

Physiological Effects of Ingesting Eucalyptus Essential Oil with Milk Casein Peptide

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This study was conducted to clarify the effect of eucalyptus essential oil mixed with milk casein peptide food for human physiological relaxation. Fifteen male university students (21.2 ± 0.9 yr) participated in study as subjects. The subjects were given one of two types of experimental drink (peptide + eucalyptus flavor (Pep + EF), and peptide + grapefruit-orange flavor (Pep + G-O), each flavor contains natural essential oil). We measured the change in salivary cortisol concentration and POMS scores before and two hours after taking experimental drink. The results of a Type A behavior pattern test were used to classify subjects. The concentration of salivary cortisol decreased significantly two hours after taking Pep + EF. And Type B showed bigger change than Type A. In conclusion, the results show that eucalyptus essential oil has the effect of relaxation, and that the effects on Type A and Type B are different.

Keywords eucalyptus essential oil, POMS, relaxation, salivary cortisol, type A behavior pattern

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1 Introduction

Casein, which represents 80% of the protein in milk, is nutritionally excellent with amino acids, and is rich in branched-chain amino acids (BCAA), which have a variety of physiological functions in the human body (Nakamura et al.

1995, Fernstrom 2005). The form of casein that is most frequently employed as a food ingredient is milk casein peptide, which is effectively absorbed in the human body. Taking milk casein peptide is reported to be effective for increasing calcium absorption (Sato et al. 1986). Therefore, it is widely used as an ingredient in health-assisting

foods. But because it has a bitter taste, milk casein peptide is used as a mixture with various flavors. Grapefruit and orange flavor are well-known additives, and they are usually added to milk casein peptide.

Recently, there have been a number of studies on the relaxation effect of essential oil extracted from wood (Miyazaki and Motohashi 1996), and essential oils and their components are widely used in the food industry as flavoring additives (Cawan 1999). Eucalyptus essential oil has been shown to have antimicrobial activity (Cawan 1999, Schelz et al. 2006). Moreover, it is used as a typical essential oil in aromatherapy.

This study was carried out to clarify the physiological effect of eucalyptus essential oil added to milk casein peptide food, with grapefruit-orange flavor added to milk casein peptide food employed as control.

Assessment of cortisol in the saliva is a widely accepted and frequently employed method in various fields of study (Park et al. 2007, Tsunetsugu et al. 2007, Park et al. 2008). The ease of sampling is one of the most obvious advantages of cortisol assessment of saliva. In general, saliva samples can be obtained in a stress-free manner at almost any desired frequency in most subjects (Kirschbaum and Hellhammer 1994).

The POMS (Profile of Mood States) is a well-established, factor-analytically derived measure of psychological distress for which high levels of reliability and validity have been documented. There is growing evidence that psychological mood state change assessed by employing POMS (McNair and Lorr 1964, Yokoyama et al. 1990).

Miyazaki and Tsunetugu (2005) have categorized hemoglobin concentration changes in the prefrontal area into certain types when gustatory stimulus is applied, and explained them by using personality characteristics (Type A behavior pattern). The result shows that physiological responses can vary according to personality characteristics.

The aim of this study was to clarify the effect of eucalyptus essential oil mixed with milk casein peptide food on the physiological relaxation of humans and to confirm the manner in which physiological responses vary according to personality characteristics.

2 Materials and Methods

2.1 Subjects

Fifteen normal male university students (21.4 ± 0.9 years old; mean value \pm standard deviation) participated in the study as subjects. None of the subjects reported any physiological or psychiatric disorders in their personal histories. The subjects' written consent to participate in the study was obtained after explaining the details of the study in advance. This study was performed under the regulations of the Institutional Review Committee of the Forestry and Forest Products Research Institute in Japan.

2.2 Measurement Items

Salivary cortisol concentration was measured for physiological response. Saliva was collected in a salivette (No. 51.1534, Sarstedt, Numbrecht, Germany) for 2 minutes (Park et al. 2007, Tsunetsugu et al. 2007, Park et al. 2008). The collected saliva was frozen, and the samples were transported to SRL, Inc. for analysis of salivary cortisol concentrations.

The POMS (McNair and Lorr 1964) has been utilized in many previous investigations in an attempt to assess transit, distinct mood states. The POMS brief form consists of 30 adjectives rated on a 0–4 scale that can be consolidated into six affective dimensions: T-A (tension-anxiety), D (depression-dejection), A-H (anger-hostility), V (vigor), F (fatigue), and C (confusion-bewilderment). It has been widely employed in the assessment of mood changes resulting from a variety of interventions due to its responsiveness (Bullington 1990, DiLorenzo et al. 1999). In the case of Japanese subjects, the Japanese edition of the POMS (Yokoyama et al. 1990) was employed for assessing the psychological response.

A Type A behavior pattern (brief questionnaire for the detection of Type A tendencies (Maeda 1985)) was measured for the classification of personality characteristics. Type A behavior pattern is reported to be associated with increased coronary heart disease, and independent of traditional risk factors such as smoking history, high cholesterol

and triglyceride levels, and hypertension (Shekelle et al. 1976). Further, the Type A behavior pattern has been defined as a chronic struggle to obtain an unlimited number of goals in the shortest possible time (Friedman and Rosenman 1974). Overt manifestations of Type A behavior pattern comprise competitive achievement-striving, impatience, a sense of time urgency, and free-floating hostility. Individuals exhibiting Type A behavior pattern are twice as likely to incur heart disease compared to individuals who are low on this dimension, or exhibiting Type B behavior pattern (Friedman and Rosenman 1974, Jenkins et al. 1974). With a brief questionnaire for the detection of Type A, we categorized the subjects whose scores were over 43.93 as Type A (9 subjects) and the subjects whose scores were under 43.93 as Type B (6 subjects).

2.3 Study Design

Two types of experimental drink were used in this study. One type per day was taken in random order. Before taking the experimental drink, we asked the subjects to fill out a POMS questionnaire, and we collected saliva from the subjects. After taking the experimental drink, the subjects were asked to stay in a waiting room for two hours. While waiting, the subjects answered questions for the Type A behavior pattern. They were allowed to read a newspaper or book. After two hours, we asked the subjects to fill out the POMS questionnaire again, and saliva of the subjects was collected for cortisol measurement.

2.4 Experimental Drinks

The experimental drinks were two types of drink, peptide + eucalyptus flavor (Pep+EF), and peptide + grapefruit-orange flavor (Pep+G-O). Casein hydrolysate (CU-2500A; Morinaga Milk Industry Co., Ltd., Tokyo) was used for the peptide. In other words, eucalyptus flavor (Takasago International Corp., Tokyo) was used as a fragrance component, and grapefruit-orange flavor (Ogawa & Co., Ltd., Tokyo) was used as the control. The eucalyptus flavor and grapefruit-orange flavor cleared the food safety evaluation. The eucalyptus

flavor contains 10.0% eucalyptus leaf essential oil; the grapefruit-orange flavor contains grapefruit (2.1%) and orange essential oil (0.2%).

The ingested amount of experimental drink varied depending on the body weight (bw) of the subject. The ingested amount of peptide was 0.2 g/kg bw, and the ingested solution was 4 g/kg bw. The concentration of the eucalyptus leaf essential oil was 0.01% (0.4 mg/kg bw) of the ingested fluid volume; and that of grapefruit and orange essential oil were 0.01% (0.4 mg/kg bw) and 0.001% (0.04 mg/kg bw) of the volume, respectively.

2.5 Statistical Analysis

As the significance test, a paired *t*-test was used for comparison of salivary cortisol concentration measurement before and after ingestion of the experimental drink. For the comparison of POMS scales before and after experimental drink ingestion, a Wilcoxon rank sum test was used. Each measured value is shown as the mean value \pm standard deviation.

3 Results

Fig. 1 shows the change in the salivary cortisol concentration before and two hours after taking the experimental drink in all subjects. The concentration of salivary cortisol before taking Pe+EF was 0.65 ± 0.42 $\mu\text{g}/\text{dl}$; the concentration of salivary cortisol two hours after taking Pep+EF was 0.35 ± 0.26 $\mu\text{g}/\text{dl}$. The concentration of salivary cortisol before taking Pep+EF was significantly decreased (46%, $P < 0.01$) within two hours after taking Pep+EF. However, there is no statistically significant difference between the observed concentrations before and after taking Pep+G-O.

Fig. 2 shows the POMS scores of the subjects before and two hours after taking the experimental drink in all subjects. There are no statistically significant differences in all POMS scores between before and after taking Pep+EF and Pep+G-O.

We were able to identify a significant difference in the salivary cortisol concentrations that were present before and after the taking Pep+EF

by the subjects. We divided the salivary cortisol concentration results into Type A and Type B only for the subjects that took Pep+EF.

Fig. 3 shows the change in the salivary cortisol concentration before and two hours after taking Pep+EF in the Type A subject group and in the Type B subject group. The results for the Type A subject group revealed no statistically significant differences in the salivary cortisol concentrations that were present before and after taking Pep+EF by the subjects. The results for the Type B subject group, however, revealed that the concentration of salivary cortisol decreased significantly (52.7%, $P < 0.01$) within two hours of the taking Pep+EF by subjects (salivary cortisol concentration before taking Pep+EF: $0.74 \pm 0.33 \mu\text{g/dl}$; salivary cortisol concentration two hours after taking Pep+EF: $0.39 \pm 0.17 \mu\text{g/dl}$).

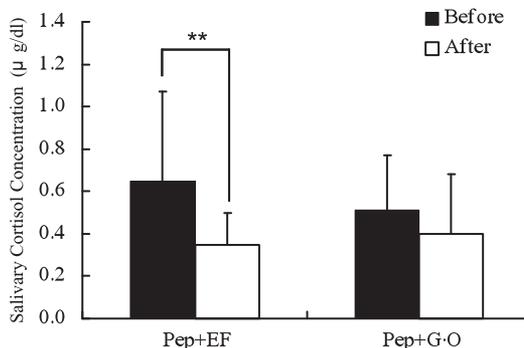


Fig. 1. Change in the salivary cortisol concentrations of the subjects before and two hours after taking the experimental drink. **: $P < 0.01$; mean \pm SD; $n = 15$; Pep+EF: Peptide+Eucalyptus flavor; Pep+G-O: Peptide+Grapefruit-orange flavor; Before: Before taking the experimental drink; After: Two hours after taking the experimental drink.

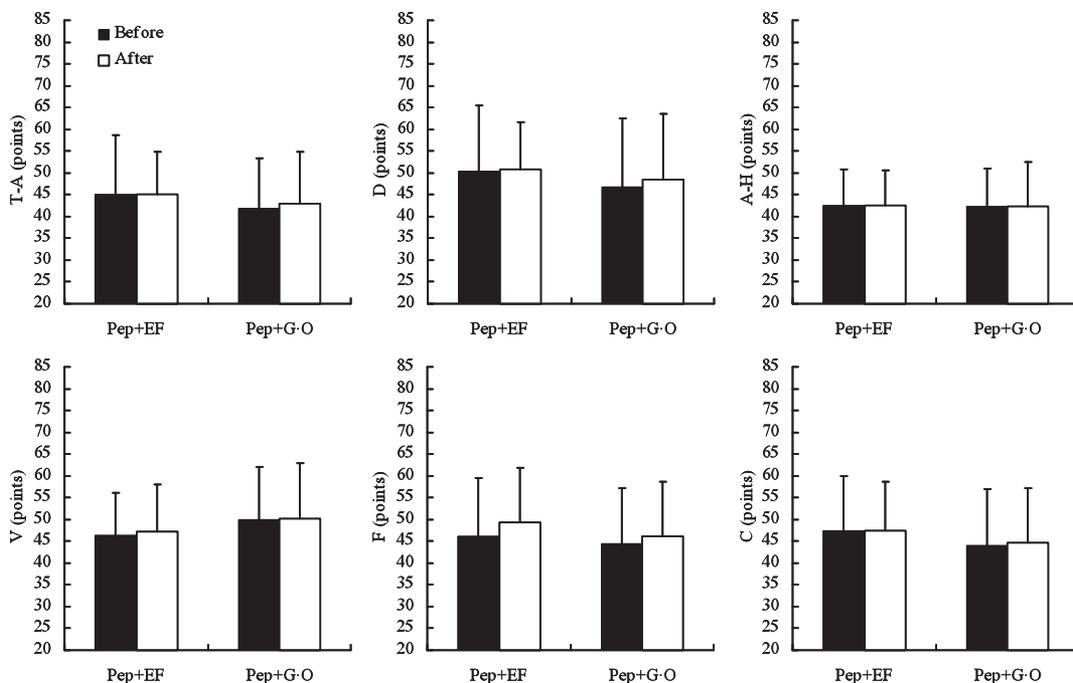


Fig. 2. POMS score of the subjects before and two hours after taking the experimental drink. T-A (tension and anxiety), D (depression and dejection), A-H (anger and hostility), V (vigor), F (fatigue), and C (confusion); mean \pm SD; $n = 14-15$; Pep+EF: Peptide+Eucalyptus flavor; Pep+G-O: Peptide+Grapefruit-orange flavor; Before: Before taking the experimental drink; After: Two hours after taking the experimental drink.

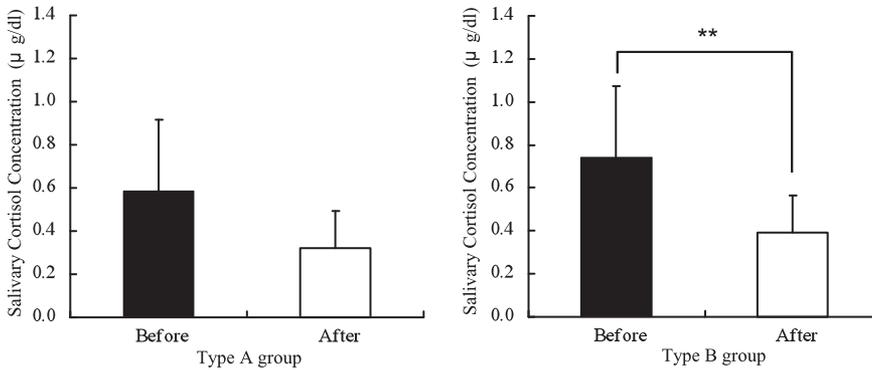


Fig. 3. Change in the salivary cortisol concentrations of the Type A subjects and Type B subjects before and two hours after taking the experimental drink (Pep+EF). **: $P < 0.01$; mean \pm SD; Pep+EF: Peptide+Eucalyptus flavor; $n=9$ (Type A group); $n=6$ (Type B group); Before: Before taking the experimental drink; After: Two hours after taking the experimental drink.

4 Discussion

The concentration of cortisol, which is a typical stress hormone, decreased significantly within two hours of the taking Pep+EF. However, there were no statistically significant differences in the concentrations of cortisol that were present before and after taking Pep+G·O by the subjects. These results clarify the fact that taking Pep+EF relaxes humans with a greater efficiency than taking Pep+G·O. The relaxation effect of essential oil extracted from wood was reported by Miyazaki and Motohashi (1996) who used Taiwan hinoki essential oil as an odoriferous stimulus to decrease systolic blood pressure.

The result of the POMS scores showed no significant change. The salivary cortisol concentration indicated that the subjects were in a relaxed state while the psychological index did not indicate not any difference. Park et al. (2007)'s field test showed the same conclusion previously. In their field test, the subject group that was scheduled to go to the forest showed significantly lower total hemoglobin concentrations in the prefrontal areas than the subject group that was scheduled to go to the city. The lower total hemoglobin concentration means that activity in the cerebral area is calmed down (Hoshi and Tamura 1993). Conversely, their psychological indexes showed no differences.

With the subjects divided into Type A and Type B, the results revealed a significant difference in the cortisol concentrations that were present before and after taking Pep+EF by Type B subjects; however, no significant difference in these concentrations were observed before and after taking Pep+EF by Type A subjects. Type A individuals who were extreme in time urgency, achievement striving, and aggressiveness were roughly twice as likely to develop heart disease as the Type B individuals (Jenkins et al. 1974). Several studies have demonstrated an association between Type A behavior pattern and autonomic nervous activity responsiveness to a variety of mental stressors such as arithmetic testing (Friedman et al. 1975, Dembroski et al. 1978, Manuck et al. 1978, Dembroski et al. 1979, Williams et al. 1982). Generally, the heart rate and blood pressure of Type A individuals, which are indicators of autonomic nervous activity, can easily be enhanced under stress conditions. However, Vermunt et al. (2007) reported that under low mental pressure, cortisol concentration of Type B subjects was higher than that in Type A, and under high mental pressure conditions, they found reverse results. In the present study, we observed a greater decrease in cortisol concentrations in Type B subjects than in Type A subjects when they take Pep+EF. This result is consistent with the report published by Miyazaki and Tsunetugu

(2005). Their study also revealed that Type B subjects exhibited a more profound change than the Type A subjects, which is consistent with our findings.

The underlying mechanism for greater decrease in cortisol concentration in Type B subjects following Pep+EF intake is unknown; however, in the present study, authors can only hypothesize that the cortisol concentration of Type B individuals decreased easily, and that the corresponding concentration in Type A hardly decreased following Pep+EF intake.

The conclusions are as follows: 1) Eucalyptus essential oil has the effect of stress control. 2) The relaxation effect of eucalyptus essential oil shows a difference between Type A and Type B; Type B showed a bigger change than Type A.

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