Changes in Physiological Indicators due to Perilla Oil Intake in Japanese People

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ABSTRACT

Objective: **The oral intake of alpha linolenic acid (ALA) has been reported to be effective in preventing vascular disease and improving blood fat status. However, the effects of ALA-rich perilla oil consumption on individuals physiological indicators have not been appropriately investigated. This study therefore investigates the effects of the oral intake of perilla oil on physiological indicators.**

Design: **A clinical study.**

Materials and Methods: **38 participants were recruited from Fukutomi-cho, Higashi-hiroshima City, Hiroshima Prefecture, Japan. The participants each took 5 ml of perilla oil daily for eight months. We calculated the mean values of the physiological index before and after the intake period and applied a paired-sample *t*-test.**

Results: **There was no change in body weight, body fat, blood lipid, or blood pressure. However, in addition to changes in the indicators of arteriosclerosis and anemia associated with aging, we observed a significant decrease—approximately 20%—in two kinds of blood oxidation markers and hormones related to bone metabolism. Participants scores on the self-rating depression scale also decreased significantly.**

Conclusion: **The study provides evidence that daily intake of perilla oil may be useful in alleviating the effects of aging and depression.**

KEY WORDS

alpha linolenic acid, perilla oil, aging

INTRODUCTION

According to the 2017 demographic statistics of Japan, the main causes of death in Japan are malignant neoplasms, heart disease (excluding hypertension), and cerebrovascular disease, with these diseases accounting for 51.4% of all deaths[1]. These are all referred to as lifestyle-related diseases; hypertension and dyslipidemia—which are risk factors for these diseases—are also considered to be lifestyle-related diseases[2]. From the above, it is clear that lifestyle-related disease is an important health issue for adults.

Alpha linolenic acid (ALA) is an essential fatty acid that is found abundantly in oils such as linseed oil and perilla oil[3]. The oral intake of ALA has been reported to potentially reduce blood pressure[4], suppress arteriosclerosis[5], lower blood lipids[6], and improve cardiovascular disease[7]. It is further said to decrease the possibility of stroke[8], alleviate allergies[9] and depression[10], improve bone density[11], and improve cognitive function[12]. Although the efficiency is very low, it has been reported that ALA is converted to docosahexaenoic acid or eicosapentaenoic acid in the body[13], which are also effective in maintaining cardiovascular health[14].

The above research considered chemically-adjusted ALA or linseed oil as a food. Standard Japanese perilla oil contains 58.3 g/100 g of ALA, compared with 56.7 g/100 g of ALA in linseed oil[3]. However, little has been reported about the effects of ingesting perilla oil. Based on these facts, we investigated whether the daily oral intake of perilla oil would have a similar effect as the oil intake studied in previous reports.

MATERIALS AND METHODS

Participants

Prior to starting the experiment, we provided a pre-briefing at a perilla plant (*Perilla frutescens*) cultivation area in Fukutomi-cho, Higashi-hiroshima City, Hiroshima Prefecture, Japan. We explained the purpose of the study, the methods, expected risks, and data handling, after which we obtained written informed consent from volunteers. Originally, 80 volunteers provided consent. However, the final study sample contained 38 individuals, please refer to the "methods" subsection for more information concerning the large dropout margin. The final study group for analysis consisted of 14 males and 24 females (n = 38) aged from 45 to 82 (mean age: 68.6; SD ± 10.6) who were not taking any medications, had no medical history of serious illness, and who were judged to be suitable candidates for the experiment by the responsible doctor. Three hypertensive volunteers on medication were excluded.

Ethics statement

This research was conducted in accordance with the Ministry of Health, Labour, and Welfare of Japan's "Ethical Guidelines for Epidemiological Research." The research plan was implemented with the approval of the Prefectural Hiroshima University Research Ethics

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Table 1: Physical characteristics and questionnaires, blood tests, and urinalysis results

	First round mean (± SD)	Second round mean (± SD)	*p*-values between 2017 and 2018	Change rate (Δ% mean (± SE)
Physical measurement				
Body weight (kg)	52.9 (± 7.6)	52.5 (± 7.3)	0.230	-0.56 (± 0.54)
Left and right brachial systolic blood pressure average (mmHg)	127.9 (± 17.8)	129.5 (± 19.1)	0.500	1.6 (± 1.8)
Left and right brachial diastolic blood pressure average (mmHg)	72.6 (± 10.8)	73.0 (± 10.7)	0.731	1.0 (± 1.6)
Right Pulse wave velocity (cm/sec)	1625.1 (± 336.1)	1710.7 (± 358.8)	0.005	5.74 (± 1.72)
Left Pulse wave velocity (cm/sec)	1662.4 (± 346.7)	1741.2 (± 367.9)	0.022	5.54 (± 2.19)
Right ankle brachial pressure index (*100)	110.8 (± 7.5)	110.7 (± 7.8)	0.948	0.11 (± 1.05)
Left ankle brachial pressure index (*100)	110.5 (± 6.3)	110.3 (± 7.7)	0.895	-0.05 (± 0.90)
Visceral fat area (cm^2)	503.2 (± 260.3)	464.8 (± 209.0)	0.122	7.92 (± 9.44)
Self-administered questionnaires				
Self-rating depression scale (point)	36.8 (± 6.5)	32.8 (± 6.4)	0.002	-9.89 (± 2.98)
Hasegawa's dementia scale (point)	28.5 (± 1.9)	29.3 (± 1.7)	0.010	3.27 (± 1.23)
Blood test				
White blood cell (/μL)	5960.5 (± 1740.4)	6394.6 (± 1633.2)	0.008	9.72 (± 2.70)
Red blood cell (/μL)	428.5 (± 33.4)	438.3 (± 40.9)	0.022	2.24 (± 0.95)
Hemoglobin (g/dL)	13.4 (± 1.1)	13.5 (± 1.2)	0.476	0.78 (± 0.97)
Hematocrit (%)	39.7 (± 3.0)	40.7 (± 3.6)	0.013	2.69 (± 1.01)
Platelet (*10□/μL)	21.7 (± 7.2)	22.8 (± 7.0)	0.100	5.21 (± 2.19)
Mean corpuscular volume (fL)	92.6 (± 3.8)	90.7 (± 14.3)	0.427	-1.96 (± 2.50)
Mean corpuscular hemoglobin (pg)	31.2 (± 1.7)	30.8 (± 1.9)	0.001	-1.39 (± 0.41)
Mean corpuscular hemoglobin concentration (%)	33.7 (± 1.0)	33.1 (± 1.2)	< 0.001	-1.79 (± 0.38)
Hemoglobin A1c (%)	6.1 (± 1.8)	6.0 (± 1.6)	0.236	-0.57 (± 0.60)
Neutrophil (%)	59.1 (± 8.8)	60.9 (± 9.4)	0.102	4.59 (± 2.40)
Total protein (g/dL)	6.86 (± 0.3)	6.89 (± 0.4)	0.732	0.32 (± 0.82)
Albumin (g/dL)	4.06 (± 0.3)	4.14 (± 0.4)	0.020	2.23 (± 0.88)
Total cholesterol (mg/dL)	190.3 (± 31.0)	192.5 (± 30.0)	0.582	1.25 (± 1.46)
Neutral Fat (mg/dL)	114.3 (± 56.0)	124.5 (± 66.6)	0.301	17.14 (± 8.33)
High density lipoprotein cholesterol (mg/dL)	63.2 (± 14.7)	65.0 (± 14.7)	0.326	2.20 (± 1.71)
Low density lipoprotein cholesterol (mg/dL)	106.1 (± 30.2)	107.2 (± 28.1)	0.620	2.22 (± 2.29)
Oxidized low density lipoprotein cholesterol (mg/dL)	99.6 (± 26.8)	79.3 (± 19.6)	< 0.001	-17.71 (± 3.17)
Aspartate aminotransferase (IU/L)	22.4 (± 7.7)	23.0 (± 11.2)	0.758	1.45 (± 3.45)
Alanine aminotransferase (IU/L)	16.8 (± 6.7)	17.9 (± 10.3)	0.266	6.22 (± 4.31)
Alkaline phosphatase (IU/L)	229.7 (± 81.2)	231.4 (± 80.5)	0.901	1.97 (± 2.94)
γ-glutamyl trans peptidase (U/L)	27.9 (± 31.0)	29.6 (± 39.2)	0.747	9.07 (± 5.70)
Creatine phosphokinase (IU/L)	119.9 (± 63.4)	118.0 (± 66.0)	0.320	2.42 (± 6.50)
Total bilirubin (mg/dL)	0.58 (± 0.2)	0.52 (± 0.2)	0.091	-4.22 (± 4.94)
Urea nitrogen (mg/dL)	17.1 (± 4.5)	16.9 (± 5.4)	0.696	-0.46 (± 3.62)
Creatinine (mg/dL)	0.76 (± 0.2)	0.78 (± 0.2)	0.320	2.26 (± 1.99)
Serum iron (μg/dL)	90.9 (± 28.6)	87.2 (± 25.5)	0.366	1.36 (± 5.72)
Total iron binding capacity (μg/dL)	308.0 (± 46.2)	316.2 (± 49.5)	0.163	2.89 (± 1.72)
High sensitivity parathyroid hormone (pg/mL)	422.4 (± 170.9)	374.9 (± 195.7)	< 0.001	-12.46 (± 2.91)
Pentosidine (μg/mL)	0.053 (± 0.009)	0.043 (± 0.017)	< 0.001	-19.95 (± 4.79)
Urinalysis				
Creatinine (mg/dL)	87.5 (± 74.1)	81.5 (± 59.3)	0.559	40.85 (± 22.91)
8-Hydroxydeoxyguanosine (ng/mL*cre)	0.16 (± 0.14)	0.15 (± 0.10)	0.626	34.23 (± 18.80)

Committee (Approval number: 17H2-03).

Perilla oil

To test our hypothesis, 150 g of commercial perilla seed oil (Hukutomi Egoma Abura 150 g, Fukutomibussan Shakunagekan Management Council, Hiroshima, Japan)—that was produced and bottled in the study area near the survey period—was distributed to the participants every month. Participants received a new bottle of oil once a month in exchange for an empty bottle.

Methods

As the first measurement prior to treatment, participants' height, weight, visceral fat cross-sectional area (HDS-2000, Omron Co., Ltd., Japan), upper arm and ankle pulse wave velocity (baPWV), and ankle joint brachial blood pressure ratio (ABI; Form BP-203 RPE, Omron Co., Ltd., Japan) were recorded. Moreover, the Revised Hasegawa's dementia scale[15] and self-rating depression scale (SDS)[16] were used to evaluate participants' cognitive function and depression status. Blood and urine were also collected and analyzed by a privately-owned clinical laboratory. These measurement items are shown in Table 1.

The participants were surveyed for one year, from September 2017

to August 2018. The participants who were judged to have no health problems at the first measurement were given perilla oil. The participants were not restricted in their intake time or intake method, except for the requirement of ingesting 5 ml per day of unheated perilla oil. Participants were also requested to keep a record of their daily perilla oil intake.

The perilla oil supply was interrupted from April to July of 2018, mainly due to the survey area being affected by the western Japan heavy rain disaster of July 2018. Therefore, the participants perilla oil intake period was shortened to eight months. Because of this disaster, more than half of the original 80 participants in the first measurement suspended research cooperation. Another reason for the shortened distribution period is the delay in annual administrative procedures for the year.

In September 2018, the same items as in the first measurement were examined in the remaining study population of 38 individuals.

Data analysis

From the obtained data, the mean value and standard deviation (SD) of each factor were calculated and the significant difference between the mean values of the first and second measurements tested using a paired-sample *t*-test. Items for which the result of the *t*-test show a p-value ≤ 0.01 were determined to be significant (Table 1). The ratio of the second measurements' average value was calculated when the first measurement average value was 100% and the difference between the measurements' average value was taken as the change rate (Δ%). Table 1 shows Δ% and the standard error (SE).

RESULTS

Most of the participants' self-reported dosage records reported "ingested" daily and no participants reported "not ingested" for more than three days during the eight-month intake period. Table 1 shows the average values, SEs, *p*-values, and change rate of the measurement items.

Physical measurements

Considering the participants' physical characteristics, there were no significant changes in body weight, visceral fat cross-sectional area, blood pressure, or ABI between the first and second measurements. However, right side baPWV increased by about 6%.

Self-administered questionnaires

There were no significant differences between the first and second round in the revised Hasegawa's dementia scale answers. The average SDS score was significantly lower, by approximately 10%.

Blood tests and urinalysis

Considering the blood and urine components, there were no significant differences in red blood cells, most blood lipids, enzymes, inorganic salts, or metabolites. Leukocytes increased significantly, by approximately 10%. The mean corpuscular volume was not significantly different; however, the mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were significantly reduced, by approximately 2%. The high sensitivity parathyroid hormone decreased significantly, by about 12%. Oxidized low density lipoprotein and pentosidine were observed to have decreased by about 20%, which is highly significant. There were no significant differences in urinary creatinine or 8-hydroxydeoxyguanosine.

DISCUSSION

Existing research points out that ALA may have various beneficial health effects; moreover, existing animal studies have reported that ALA-rich linseed oil and perilla oil have the potential to improve blood lipids[17,18]. As standard Japanese perilla oil contains 16 g of oleic acid, 12 g of linoleic acid, and 58 g of ALA per 100 g[3], this study aimed to determine whether the oral intake of perilla oil would exert similar effects[4-12].

Although the participants consumed the perilla oil consciously, their body weight, body fat cross-sectional area, and blood lipid-related indicators did not change significantly. As perilla oil contains 9.21 kcal/g[3] and has a specific gravity of 0.93[19], the amount of oil consumed in this study equals approximately 43 kcal per 5 ml; however, based on the results, it is thought that fat accumulation was not influenced. Because the participants diet was not restricted, it may be possible that consuming the perilla oil prevented other calorie intake.

As mentioned earlier, the study population suffered landslides and floods in July 2019 due to heavy rainfall in western Japan. The disaster polluted the study area with a sewer spill. The significant increase in leukocytes may be due to the stress associated with the disaster, as it has been shown that stress causes an increase in erythrocytes[20]. This can be attributed to a significant decrease in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration.

This study did not confirm the reduction in blood lipids reported in previous studies[6]. Moreover, no significant changes in blood pressure and ABI were observed. However, the right side baPWV increased significantly. Previous studies have shown that baPWV increases with age[21], which are contrary to other reports which state that ALA is effective for the treatment of vascular diseases[4-7]. However, both oxidized LDL and pentosidine in the blood decreased by approximately 20% and their *p*-values were very small. These elements are known as oxidative stress markers and are said to reflect the amount of active oxygen in the body[22,23]. Oxidized LDL is considered to be a cause of arteriosclerosis[24] and pentosidine is considered to promote arteriosclerosis and bone deterioration[25,26]. The fact that these were significantly reduced in the second test means that oxidative stress—which is considered to be the cause of aging[27]—was reduced by the intake of perilla oil. Urinary 8-hydroxydeoxyguanosine values, which are also known as oxidative stress markers, were not significantly different; however, this can vary according to the participant's health condition, for example, the amount of exercise the participant gets[28,29]. As stated earlier, the baPWV increased during the survey but there were no differences in blood pressure or ABI; however, it can be expected that these values will decrease when perilla oil is ingested for a longer period. The causative agent of the antioxidant action of perilla oil obtained in this survey can be considered to be ALA, but tocopherol (vitamin E) may also play a role. Standard Japanese perilla oil contains 66.2 mg per 100 g of tocopherol, of which 2.4 mg is α-tocopherol[3]. As tocopherol has been reported to have an antioxidative effect[30], it may be that ALA and tocopherol in perilla oil act in combination.

Previous research also states that ALA may maintain bone density[11,14]. Although bone density was not directly measured in this study, a significant decrease in high sensitivity parathyroid hormone supports this.

There was a significant difference in the average score of the SDS questionnaire. This result may support previous research stating that ALA has antidepressant action[10]. However, it must be noted that our study started with 80 people and more than half suspended research cooperation due to the heavy rain disaster in July 2019. This may have created bias concerning participants' mental state, assuming that participants with increased depression due to the disaster dropped out and only participants with good psychological recovery from the disaster remained. It can therefore not be said that the antidepressant effects of perilla oil have been confirmed.

CONCLUSION

This study has certain limitations. For a strictly comparative study, the results of this survey should be compared with a group ingesting another oil or a group not ingesting oil at all, under the same conditions in the same region, and with the same age composition and sex ratio. However, in this study, this approach was abandoned because of ethical and sample size restrictions. Therefore, the mean values were compared before and after the perilla oil intake period in a single participant group. Moreover, because of the interruption of the perilla oil supply, the intake was imposed only for eight months. By setting the survey period to one year, the factors of seasonal fluctuation such as lifestyle habits were excluded.

The results of this study strongly suggest that continuous intake of perilla oil reduces oxidative stress, thereby alleviating the effects of aging. It should be noted that—as recommended—the researchers provided fresh oil every month. This is because care should be taken as the intake of oxidized perilla oil or oxidized ALA increases oxidative stress in the body[17,31]. A longer-term survey that considers this aspect is necessary in the future.

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