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Research Note

The Combined Effect of Caffeine and Ornithine on the Mood of Healthy Office Workers

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ABSTRACT: Caffeine is widely consumed and well known for stimulating the central nervous system. When developing new foods and beverages that contain caffeine, it is important to explore the potential synergistic effects of consuming amino acids and other food ingredients with caffeine on humans. Given the physiological pathways affected by the amino acid ornithine, consumption of ornithine with caffeine may have synergistic effects. The purpose of the present study was to examine the effect of consuming caffeine with ornithine in humans. The study used a randomized, placebo-controlled, double-blinded crossover design. The subjects were all healthy office workers who ingested the placebo, 100 mg caffeine, or 100 mg caffeine plus 200 mg ornithine in the morning and completed questionnaires about their mood. Office workers who consumed the combination of caffeine and ornithine had higher mood ratings 8 h after consumption than office workers who consumed caffeine alone. The results of the present study suggest that there is a unique synergistic effect between caffeine and ornithine on the mood of healthy office workers and that ornithine may potentiate the effects of caffeine.

Keywords: caffeine, ornithine, psychomotor stimulants, mood

INTRODUCTION

Caffeine is a type of purine alkaloid that is abundant in coffee, green tea, red tea, oolong tea, cola, and chocolate. Generally, caffeine is known for stimulating the central nervous system and promoting mental function (1). Thus, many people consume caffeine-containing food and drink products to ameliorate mental fatigue, prevent drowsiness, and maintain concentration when working, driving, or participating in cognitively demanding activities.

Previous studies have demonstrated that caffeine combined with various food ingredients significantly improves cognitive function. Haskell et al. (2) reported a significant positive interaction between caffeine and L-theanine, an amino acid found naturally in tea, on some cognitive and mood scores and suggested that beverages containing L-theanine and caffeine may have a unique pharmacological profile. Commercial energy drinks often include caffeine and other ingredients such as taurine, sugars, and B vitamins. Recently, it was suggested that caffeine acts synergistically with these other ingredients (3,4). In contrast, Peacock et al. reported that the co-administration of caffeine with taurine, a key energy drink ingredient, attenuates the facilitative effects of caffeine on the stimulus degradation task (5). Efforts to identify novel combinations of ingredients that work synergistically with caffeine to promote mental and physical activity or work to counteract the effects of caffeine have increased.

L-ornithine is an amino acid that is not incorporated into proteins but plays a role in the urea cycle in the liver. Ornithine promotes the release of growth hormone by stimulating the pituitary gland, which accelerates the metabolism of carbohydrates, proteins, and lipids (6,7). Ornithine has been shown to improve subjective feelings of fatigue and fatigue-related sleep quality (8-10). Researchers have suggested that the anti-fatigue effect of ornithine is due to ornithine's ability to reduce hypothalamic-pituitary-adrenal (HPA) axis activity, increase the efficiency of energy consumption, and promote the excretion of ammonia. The HPA axis is a stress-sensitive system and plays a central role in stress resistance. The HPA system begins with the release of corticotrophin-releasing hormones and culminates in the production of cortisol. It has also been shown that human cortisol concentrations are higher on working

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days than on non-working days (11). Therefore, it is important for office workers to decrease HPA axis activity as a means of preventing excessive stress.

L-arginine has also been used as a caffeinated energy drink ingredient and has recently been reported to potentiate the effect of caffeine in an animal study (12). Since L-arginine is a urea cycle intermediate and is metabolized to ornithine, we hypothesized that combining caffeine and ornithine would have synergetic effects that were similar to those observed when combining caffeine and L-arginine. Therefore, the aim of the present study was to examine the effect of the consumption of caffeine with ornithine on the mood (i.e., physiological and psychological parameters of arousal) of office workers.

SUBJECTS AND METHODS

Subjects

This study used a randomized, placebo-controlled, double-blinded crossover design. Healthy Japanese office workers, 14 males and 5 females, with an average age of 34.4 years (standard deviation=6.0 years), were invited to participate in the study. After an explanation of the purpose of the experiment, all subjects signed an informed consent form. The study was approved by the Kirin Co., Ltd. Ethics Committee and was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Questionnaires

A visual analog scale (VAS) and the Profile of Mood States (POMS) were used to collect data in this study (13,14). The VAS included six questions that related to drowsiness, current mood, feelings of fatigue, concentration, vigor, and willingness to work. For each question, the subjects were instructed to mark a spot on a 100-mm, horizontal, straight line that corresponded to their feelings upon awakening (left end/0=best, right end/100=worst). The distance of the mark from the left end of each line was measured and used as the VAS score, with a low score indicating a good mood.

The POMS test (brief version) is composed of 30 questions, each with a five-point rating scale focusing on the current mood state. The current mood states are classified into tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment subscales. Except for the vigor-activity factor, a lower POMS score indicates a better mood state.

Questionnaires were used to calculate the daily liquid caffeine dose of each subject. Subjects reported how many cups of coffee, tea, or green tea were consumed per day. The caffeine dose was calculated using the following data: 85 mg caffeine per cup of percolated coffee (15), 30 mg caffeine per cup of brewed leaf tea (16), and 30 mg caffeine per cup of green tea (17).

Experimental design

All subjects participated in all three experiments. In each experiment participants were given a single dose of the placebo or the test supplement. To ensure that the subjects and investigators remained unaware of the treatment assignments, the order of supplements was randomly determined for each subject and kept confidential by an independent party until the data analysis had been completed. To prevent the previous test sample from affecting the results, each experiment was conducted at least two days after the previous one.

The placebo capsules contained microcrystalline cellulose (Asahi Kasei Chemicals, Tokyo, Japan). The caffeine capsules contained 100 mg caffeine (Shiratori seiyaku, Narashino, Japan). The caffeine plus ornithine capsules contained 100 mg caffeine and 250 mg ornithine hydrochloride (Kyowa Hakko Bio, Tokyo, Japan), which corresponds to 200 mg ornithine. The weight of each capsule was adjusted to be uniform by adding crystalline cellulose and all capsules were identical in appearance.

Subjects were allowed free access to water, but were instructed to abstain from food after the last meal on the day before the experiment until noon on the day of the experiment. Caffeine consumption was prohibited from the night before the experiment until the termination of the experiment. A schematic representation of the study timeline is shown in Fig. 1. On the day of the experiment, each subject reported to the lab by 09:00, where they were asked to rest for 30 min or more. Saliva samples were collected at 09:30. Then, each subject completed the VAS and POMS questionnaires and ingested the test sample. Thirty minutes later, at 10:00, each sub-

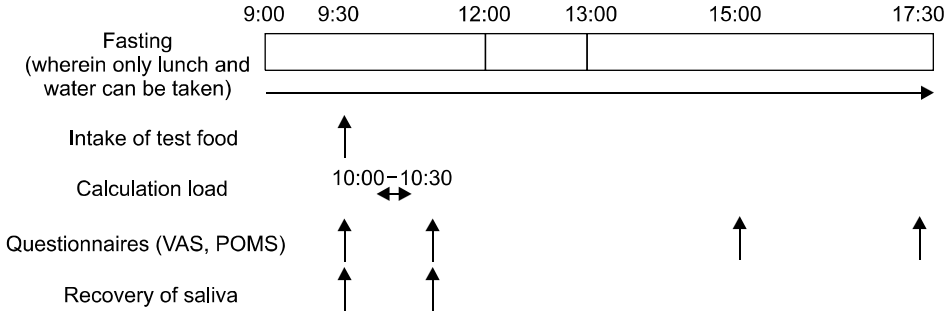

Fig. 1. A timeline of the supplement (i.e., capsule) intake, calculation test administration, and data collection carried out in the present study. Fasting began after the dinner on the night before the experiment. Subjects were provided *ad libitum* access to water and consumed a standardized lunch at 12:00.

ject took a simple, 30-minute calculation test (Uchida-Kraepelin Psychodiagnostic Test; UKT) (18). The purpose of this test was to impose a mental workload and generate a cognitive effort. After completion of the UKT (i.e., at 10:30), saliva was collected from each subject, and subjects completed the VAS and POMS questionnaires again. At 12:00, the subjects were given a standardized meal. The subjects completed the VAS and POMS questionnaires again at 15:00 and 17:30.

Saliva collection and measurement

The saliva samples were collected and stored, and the salivary cortisol, secretory immunoglobulin A (s-IgA), and α-amylase contents were measured as described by Kokubo et al. (10).

Statistical analysis

The data are expressed as mean±SE. Differences among the three groups were evaluated by analysis of variance (one-way ANOVA) followed by Tukey's test. Statistical analyses were performed using PASW Statistics (Version 18.0.0., IBM, Chicago, IL, USA). A P value of <0.05 was considered statistically significant.

RESULTS

Caffeine intake

The caffeine consumption questionnaires revealed that the mean daily caffeine consumption for the 19 subjects (14 males and 5 females) included in this study was 205.7±34.9 mg. Eighteen subjects reported consuming caffeine every day.

Questionnaires

Table 1 shows the VAS score changes. In the placebo group, all of the VAS scores except for the "drowsiness" score deteriorated after completing the calculation task (i.e., the UKT). Consumption of caffeine and the combination of caffeine with ornithine improved VAS scores. The degree of improvement was greater after consumption of caffeine with ornithine than it was for caffeine alone. However, the only significant between-groups difference was noted in the "concentration" category; the mean change of the VAS scores of the caffeine plus ornithine group was significantly more improved than the mean change of the VAS scores of the placebo group at 10:30.

Compared with the placebo group, consuming caffeine plus ornithine significantly improved the VAS scores for "drowsiness", "current mood", "willingness to work", and "concentration" in the afternoon (i.e., at 15:00 and 17:30), with the exception of "willingness to work" at 15:00. Compared with the consumption of caffeine alone, the combination of caffeine with ornithine significantly improved the scores for "feelings of fatigue" at 17:30, "willingness to work" at 15:00 and 17:30, and "vigor" at 15:00 (Table 1).

Table 1. Mean change (from the value measured at 09:30) in VAS scores at three time points

	10:30	15:00	17:30
Drowsiness			
Placebo	−33.0±36.2	101.8±36.6	63.5±50.4
Caffeine	−55.4±24.0	30.8±43.6	−16.5±31.7
Caffeine+Ornithine	−76.6±48.0	−66.1±54.7*	−86.8±48.3**
Current mood			
Placebo	68.8±35.4	86.8±32.1	79.9±33.0
Caffeine	8.4±30.0	19.4±29.9	9.7±34.0
Caffeine+Ornithine	−19.9±37.8	−39.7±32.2**	−74.5±29.7**
Feeling of fatigue			
Placebo	80.7±27.8	34.5±28.3	60.1±32.5
Caffeine	80.1±34.4	37.7±24.0	80.5±21.6
Caffeine+Ornithine	53.3±36.3	−25.3±27.0	−30.8±35.8#
Concentration			
Placebo	110.8±39.4	66.1±35.2	94.2±42.2
Caffeine	25.2±38.5	32.4±31.6	45.1±27.4
Caffeine+Ornithine	−23.5±53.0*	−61.3±31.6**	−51.2±31.8**
Willingness to work			
Placebo	89.0±39.9	54.1±30.6	69.3±34.1
Caffeine	72.8±31.8	73.0±31.0	74.9±26.5
Caffeine+Ornithine	6.5±37.4	−21.0±23.1#	−22.6±29.7#,*
Vigor			
Placebo	66.6±41.3	38.4±36.5	50.8±34.4
Caffeine	27.6±37.2	61.9±32.2	54.3±33.3
Caffeine+Ornithine	−24.6±39.1	−36.8±24.0#	−32.2±27.7

Values are mean±SE (n=19).
A lower score means improved.
Tukey's test indicates that the value is significantly different (*$P<0.05$, **$P<0.01$) from that of the placebo group.
Tukey's test indicates that the value is significantly different (#$P<0.05$) from that of the caffeine group.

Table 2. Mean change (from the value measured at 09:30) in POMS scores at three time points

	10:30	15:00	17:30
Tension-Anxiety			
Placebo	1.2±1.1	−1.6±1.2	−2.2±1.5
Caffeine	2.2±1.0	0.2±0.7	−0.4±1.0
Caffeine+Ornithine	0.3±1.6	−1.3±1.0	−1.4±1.1
Depression-Dejection			
Placebo	−0.8±0.7	−2.2±1.0	−2.3±1.3
Caffeine	−0.3±0.7	−1.0±0.5	−1.6±0.6
Caffeine+Ornithine	−1.1±0.7	−0.9±0.4	−1.2±0.4
Anger-Hostility			
Placebo	0.1±0.4	−2.2±0.9	−1.8±1.1
Caffeine	−0.5±0.4	−1.2±0.6	−2.1±0.8
Caffeine+Ornithine	−1.2±1.1	−1.3±0.7	−1.6±0.6
Vigor-Activity			
Placebo	−4.1±1.7	−5.1±1.6	−6.4±1.6
Caffeine	−3.9±1.7	−4.3±1.5	−4.7±1.6
Caffeine+Ornithine	−0.8±1.7	0.1±1.4*	−1.7±1.4*
Fatigue-Inertia			
Placebo	5.0±1.9	2.7±1.7	3.6±2.6
Caffeine	5.5±1.9	1.2±1.4	1.9±1.6
Caffeine+Ornithine	3.8±2.4	−0.7±1.5	0.4±2.0
Confusion-Bewilderment			
Placebo	2.6±1.4	1.1±1.7	2.2±2.0
Caffeine	1.3±1.0	0.6±1.3	0.6±1.3
Caffeine+Ornithine	−0.1±1.9	−0.6±1.6	−3.2±1.7**

Values are mean±SE (n=19).
A lower score means improved, except for vigor.
Tukey's test indicates that the value is significantly different (*$P<0.05$, **$P<0.01$) from that of the placebo group.

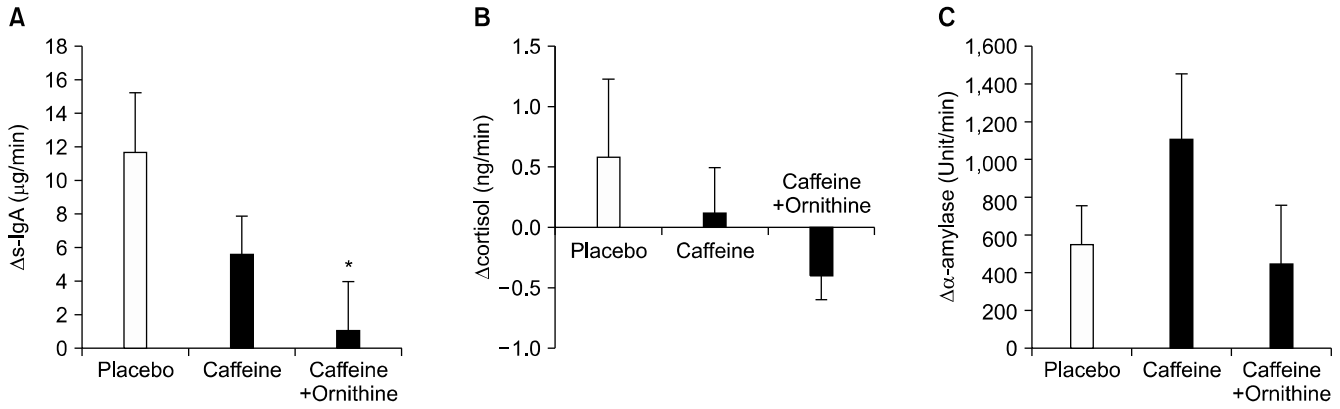

Fig. 2. Changes in salivary contents of (A) secretory IgA (s-IgA), (B) cortisol, and (C) α-amylase in saliva following ingestion of placebo, 100 mg caffeine, and 100 mg caffeine plus 200 mg ornithine. *$P<0.05$ (vs. control, Tukey's test).

Table 2 shows the changes in POMS scores. The combination of ornithine with caffeine significantly improved the scores for "vigor" at 15:00 and 17:30 and "confusion" at 17:30 compared with the placebo. There were no significant differences between the POMS scores of the caffeine and the caffeine plus ornithine groups.

Changes in salivary stress markers

Assay kits were used to determine the cortisol, s-IgA, and α-amylase content of the saliva samples. The calculation test-induced increase in the s-IgA concentration was suppressed more strongly in the caffeine with ornithine group than in the other groups (Fig. 2A). In addition, there was a significant difference between the s-IgA concentration of the placebo group and the caffeine with ornithine group. There were no differences in salivary cortisol and salivary α-amylase among the three treatment groups (Fig. 2B, 2C).

DISCUSSION

We expected that the combination of caffeine with ornithine would have some physiological effects in humans. It is well known that caffeine has a physiological action that stimulates the central nervous system to enhance self-reported alertness and mood. However, this effect was not thought to persist for extended periods of time.

Kaplan et al. (19) reported that the subjective effects of caffeine peaked during the first four hours of ingestion. In the present study, we examined the effect of caffeine with ornithine on the mood and mental state of healthy office workers for 8 hours after test sample intake. We found that consuming caffeine with ornithine in the morning had a positive effect on self-reported mood (especially "feelings of fatigue", "willingness to work", and "vigor") in the late afternoon (at 17:30). Although previous reports indicate that ornithine improves feelings of fatigue in the morning after alcohol consumption (10), ornithine alone has not been shown to have an effect on "vigor" immediately or several hours after its intake. Hence, we speculate that ornithine potentiated the physiological action of caffeine, although the effect of ornithine alone needs to be confirmed in a future study.

Stressful events are known to affect various biological responses in the nervous system, the HPA axis, and the immune system. We measured three typical stress markers, salivary α-amylase, salivary cortisol, and s-IgA, which are reported to increase under acute stress conditions through activation of the nervous system, the HPA axis, and the immune system, respectively (20-22). Hibino et al. (23) demonstrated that caffeine enhances the modulation of parasympathetic nerve activity and increases vagal autonomic nerve activity. The caffeine-induced enhancement of parasympathetic nerve activity is expected to reduce this stress reaction. In addition, previous reports have indicated that ornithine improves the subjective feelings of fatigue experienced by Asian-flushers the morning after alcohol consumption by reducing the amount of salivary cortisol (10). Taking ornithine for 8 weeks has also been shown to reduce serum cortisol in office workers (9). These findings suggest that ornithine ameliorates the activity of the HPA axis to reduce the stress reaction. Therefore, we hypothesized that caffeine and ornithine may act synergistically to reduce stress.

As expected, a calculation test-induced increase in the amount of s-IgA was suppressed more in the subjects that consumed a combination of caffeine with ornithine than in the other groups, corresponding to the improvement of subjective feeling shown in the POMS and VAS scores. Although there were no significant differences in salivary cortisol and α-amylase levels between the three groups, the combination of caffeine and ornithine tended to reduce cortisol levels. Many office workers feel some stress at the office, and the level of cortisol on working days is higher than the level of cortisol on non-working days (11). There is a possibility that the stress-reducing effect of ornithine combined with caffeine could positively affect people's mental state and mood.

Dopamine is known to have close relationship with mood improvement (e.g., increased feelings of energy and alertness). Caffeine has been shown to enhance dopaminergic activity, presumably by acting as a competitive antagonist of the adenosine receptors that are co-localized and functionally interact with dopamine receptors (24). Moreover, it has been revealed that prolonged treatment with caffeine reduces liver arginase activity in rats (25). L-arginine is a substrate of arginase and nitric oxide (NO) synthase, so inhibiting arginase could lead to an increase in NO generation by NO synthase. Some pharmacological studies have demonstrated the possible involvement of NO in the dopamine release process in the rat striatum (26,27).

Ornithine is converted to L-arginine in the urea cycle. Therefore, the L-arginine level of the combination group could be higher than that of the group treated with caffeine alone. Thus, we speculate that increased dopamine levels may be responsible for the subjective mood improvement reflected in the VAS and POMS scores of subjects in the caffeine with ornithine group.

Two questionnaires, VAS and POMS, were used in the present study. However, only the VAS score was significantly improved by the combination of caffeine and ornithine compared with caffeine alone. We speculate that this result is due to the fact that the measurement system of the VAS allows subjects to easily indicate their change in mood. Our results strongly suggest that there was a unique interaction between the caffeine and ornithine consumed by healthy office workers, and that ornithine may potentiate the effect of caffeine. Future studies are needed to identify the specific mechanisms of action that underline the unique relationship between caffeine and ornithine. These studies will be important for providing guidance on the interactions of supplements with caffeine when preparing foods and beverages to help prevent excessive stress in office workers. We hope the present study will be the basis for future studies.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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