

Effects of Essence of Chicken on Cognitive Brain Function: A Near-Infrared Spectroscopy Study

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The aim of this study was to investigate the effects of the essence of chicken on brain function by near-infrared spectroscopy. Twelve healthy elderly subjects took the essence of chicken or a placebo for 7 d in a double-blind cross-over design study. Changes in oxy-hemoglobin concentrations in the bilateral prefrontal areas of the brain were measured while the subjects performed the simple reaction task, the Groton Maze Learning Test, and the working memory task. In the latter case, there were significant interactions in the changes in oxy-hemoglobin concentrations between treatment and period of intake according to two-way repeated ANOVA. The changes in oxy-hemoglobin concentrations significantly increased in several regions of the prefrontal areas of the brain in those taking essence of chicken for 7 d. These results suggest that essence of chicken is useful as a nutritional supplement to enhance or maintain brain function in the elderly.

Key words: essence of chicken; near-infrared spectroscopy (NIRS); oxy-Hb concentration; working memory task

Essence of chicken (EOC), a chicken meat-extract, is a popular beverage in Southeast Asia, and is consumed as a traditional remedy for several ailments. The elderly use it as a nutritional supplement, and students use it to ease the anxiety associated with studying for examinations. EOC is produced by subjecting chicken meat to a water extraction process for several hours under high-temperature and high-pressure conditions. After the fat is removed, the EOC is concentrated and bottled. The solid content consists mainly of proteins, peptides, and amino acids. Previous studies on humans have demonstrated that EOC increases the thermic response,¹⁾ stimulates hematopoiesis,²⁾ affects the protein compositions of colostrums in lactating women,³⁾ and enhances the elimination of post-exercise plasma lactate and ammonia.⁴⁾ Patients with anxiety disorder diagnosed according to DSM-IV criteria⁵⁾ experienced significant improvements not only in their anxiety levels but also in their systolic blood pressures and pulse rates when given EOC in combination with psychotherapy.⁶⁾ Moreover, anxiety in stressed normal students was reduced after

taking EOC every day for 2 weeks.⁷⁾ Recently, Azhar *et al.* found that EOC had positive effects on cognitive function in distressed medical students.⁸⁾ Daily consumption of EOC for 2 weeks significantly improved attention and working memory as assessed by a combination of psychological tests, Digit Span, Arithmetic, and Letter-Number Sequencing. However, it was not determined physiologically whether these effects of EOC were due to improvements in brain function.

Of the functional neuroimaging techniques based on cerebral hemodynamic levels associated with brain activities, near-infrared spectroscopy (NIRS) was chosen to evaluate the effects of EOC on brain function. NIRS is a noninvasive, stress-free method that, unlike positron emission tomography (PET), is not influenced by slight body movements.^{9–11)} The NIRS technique is sensitive to hemoglobin changes in the cerebral cortex, because near-infrared light can penetrate the scalp and skull and reach to a depth of 5–10 mm into the brain tissue, and it is absorbed by oxy-hemoglobin (oxy-Hb) and deoxy-hemoglobin (deoxy-Hb), which have different absorption spectra.¹²⁾ It can measure regional cerebral blood volume changes in terms of changes in oxy-Hb and deoxy-Hb concentrations in a subject performing a cognitive task. Thus it is suitable for noninvasive assessment of the neurophysiological changes induced by taking food or beverages, especially in elderly subjects.

Several studies have indicated that cerebral oxy-Hb and total-hemoglobin (total-Hb) concentrations increased in healthy subjects performing cognitive and working memory tasks.^{13–16)} Also, NIRS studies indicate that hemoglobin oxygenation in response to brain activation is influenced by physiological aging.^{17–19)} Elderly subjects showed significantly lower increases in oxy-Hb and total-Hb concentrations in the frontal cortex than young subjects during a calculation task.¹⁷⁾ The elderly subjects exhibited deactivation in the frontal lobe during cognitive tasks.¹⁸⁾

In the study reported here, to determine the effects of EOC on brain function in normal elderly subjects, we examined changes in oxy-Hb concentrations by NIRS while the subjects performed three tasks: the simple reaction task, the Groton Maze Learning Task (GMLT),

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Abbreviations: BDI, Beck Depression Inventory; BMI, body mass index; CgA, chromogranin A; deoxy-Hb, deoxy-hemoglobin; DLPFC, dorsolateral prefrontal cortex; EOC, essence of chicken; GMLT, Groton Maze Learning Task; NIRS, near-infrared spectroscopy; oxy-Hb, oxy-hemoglobin; PET, positron emission tomography; total-Hb, total-hemoglobin

and the working memory task (2-back paradigm). In addition, a psychological test and measurement of salivary chromogranin A (CgA) were carried out to evaluate the effects of EOC as to stress reduction.

Materials and Methods

General. The present study was carried out in compliance with the Helsinki Declaration. All protocols were accepted by the Ethics Committee of Kyorin University School of Medicine. All subjects provided written informed consent before enrollment in the study.

Subjects. Twelve healthy right-handed paid volunteers of ages ranging from 60 to 68 years (six males and six females, mean age 62.3 ± 2.5 years) participated in the study (Table 1). No subjects had previously taken EOC. All the subjects were restricted from taking soups made from chicken bouillon cubes at all times during the test period, because they might have contained substances that would affect the experimental results.

Study products. EOC and placebo beverages were kindly provided by Cerebos Pacific Limited (Singapore). One bottle of EOC (70 mL) contained protein and peptide (83 mg/mL), free amino acid (3.1 mg/mL), hexose (0.8 mg/mL), fat (0.4 mg/mL), and caramel (3 mg/mL).³⁾ EOC also contained β -alanyl-histidine (carnosine) and β -alanyl-methylhistidine (anserine) as active di-peptides.^{20,21)} One bottle of placebo (70 mL) contained milk casein (83 mg/mL), caramel (3 mg/mL), and flavoring such as to yield proteins, calories, and color similar to EOC.

Experimental design. A randomized, double-blind, placebo-controlled, cross-over study was conducted. The volunteers were divided randomly into two groups, EOC treated and placebo treated. The subjects took two bottles of either EOC or placebo per day for 7 d, one bottle immediately after breakfast and dinner. After a 7-d wash-out period, each volunteer took the opposite beverages for one further week.

Before and after taking the beverages for 7 d, the changes in oxy-Hb concentrations in the bilateral prefrontal areas of the brain were measured by NIRS during the performances of three tasks.

NIRS. NIRS measurements were done using the ETG-4000 system (Hitachi Medical, Tokyo). Emitter and detector probes set in fixed holders were located at the bilateral prefrontal areas of the subject's head. The front row and inner optodes were positioned 0.5 cm outside of Fp₁ and Fp₂ placement according to the International EEG 10–20 system. The distance between the pair was set at 3.0 cm, and each measurement area between the pair was designated a channel (Fig. 1). The concentrations of oxy-Hb and deoxy-Hb were calculated from the absorption of near-infrared light at wavelengths of 695 and 830 nm. Mean changes in hemoglobin concentrations were calculated against the mean concentration during a 10-s period for the prestimulus baseline.

Simple reaction task. Numbers from 1 to 9 appeared randomly on a computer screen placed 150 cm in front of the subject's eyes for 500 ms at an interstimulus interval of 2,500 ms. The subject was instructed to push the button using his or her right thumb whenever a number appeared. One hundred and fifty numbers were presented. The task was performed over 375 s.

Groton Maze Learning Test. The GMLT is a hidden maze task designed by Milner²²⁾ and developed by Snyder.²³⁾ It is recognized as a human analog of the Morris Water Maze Test.²⁴⁾ The subjects were shown a 10×10 grid of tiles on a computer touch screen. A 28-step pathway was hidden among the 100 possible locations. The start was indicated by a blue tile at the top left and the finish by a tile with red circles at the bottom right of the grid. Subjects were instructed to move one step from the start and then to continue, one tile at a time, toward the end. They repeated the same task 5 times in total. Speed and accuracy were required, and the total time and number of errors were calculated.

Table 1. Characteristics of the Subjects

| | | Total (n = 12) |
|-------------|----------------------|-----------------|
| Age | (years) | 62.3 ± 2.5 |
| Height | (cm) | 159.2 ± 8.6 |
| Body weight | (kg) | 55.8 ± 9.3 |
| BMI | (kg/m ²) | 22.0 ± 3.0 |

Values are means \pm SD. BMI, body mass index.

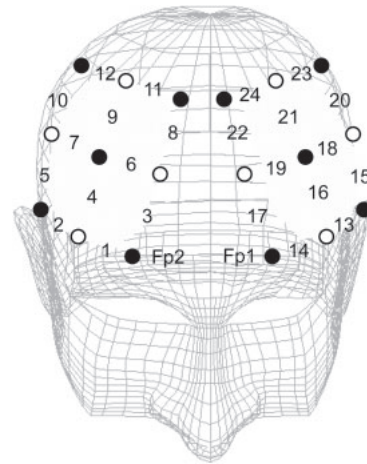


Fig. 1. Location of Near-Infrared Spectroscopy Optodes and Channel Positions.

Changes in hemoglobin concentrations were measured at 24 sites in bilateral anterior areas of the brain. Solid circles and hollow circles indicate emitter and detector probes respectively. Numbers designate the locations of measured channels.

Working memory task (2-back paradigm). Numbers from 1 to 9 appeared randomly on a computer screen for 500 ms at an interstimulus interval of 2,500 ms. The subject was instructed to push the button using his or her right thumb when shown the number 3 only when the 2-back number was even, not when it was odd. One hundred and fifty numbers were presented in total, and the target 3 was presented 10% of the time. The task was performed for 375 s. The average reaction time was calculated.

The tasks were conducted consecutively in the order simple reaction task, GMLT, and working memory task. Subjects had a rest of 5 min after each experimental block.

Beck Depression Inventory. The Beck Depression Inventory (BDI) is a 21-question multiple-choice self-report inventory, one of the most widely used instruments for measuring the severity of depression.²⁵⁾ A score of more than 21 out of 63 points is considered to indicate depression.

Chromogranin A. The salivary CgA concentration was chosen as an index of a subject's stress level.²⁶⁾ Before, immediately after, and 10 min after performance of the tasks, the saliva of each subject was collected and the concentration of CgA was measured by Prevention International (Tokyo).

Statistical analysis. All statistical analyses were performed using SPSS statistical computer software version 16.0. Changes in oxy-Hb concentrations were analyzed by two-way repeated ANOVA (treatment \times period of intake). In the case of significant interaction, the *post hoc* paired *t*-test was used for further analysis. The BDI score was analyzed by Friedman one-way ANOVA and the *post hoc* Wilcoxon signed-rank test.

Results

With regard to the simple reaction task, no interactions were found between treatment and period of

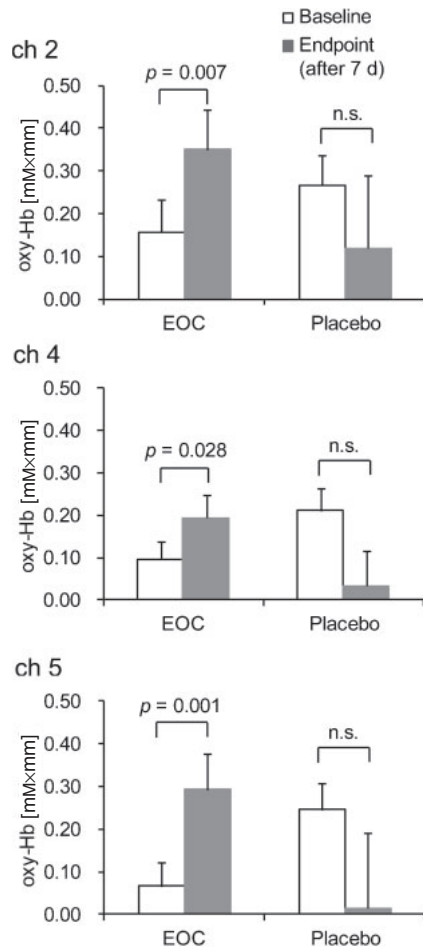


Fig. 2. Changes in Oxy-Hemoglobin (oxy-Hb) Concentrations during the Working Memory Task (means \pm SEM) at Baseline and after 7 d of Twice-Daily Intake of Essence of Chicken (EOC) and Placebo.

intake as to changes in the oxy-Hb concentrations in any channel. In the case of the GMLT, no interactions were found between treatment and period of intake as to changes in the oxy-Hb concentrations in any channel. In contrast, in the case of the working memory task, significant interactions were found between treatment and period of intake as to changes in the oxy-Hb concentrations in several channels (ch2: $p = 0.038$, ch4: $p = 0.003$, ch5: $p = 0.010$). In the EOC treated subjects, the changes in the oxy-Hb concentrations at the endpoint significantly increased as compared with the baseline (Figs. 2 and 3).

With respect to the performance of the GMLT, no significant differences were found between baseline and endpoint as to total time or number of errors under the EOC and placebo conditions. With regard to the working memory task, there were no significant differences between baseline and endpoint as to the reaction time under the two conditions.

BDI scores decreased under both treatments at 7 d. The score of every subject was less than 15 before and after the period of intake. Significant differences were found in the scores between baseline and endpoint while the subjects took EOC ($p = 0.028$, by Wilcoxon signed-rank test) (Fig. 4).

There were no significant differences in the concentrations of salivary CgA between the EOC and the placebo condition at any point of collection.

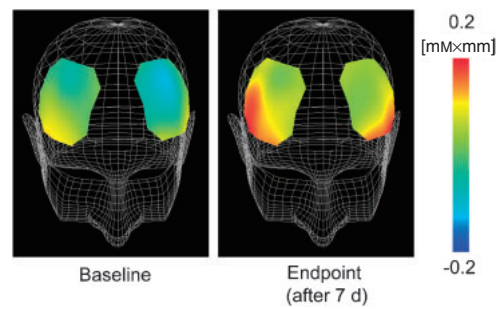


Fig. 3. Comparison of Changes in Oxy-Hemoglobin (oxy-Hb) Concentrations at 30.0 s during the Working Memory Task ($n = 12$) at Baseline and after 7 d of Twice-Daily Intake of Essence of Chicken (EOC).

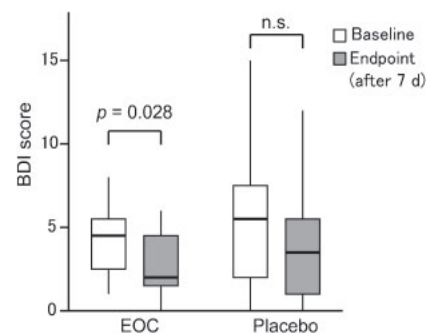


Fig. 4. Changes in the Beck Depression Inventory (BDI) Score at Baseline and after 7 d of Twice-Daily Intake of Essence of Chicken (EOC) and Placebo.

Discussion

We examined the effects of EOC on changes in the oxy-Hb concentrations of the bilateral anterior areas of the cerebral cortex in healthy elderly subjects during the simple reaction task, the GMLT, and the working memory task by NIRS. The results indicate that changes in the oxy-Hb concentrations in the prefrontal areas during the working memory task increased significantly after the subjects took EOC for 7 d. In contrast, no significant increase was observed after they took the placebo. With regard to the other tasks, no significant differences were found between baseline and endpoint as to the changes in the oxy-Hb concentrations in those areas under either treatment. The absence of significant differences in the simple reaction tasks might have been due to the fact that these tasks were easy and demanded little mobilization of working memory.

Working memory involves subsystems that are thought to be located in specific areas of the brain. Neuroimaging studies have revealed that the dorsolateral prefrontal cortex (DLPFC) plays a significant role in the performance of a working memory task.^{27,28} In our study, as shown in Fig. 1, NIRS optodes were placed mainly at the bilateral DLPFC. In other words, greater numbers of optodes were placed so as to measure changes in the oxy-Hb concentrations induced in those areas in the performance of the working memory task. The 2-back paradigm used in this study is considered to demand a great deal of mobilization of working memory. Hence, the results for this task suggest that EOC increases the activity of the DLPFC when the task demands much mobilization of working memory.

Reaction time reflects a variety of processes, including cognition, judgment, and motor control. As shown by the results for changes in the oxy-Hb concentrations on the working memory task, EOC activates the cognitive process, but it might not influence the whole processes, including judgment and motor control, because reaction times did not change on taking EOC.

With regard to the GMLT, DLPFC activation was not observed under the EOC condition. GMLT reflects the ability of spatial memory, which is relevant to the functioning of the parietal areas of the brain. Since NIRS of our system did not measure changes in oxy-Hb concentrations in those areas, studies should be done to investigate the effects of EOC on spatial memory by measuring changes in oxy-Hb concentrations in broader areas of the brain.

Since EOC consists of many ingredients, it is difficult to identify the active ingredients that increase oxy-Hb concentrations during the performance of the working memory task. EOC consists mainly of proteins, peptides, and free amino acids.²¹⁾ Among the peptides, carnosine, and anserine are present at relatively high concentrations in the human brain,²⁹⁾ are conjugated to contribute to protection of neuronal cells,³⁰⁾ and have antioxidant activity.^{29,31)} With regard to memory function, carnosine has the effect of elevating the long-term potentiation of the dentate gyrus in rats,³²⁾ but the effect on the frontal cortex has not been investigated. It has also been reported that carnosine improves the performance on the Morris Water Maze Test by aged rats,³³⁾ but no human study of the effect of carnosine on memory function has yet been conducted. Our results indicate that carnosine is one of the active ingredients that increase the concentration of oxy-Hb. Moreover, an antidepressant-like effect of carnosine has been reported.³⁴⁾ Hence, our results for the BDI may be related to that effect of carnosine. Further investigation should be conducted to identify the active ingredients, because it is possible that ingredients of EOC not yet identified play significant roles in activating brain function.

The level of salivary CgA provides a sensitive and reliable index for evaluating acute stress.^{35,36)} Our results for salivary CgA analysis indicate that EOC does not reduce acute stress levels in aged human subjects.

Our results suggest that supplemental EOC is useful for healthy elderly subjects to activate working memory, but our findings should be taken as preliminary. This study had several limitations, including small sample size and the fact that we did not identify the active ingredients of EOC. Moreover, we measured only changes in oxy-Hb concentrations in restricted areas of the brain. Further studies should be done to confirm the effects of EOC using the identified active ingredients.

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