increased stress significantly increases average levels of prolactin and consumption of coffee, chocolate and cigarette (Tomei et al., 2012). From the above, we considered the possibility of using taste changes for stress management.

If caffeine and quinine have different bitterness intensities, we cannot track the change in bitterness threshold due to stress. Therefore, it is necessary to estimate the change in bitterness threshold due to stress before proceeding with this study. The bitterness of the taste test kit is quinine and the bitterness component contained in matcha is caffeine. The threshold for bitterness in quinine is 0.03 mM, which increases to 0.09 mM with stress (Heath et al., 2006). The bitterness threshold of caffeine is 1.2 mM (Keast & Roper, 2007), but the effect of stress addition is unknown. Therefore, taking advantage of the fact that the change in bitterness threshold due to stress in quinine is 3-fold, we multiplied 1.2 by 3 to calculate a threshold of 3.6 mM. The caffeine content of matcha was estimated to be 3.2 g (Ministry of education, 2015), and based on the molar concentration calculation,

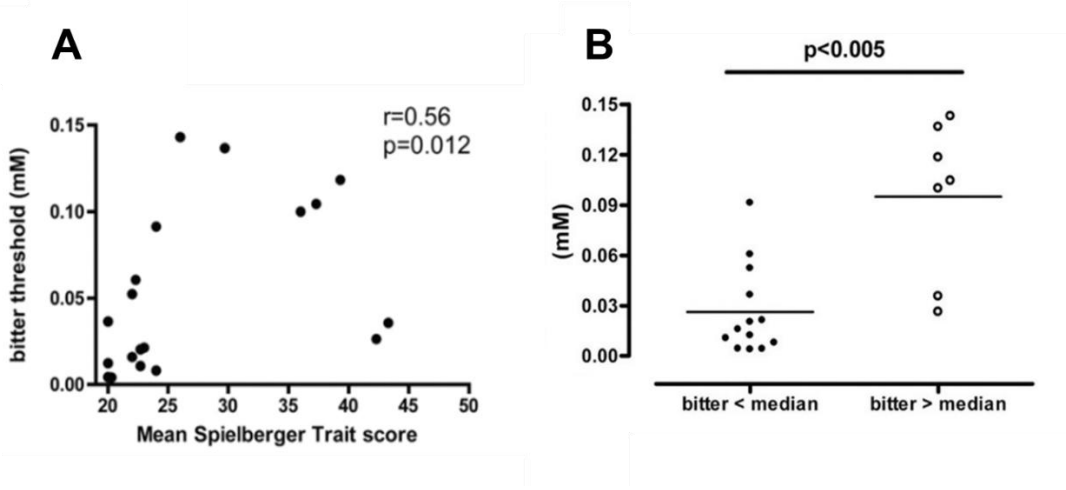

Figure 1, A; Results of correlation between anxiety score and bitterness threshold by Spielberger Trait. There is a significant difference, indicating that the bitterness threshold changes with stress (Heath et al., 2006)., B; Differences in bitterness thresholds when divided into high and low stress according to the intensity of the anxiety score of the Spielberger Trait. It can be seen that the higher the stress, the higher the bitterness threshold (Heath et al., 2006).

the caffeine concentration in matcha was estimated to be 2.3 mM. Since it is above the threshold before stress addition and below the threshold after stress addition, it is thought that the perceived bitterness of matcha changes with stress (Figure 2).

The aim of the present study was to investigate the effect of acute mental stress on bitterness perception in matcha. Since it is expected that the caffeine-related bitterness in matcha is higher than normal bitterness threshold and lower than the estimated bitterness threshold following mental stress (Figure 2), it is hypothesized that bitterness perception in matcha reflect the acute mental stress.

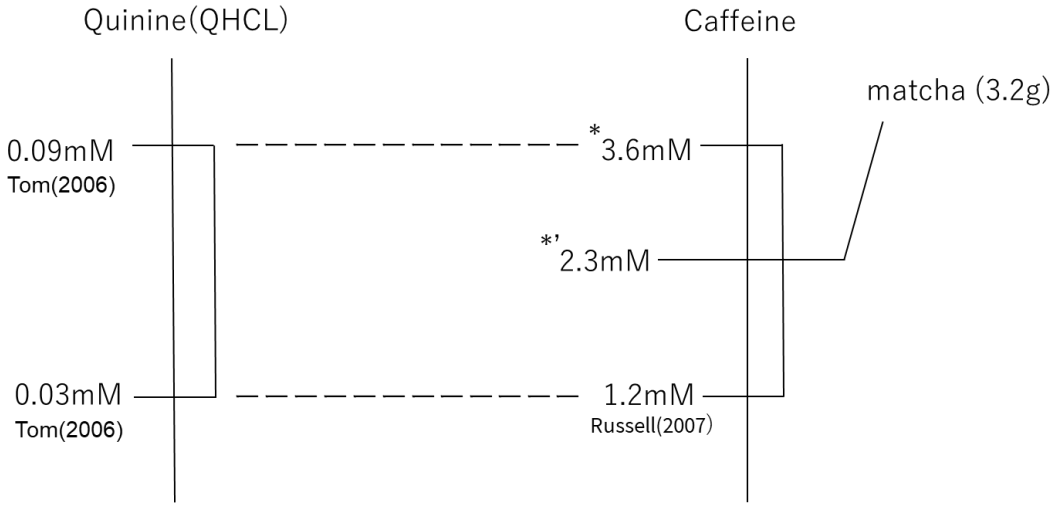

Figure 2, Bitterness thresholds of quinine and caffeine its changes with mental stress and bitterness threshold of matcha. *, Effect of mental stress on the Bitterness threshold in caffeine was calculated from changes in bitterness threshold in quinine following mental stress (Heath et al., 2006). *', The caffeine concentration of matcha was calculated from the caffeine content of matcha and molar concentration calculation. (Ministry of education, 2015)

Materials and methods

Participants

Nine healthy young man and women (age: 20 to 39 years) participated in this study. The participants gave written informed consent for this study after receiving a detailed explanation of the purposes, potential benefits, and risks associated with participation in this study.

Experimental design

The experimental protocol is shown in Figure 3. Participants came to the laboratory on 2 days separated by at least 24h and were randomly assigned the days for the trials. Following the preparation of the experiment, participants drank matcha and were asked bitterness perception by using visual analogue scale (VAS). Bitterness threshold were also measured from Taste disc. We used the Uchida-Kraepelin test (35min) sheet as way to give participants psychological stress. One of the two days was for control, so they were relaxed in the same posture for the same time as when they was undergoing the Kraepelin test. Before and after the Kraepelin test and rest, we asked them to answer a stress subjective questionnaire using the VAS evaluation to see how much they felt stress. After that, taste measurement (POST) was performed. The concentration of matcha used in the taste test is constant.

Measurement of the subjective bitterness of matcha

The ingredients for matcha are matcha powder (Hoshino Tea Garden Co., Ltd.) 2g with

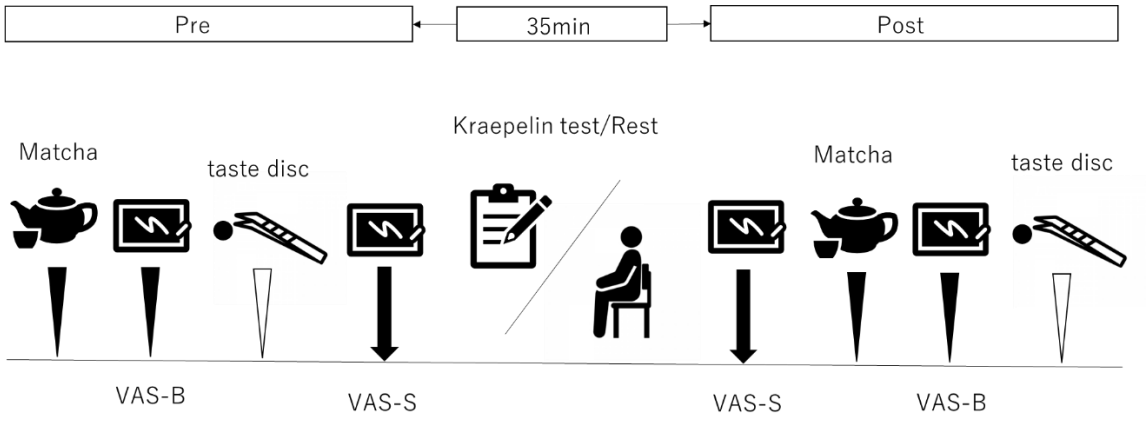

Figure 3, Schematic overview of the experimental protocol.; VAS-B, visual analogue scale for bitter taste.; VAS-S, visual analogue scale for stress.; Matcha, Matcha intake.

90 ℃ hot water 70ml. The reason for matcha intake before the taste disc test is to prevent the subjective taste of matcha from being influenced by the results of the bitterness threshold.Bitterness perception in matcha was measured by using VAS ranging from 0 to 10 which are corresponds to from don't feel bitterness to very bitter (Figure 4).

Measurements of bitterness threshold using taste disc

For measurements of bitterness threshold, traditional testing for taste thresholds, i.e., Taste Disc (Sanwa Kagaku Kenkyujyo Co., Ltd.), were applied with modification to increase sensitivity of the test. A 5 mm circular filter paper soaking the solution were placed on the measurement points in mouth with tweezers. After a few seconds with mouth open, remove the filter paper and ask if there is a bitterness reaction. Gradually increase the concentration until participants feel a bitterness reaction from the lowest concentration. There are 5 levels of solution concentration. (0.001%, 0.02%, 0.1%, 0.5%, 4%). However, this intensity is coarse, and if the change in the bitterness reaction is small, accurate results cannot be obtained. Therefore, it is necessary to narrow (dilute) the concentration interval of the aqueous solution. Preliminary experiments have shown that there are 23 levels of solution concentration. This is the minimum value at which changes in bitterness intensity can be recognized. The detailed solution concentrations are shown in Figure 5.

Measurement point,

The point connecting 1 cm from the retromolar trigone, which is behind the second molar

Figure 4, Visual analogue scale for measurement of bitterness perception in matcha.

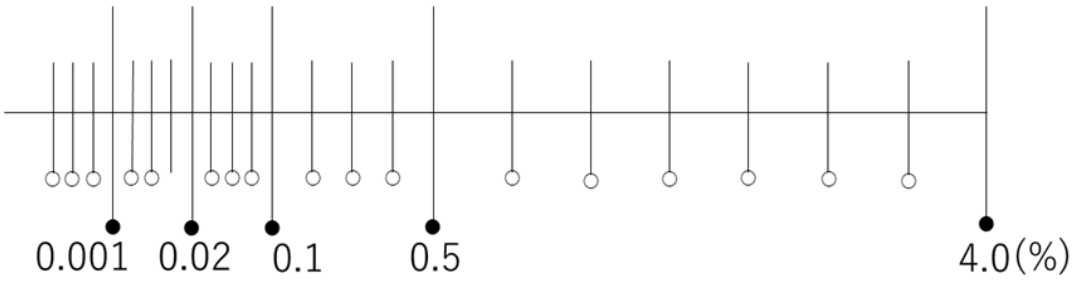

Figure 5, Concentration of quinine used in this study.; ●, Original scale in taste disc.; ○, Modified scale in taste disc.

alveolar, the sloping triangular bone surface that continues to the inner surface of the ramus of the mandible, to the front (End of the tongue). The measurement point was determined by preliminary experiments. In preliminary experiment, four measurement point were considered, i.e., 1) midpoints between right and left retromolar trigone on the center of the tongue, 2) midpoints between right and left retromolar trigone on the lateral edge of the tongue, 3) midpoints between right and left to the front from retromolar trigone on the tongue, and 4) midpoints between right and left to the front from retromolar trigone on the lateral edge of the tongue (Figure 6).

Kraepelin test

We used Uchida-Kraepelin for loading acute mental stress to the participants, which is consists of two sets of 15 min of calculation test with 5 min of break between them. Participants added two single-digit numbers, and repeated the task of entering the last digit of the calculation result. In the previous studies, this test was used for inducing mild acute mental stress (Li et al. 2004, Kanehira et al. 2011).

We used VAS evaluation to whether the stress changed due to Kraepelin test. One point is 1 mm, and 10 cm can be evaluated on a 100-point scale. There is evidence in favour of effective for stress assessment.

Statistics

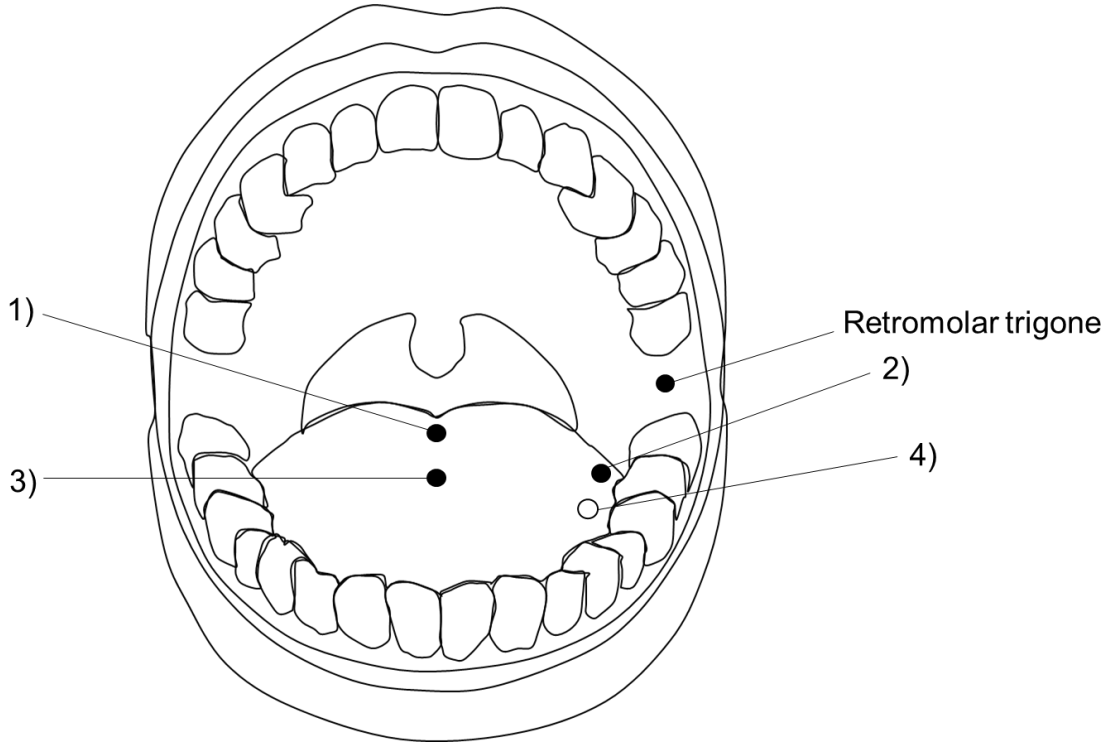

Figure 6, Retromolar trigone and preliminary experiment measurement position.; 1), Midpoints between right and left retromolar trigone on the center of the tongue.; 2), Midpoints between right and left retromolar trigone on the lateral edge of the tongue.; 3), Midpoints between right and left to the front from retromolar trigone on the tongue.; 4), Midpoints between right and left to the front from retromolar trigone on the lateral edge of the tongue.; ○, Measurement points determined by preliminary experiments.

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Figure 7, Visual analogue scale for measurement of subjective mental stress.

Each result is given as the mean and standard deviation. The non-parametric analysis was used in this study from the results of Shapiro-Wilk test. Bitterness perception in matcha, bitterness threshold, stress perception were compared between Pre and Post for Kraepelin test and rest and between Kraepelin test and rest at Pre and Post using Wilcoxon sum-rank test. The level of statistical significance was set at $p < 0.05$. Statistical analyses were performed using SPSS software (version 25.0; SPSS, Tokyo, Japan).

Results

Significant increase in stress was observed following Kraepelin test ($p < 0.05$), but not following rest ($p > 0.05$) (Figure 8). Bitterness perception in matcha and bitterness threshold were not significantly changed following both Kraepelin test and rest ($p > 0.05$) (Figure 9). There were no significant differences in bitterness perception in matcha and bitterness threshold between Kraepelin test and rest at Pre and Post ($p > 0.05$) (Figure 10).

Discussion

In this study, participants were subjected to acute psychological stress using the Kraeperin test. The results showed a significant increase in subjective stress level after the Kraeperin test, which means that the study was successful in creating an experimental acute stress state. It has also been used

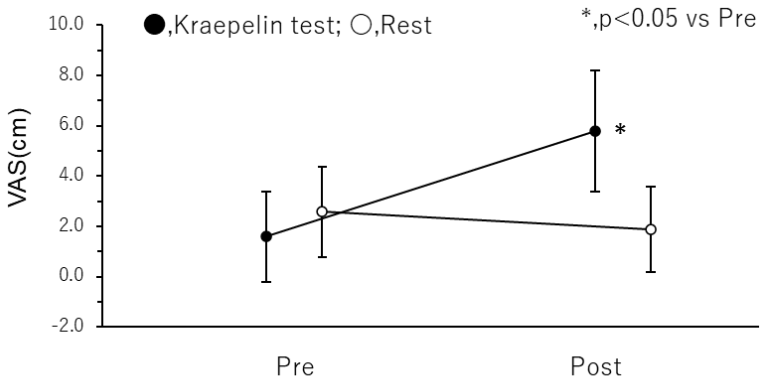

Figure 8, Subjective stress level following Kraepelin test and rest.; *, $p < 0.05$ vs Pre

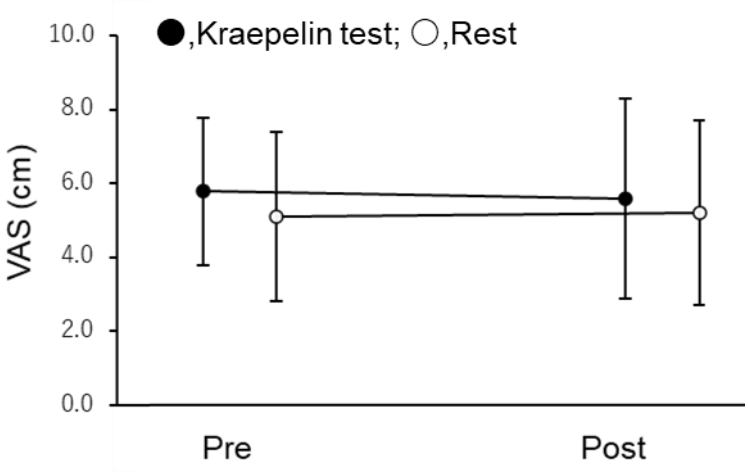

Figure 9, Bitterness perception in matcha following Kraepelin test and rest.

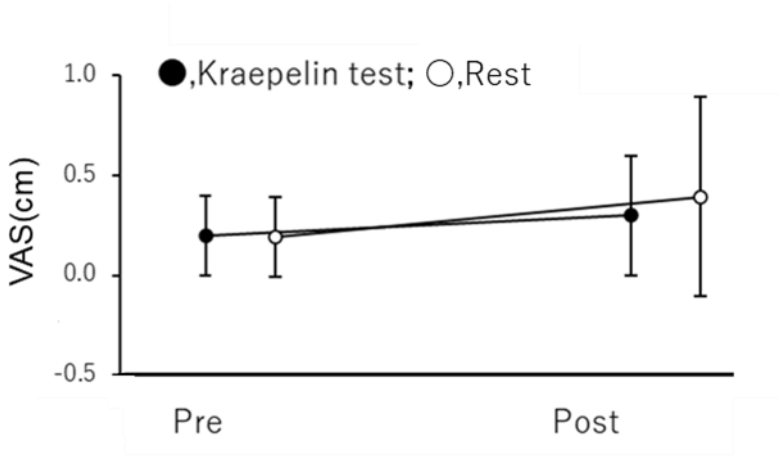

Figure 10, Bitterness threshold following Kraepelin test and rest.

in previous studies at 15-5-15 minutes. This test was able to provide mild acute psychological stress and may have lacked a little stress to affect how the bitter taste of matcha was perceived. It was necessary to revise the condition settings for long-term testing, changing the temperature of the matcha, and diluting the taste test kit (taste disc). Therefore, acute stress in this study is not sufficient to alter the perception of bitterness. Although subjective stress level was increased following Kraepelin test, changes in bitterness perception in matcha and bitterness threshold were not found following Kraepelin test. These results don't support the hypothesis that bitterness perception in matcha reflect the acute mental stress.

Amount of stress

Figure 8 shows that the Kraepelin test was effective in exerting stress load. Figures 9 and 10 shows that the stress load was not enough to change the taste. So how much stress did the taste change? Focused on the implementation time of the Kraepelin test. Which is more effective in giving more mental stress, the normal method (15 min-5 min-15 min) or the method performed continuously for 30 minutes. Sakamoto(2016) describes that if the change in the sAMY value during the test can be tracked, it may be a material for considering the implementation time of the test.

Relationship between Stress and Matcha Bitterness and Bitterness Threshold

As can be seen from the results, stress was applied to the test subjects, so why did no change in taste occur? In order to analyze the results in more detail, we examined whether there was a

correlation between the amount of stress on the Kraepelin test or rest and the amount of change in the VAS of matcha bitterness and bitterness threshold for each individual (Figure 11 & 12). It can be seen that the correlation results are not linear, indicating that there are individual differences in the perception of stress despite the same load of Kraepelin test. Therefore, it cannot be said that the stress load has a proportional effect on the change in bitterness threshold.

Proving a hypothesis

Calculate the quinine and caffeine thresholds and whether the hypothesis of change due to the effects of stress is correct using the 23 levels taste disc used. Multiply the taste disc thresholds by 3, just as we calculated the post-change values for the caffeine thresholds. The average of the 23 levels teste disc thresholds is 0.2%. 0.2 x 3 = 0.6 (%), so if the average of the 23 levels taste disc thresholds after the effects of stress is 0.6%, then the hypothesis is correct. However, since the result obtained in this study was 0.3%, the calculation of the hypothesis may be incorrect.

Matcha water temperature

Fujiyama (2017) revealed that a statistical comparison of taste recognition thresholds before and after cold stimulation showed increased taste sensitivity for all four basic tastes (Fujiyama & Toda, 2017). Decreased at bitterness threshold 1.9 ± 0.4. Fujiyama (2017) revealed that it was easier to feel the bitter taste if the drink was taken after cooling the mouth before drinking (Fujiyama & Toda, 2017). From the above, it is expected that the bitter taste of matcha will be easier to feel if you drink it after

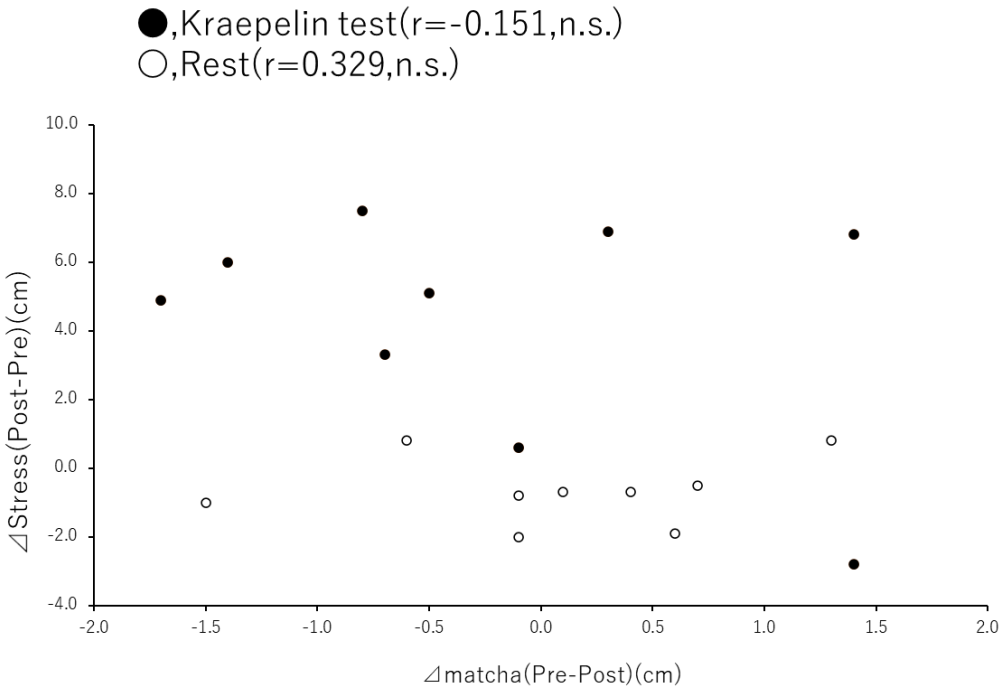

Figure 11, Correlation between changes following Kraeperin test and rest in bitterness perception in matcha and in subjective stress level.; ●, Kraepelin test.; ○, Rest.

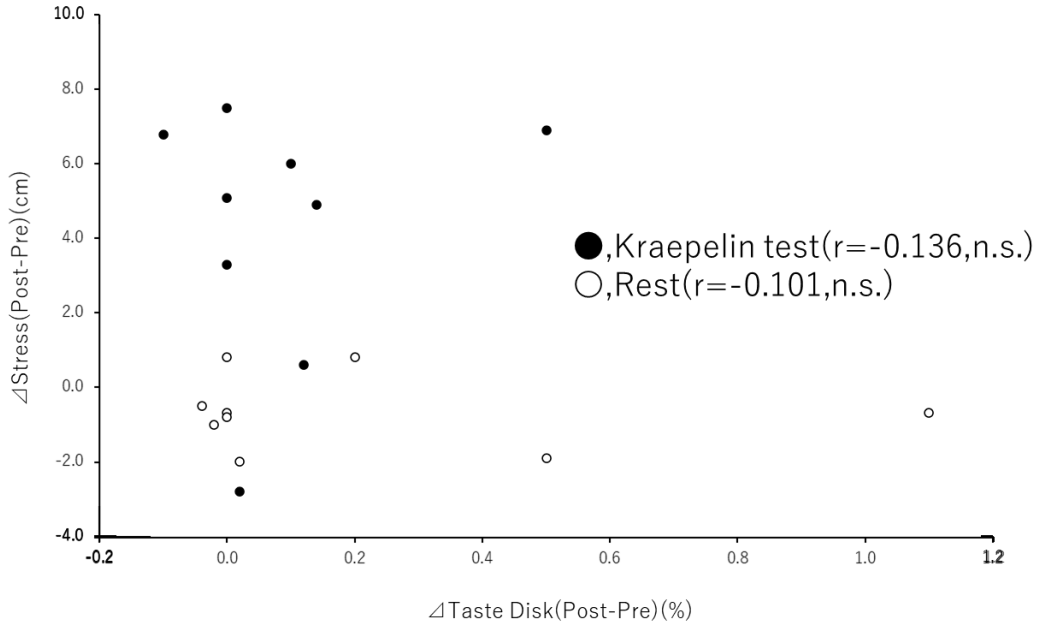

Figure 12, Correlation between changes following Kraeperin test and rest in bitterness threshold and in subjective stress level.; ●, Kraepelin test.; ○, Rest.

cooling your mouth.

Restrictions on participants

This expeimental did not limit the participants, but it may have been necessary to limit them. Mura (2018) revealed that a single exposure prolongs the taste until 120 minutes later (Mura, E et al. 2018). It is considered that the results were more remarkable when the subjects were restricted to refrain from ingesting bitterness for 2 hours before the experiment.

Conclusion

In conclusion, this study investigated the effect of acute psychological stress on the perception of bitterness in matcha. We were able to apply psychological stress, but we did not find any change in the perception of bitterness in matcha. This result suggests that more intense stress may have an effect on the taste buds to the extent that they change.

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