ORIGINAL

Effect of olive oil consumption on aging in a senescence-accelerated mice-prone 8 (SAMP8) model

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Abstract : Background : Mediterranean diets have been linked to a reduced risk of cancer, vascular illnesses, Parkinson's and Alzheimer's disease. Olive oil is the primary fat source in the Mediterranean diet ; however, only a few studies have investigated the effect of olive oil on aging. In the present study, we aimed to determine whether consumption of olive oil significantly influences aging and memory in senescence-accelerated mouse-prone 8 (SAMP8). Methods : SAMP8 and senescence-accelerated mouse resistant 1 (SAMR1) mice were fed either 7% soy oil or 1% olive oil and 6% soy oil during a six-month study period. Reduction in memory in passive avoidance learning was examined after two months from the initiation of the experiment. Results : The weight of organs including the liver, kidney, spleen, and fat tissue changed significantly and memory performance was reduced in SAMP8 than in SAMR1 mice. There were no significant differences in SAMP8 and SAMR1 mice; however, blood triglyceride level decreased significantly in SAMP8 mice fed on olive oil. Conclusions : These results suggest that consuming olive oil may not have a protective role in aging and memory recall, but beneficial effects may be related to improvement in lipid metabolism. J. Med. Invest. 66 : 241-247, August, 2019

Keywords **:** *Mediterranean diet, olive oil, MUFA, SAMP8, aging*

INTRODUCTION

The consumption of a Mediterranean diet has extensively been reported to be associated with favorable health and an improved quality of life, and recently, an increased interest in a possible relationship between diet and cognitive health has also been noted. Intake of a Mediterranean diet has been linked with the reduced risk of developing cancer and vascular illnesses and also with decreased incidences of chronic diseases, such as Parkinson's disease (1, 2, 3, 4). Several studies have revealed the protective effects of this dietary pattern against numerous diseases, including neurodegenerative disorders like Alzheimer's disease (5). Recent research suggests that the intake of Mediterranean diets enriched with coconut oil improves cognitive function in patients presenting with Alzheimer's disease (6). Thus, the benefits Mediterranean diets have on the health of both the body and the mind are evident.

The Senescence-Accelerated Mouse-Prone 8 (SAMP8) is a good model for studying the effects of aging. The SAMP8 mouse strain is a mouse model exhibiting accelerated aging, phenotypically selected from the AKR/J strain by Dr. Takeda's lab at Kyoto University (7). These SAMP8 mice are also used to study brain aging and neurodegeneration (8). While these mice are an accepted model to investigate the aging process, most phenotypes in the SAMP8 mice are not fully explained by genetic factors (9). In contrast, the Senescent-Accelerated Mouse-Resistant 1 (SAMR1) model, with a similar genetic background to SAMP8 mice, has been used as an appropriate control model displaying normal aging characteristics.

These SAMP strains have been used widely in research focusing on the effects of feeding and various food constituents on aging. For instance, SAMP8 mice were used to investigate the protective effect that Antarctic Krill Oil, rich in polyunsaturated fatty acids (PUFA), has on memory (10). Similarly, dietary resveratrol was shown to decrease the progressive accumulation of extracellular brain β-amyloid (Aβ) aggregates in Alzheimer's disease and prolonged survival in SAMP8 mice (11). Sesaminol, a component of sesame oil, reduced brain Aβ accumulation and decreased serum 8-hydroxydeoxyguanosine, an indicator of oxidative stress in the same model (12).

A Mediterranean diet is characterized by high olive oil content. Olive oils are predominantly composed of mono-unsaturated fatty acids (MUFA) (13). Diets rich in MUFAs are associated with a reduction in the risk of developing diabetes and cardiovascular diseases (14). For instance, a Mediterranean diet supplemented with either olive oil or mixed nuts for approximately five years, reduced the incidence of cardiovascular diseases in older individuals (15). Recent research shows that dietary MUFAs, but not PUFAs, extends lifespan of *Caenorhabditis elegans* (16). Other studies have focused on identifying a possible link between the consumption of fish oil containing PUFAs and dementia (17, 18). However, only a few studies have investigated the effect of olive oil on cognitive function. As such, in this study, we investigated the effect of olive oil on aging and memory in SAMP8 mice.

We first investigated the characteristic aging differences between SAMR1 and SAMP8 models that were either provided a diet consisting of soy oil (saturated fatty acids) or olive oil (MUFA) for a period of six months. Memory performance was investigated through passive avoidance tests at two months from the start of feeding. At the end of the feeding period, body and organ weight were taken

METHODS

Animals and experimental design

Ten-week old SAMR1 and SAMP8 male mice were purchased from Japan SLC Inc. In terms of the process of senescence, SAMR1 mice exhibited normal development and maturation,

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while SAMP8 mice spontaneously develop accelerated senescence (19, 20). Mice were maintained under a 12-h light/dark cycle with food and water available *ad libitum*. From 11 weeks old and throughout the 24 week study period, SAMR1 and SAMP8 mice were fed either a Control diet, Western diet D10012GM (Table 1)-adjusted Calories Diet (Research Diets, Inc., NJ) or an Olive Oil diet, Western diet D17052602px (Table 1) supplemented with 1% olive oil (Nippon Suisan Kaisha, Tokyo, Japan). Body weight and food intake were recorded twice per week. All experimental procedures conformed to the guidelines for animal experimentation of Tokushima University.

Table 1. Composition of the experimental diets for SAMR1 and SAMP8 mice.

Ingredient (g/kg)	Control diet	Olive oil diet
Casein	200	200
L-Cystine	3	3
Corn Starch	397.486	397.486
Maltodextrin 10	132	132
Sucrose	100	100
Cellulose, BW200	50	50
Soybean Oil	70	60
olive oil	0	10
t-Butylhydroquinone	0.014	0.014
Mineral Mix S10022G	35	35
Vitamin Mix V10037	10	10
Choline Bitartrate	2.5	2.5
Nutritional values		
Energy content, *Kcal/g*	4	4
Protein, % *of energy*	20	20
Carbohydrate, % *of energy*	64	64
Fat, % *of energy*	16	16

Plasma analysis

The fasting plasma level of aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), total cholesterol (T-CHO), free cholesterol (F-CHO), cholesterol ester (E-CHO), triglycerides levels (TG), phospholipids (PL), non-esterified fatty acid (NEFA), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and blood glucose level (GLU) were quantified using routine laboratory methods (Nagahama Life Science Laboratory, Shiga, Japan). Mice from each group were sacrificed via decapitation, and their blood was collected in heparinized capillary tubes. Plasma was separated from blood samples by centrifugation and stored in aliquots at -80°C. All experimental procedures conformed to the guidelines for animal experimentation of Tokushima University.

Passive avoidance test

Passive avoidance tests were performed using the shuttle box system (Shin Factory Corporation, JAPAN). This test was used to study memory performance in mice and is carried out over a period of two days (21, 22, 23, 24). The system consists of the two boxes centrally divided by a wall with sliding door that can be either open or closed. One box is a bright compartment without a top covering (bright box), while the other is a dark compartment in which all sides enclosed (dark box). The floor of the box was made of a stainless-steel grid, which in the dark box can produce a mild electric shock under certain stimuli. On the first day, after a mouse was released into the bright box and tended to migrate to the dark box through the central door opening. When the mouse entered the dark box, the central door was closed, and the mouse was exposed to a 0.5 mA electric shock. On the second day, the mouse was released into the bright box again, and the choice made by the mouse whether or not to enter the dark box was recorded each day (maximum assay time is 180 s). A longer interval or the lack of entry on the second day indicates a memory response, as described previously (24).

Grading score of senescence

The grading score of the senescence system (20, 25) represents the senescent status according to the animals' appearance and behavioral changes with regard to 11 items (reactivity, passive flight reaction, skin glossiness, coarseness, hair loss, ulcer, catarrhal change in the periophthalmic lesions, corneal opacity, corneal ulcer, cataract, and kyphosis). Each item was graded with a score between 0 and 4 according to the intensity of changes (grade 0 represented no particular change and grade 4 represents the most severe changes). In this study, we used six items, reactivity, passivity, glossiness, coarseness, hair loss, and catarrhal change in the periophthalmic lesions.

Statistical analysis

For comparison of the two groups, the Student's t-test for independent samples was applied for categorical data. The results are presented as mean ± standard error. All statistical analysis was performed using the 4-Steps Excel Statistic Third Edition (The Publisher OMS Ltd., Tokyo, Japan). A p-value less than 0.05 was considered statistically significant.

RESULTS

Organ weight and blood analysis in SAMR1 and SAMP8 mice

SAMR1 and SAMP8 mice were fed a diet supplemented with 7% soy oil throughout the 24-week study. At the end of the feeding period, mice of each strain were sacrificed to measure organ weight and intestinal length. Although there were no significant differences in body weights between SAMR1 and SAMP8 mice, the liver and spleen weight did increase, and kidney and epididymal fat weight was significantly lower in SAMP8 mice (Table 2). The result of blood analysis demonstrated that AST, ALT, and LDL-C were significantly higher and T-CHO, NEFA, and HDL-C were significantly lower in SAMP8 mice when compared with SAMR1 mice. There were no significant differences in TG, GLU, and PL between the groups (Table 3).

Table 2. Body and organ weight in SAMR1 or SAMP8 mice after 24 weeks of feeding on 7 % soy oil.

	SAMR1	SAMP8	*P* values
Body weight (g)	39.57 ± 1.38	39.75 ± 1.51	NS
Liver (g)	1.798 ± 0.074	2.033 ± 0.082	< 0.05
Kidney (g)	0.574 ± 0.022	0.475 ± 0.026	< 0.05
Spleen (g)	0.103 ± 0.004	0.156 ± 0.020	< 0.05
Mesenteric fat (g)	0.786 ± 0.056	0.597 ± 0.079	NS
Perirenal fat (g)	0.954 ± 0.093	0.728 ± 0.097	NS
Epididymal fat (g)	2.181 ± 0.210	1.170 ± 0.071	< 0.001
Intestinal length (cm)	6.9 ± 0.2	7.1 ± 0.2	NS

Scores are presented as the mean ± standard error ; NS = nonsignificant.

Table 3. Result of analyzing the plasma in SAMR1 or SAMP8 mice after 24 weeks feeding on 7% soy oil.

	SAMR1	SAMP8	*P* values
AST (IU/L)	50.2 ± 4.2	77.1 ± 5.4	< 0.001
ALT (IU/L)	44.7 ± 7.5	74.7 ± 11.0	< 0.05
T-CHO (mg/dL)	148.6 ± 3.2	116.4 ± 8.1	< 0.01
F-CHO (mg/dL)	38.5 ± 1.4	25.2 ± 1.6	< 0.001
E-CHO (mg/dL)	110.2 ± 2.1	91.2 ± 6.8	< 0.05
TG (mg/dL)	91.6 ± 6.8	95.7 ± 9.1	NS
PL (mg/dL)	255.0 ± 7.7	228.0 ± 16.0	NS
NEFA (μEq/L)	1359.5 ± 70.5	1015.5 ± 91.0	< 0.01
LDL-C (mg/dL)	3.5 ± 0.4	6.0 ± 0.9	< 0.05
HDL-C (mg/dL)	88.6 ± 2.1	65.7 ± 5.2	< 0.01
GLU (mg/dL)	184.6 ± 5.7	183.3 ± 25.6	NS

Scores are presented as the mean ± standard error ; NS = nonsignificant.

Grading score and passive avoidance test in SAMR1 and SAMP8 mice

To investigate the difference in aging between SAMR1 and SAMP8 mice, we recorded the grade score of reactivity, passivity, glossiness, coarseness, hair loss, and catarrhal change in the periophthalmic lesions at the end of the feeding period (Fig. 1, 2). SAMR1 mice were granted grade 1 for reactivity (restless movement) and passivity (getaway speed was slow) while SAMP8 mice were granted grade 2 (walk slowly and did not getaway) for the same traits. Grades for coarseness, reduction in hair size, and catarrhal change in the both periophthalmic lesions were lower in SAMR1 than in SAMP8 mice.

The passive avoidance test was performed at the 8th-week since initiation of the experiment. SAMR1 mice spent, on average, 60 seconds entering the dark box from the bright box, while SAMP8 group mice spent 15 seconds on the task, before they were exposed to an electric shock on first day (data not shown). On the second day, after being released into the bright box again, SAMR1 and SAMP8 mice spent 133 and 56 seconds entering the dark box, respectively (Fig. 3).

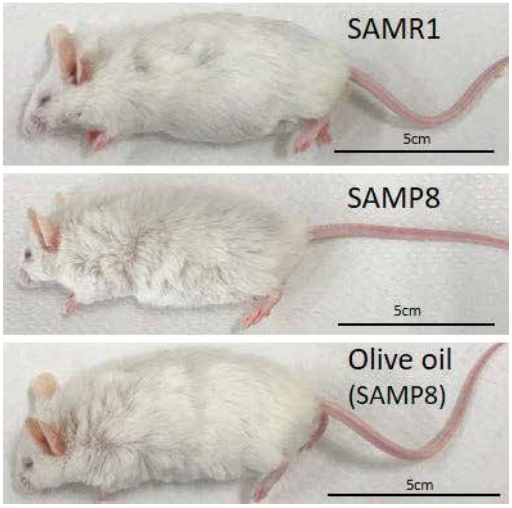

Fig 1. SAMR1, SAMP8 mice, and the olive oil group (SAMP8 mice) at end of the feeding period.

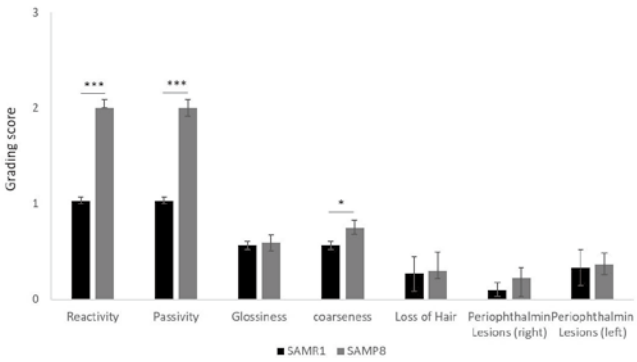

Fig 2. Grading scores of SAMR1 and SAMP8 mice at end of the feeding period.
Black bar represents SAMR1 mice and gray bar indicates SAMP8 mice. Scores are presented as the mean ± standard error ; * : $p < 0.05$, *** : $p < 0.0001$

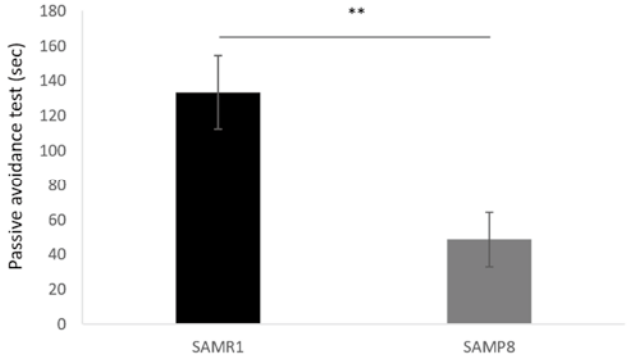

Fig 3. SAMR1 mice spent more time entering the dark box from bright box than did SAMP8 mice on the second day.
Black bar represents SAMR1 mice and gray bar indicates SAMP8 mice. Scores are presented as the mean ± standard error ; ** : $p < 0.01$.

Organ weight and blood analysis in SAMP8 mice feeding on olive oil

SAMP8 mice were fed a diet consisting of either a western diet D17052602px including 6% soy oil, supplemented with 1% olive oil (olive oil group) or 7% soy oil. No significant differences in organ weight or blood markers (except in the case of TG level) were noted between the two groups (Tables 4 and 5, respectively).

Table 4. Body and organ weight of SAMP8 mice fed on different diets, after 24 weeks.

	Control	Olive	*P* values
Body weight (g)	39.75 ± 1.51	37.44 ± 0.99	NS
Liver (g)	2.03 3 ± 0.082	2.03 5 ± 0.197	NS
Kidney (g)	0.475 ± 0.026	0.499 ± 0.014	NS
Spleen (g)	0.156 ± 0.020	0.194 ± 0.077	NS
Mesenteric fat (g)	0.597 ± 0.079	0.446 ± 0.053	NS
Perirenal fat (g)	0.728 ± 0.097	0.600 ± 0.085	NS
Epididymal fat (g)	1.170 ± 0.071	1.10 7 ± 0.156	NS
Intestinal length (cm)	7.1 ± 0.2	7.0 ± 0.2	NS

Scores are presented as the mean ± standard error ; NS = nonsignificant.

Table 5. Analyses of the plasma from SAMP8 mice after 24 weeks

	Control	Olive oil	*P* values
AST (IU/L)	77.1 ± 5.4	127.2 ± 54.9	NS
ALT (IU/L)	74.7 ± 11.0	81.6 ± 23.0	NS
T-CHO (mg/dL)	116.4 ± 8.1	106.2 ± 9.6	NS
F-CHO (mg/dL)	25.2 ± 1.6	25.5 ± 2.3	NS
E-CHO (mg/dL)	91.2 ± 6.8	80.7 ± 8.6	NS
TG (mg/dL)	95.7 ± 9.1	63 ± 6.6	< 0.05
PL (mg/dL)	228 ± 16.0	211.2 ± 16.6	NS
NEFA (μEq/L)	1015.5 ± 91.0	889.8 ± 114.0	NS
LDL-C (mg/dL)	6 ± 0.9	6.3 ± 1.1	NS
HDL-C (mg/dL)	65.7 ± 5.2	60.9 ± 7.2	NS
GLU (mg/dL)	183.3 ± 25.6	183 ± 12.9	NS

Scores are presented as the mean ± standard error ; NS = nonsignificant.

Grade score and passive avoidance test in olive oil-fed SAMP8 mice

Grade scores were recorded in order to investigate the effect of olive oil on aging. Mice from both the control (soy oil) and olive oil groups were graded a score of 2 for reactivity and passivity. This grade for reactivity represents slow movements when mice leave the cage, and a grade of 2 for passivity suggests that mice do not try to get away when their neck is held. Glossiness, coarseness, hair loss, and catarrhal change in periophthalmic lesions were not significantly different between the two groups (Fig. 1, 4).

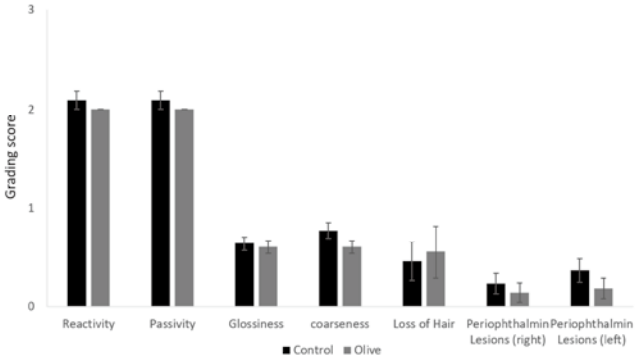

Fig 4. Grading scores of the control group and olive oil group at end of the feeding period.
Black bar represents control group mice and gray bar represents olive oil group mice. Scores are presented as the mean ± standard error.

The passive avoidance test was performed in both feeding groups at the 8th week since initiation of the experiment. Mice from the control or olive oil group spent 15 seconds entering the dark box from the bright box before an electric shock on first day (data not shown). On the second day, the time taken to enter the dark box increased to 56 and 37 seconds for the control and olive oil-fed mice, respectively (Fig. 5). Although in both cases, the time taken to enter the dark box was extended, there was no significant difference between the groups.

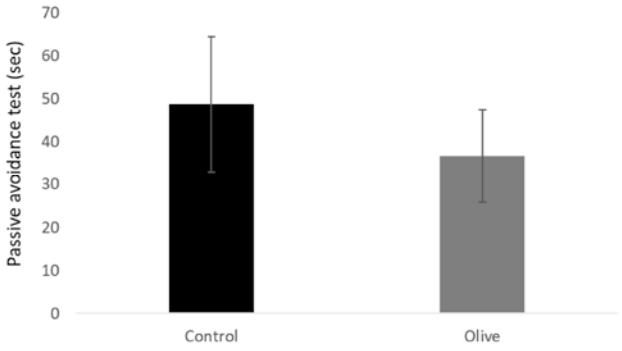

Fig 5. Time taken by mice to enter the black box.
Black bar represents control group mice and gray bar represents olive oil group mice. Scores are presented as the mean ± standard error.

DISCUSSIONS

To investigate the difference of aging between SAMR1 and SAMP8 mice, the both mice were fed on the food including 7% soy oil during 24 weeks. In general, the primary identifiable causes of death in these SAM mice strains include contracted kidney, abscess formation, pneumonia and lymphoid cell neoplasms. SAMP8 mice most likely died of lymphoid cell neoplasms and contracted kidney (26). Compared with SAMR1 mice, kidney weight was obviously decreased in SAMP8 mice (Table 2). Furthermore, the blood test for AST and ALT are usually used as reasonably sensitive indicators of liver damage. The elevated AST and ALT level in SAMP8 mice in the blood sample may indicate liver damage or injury (Table 3). Compared with SAMR1 mice, T-CHO and HDL-C level significantly decreased in SAMP8 mice (Table 3). Hepatic dysfunction by aging in SAMP8 mice might also lead to lower blood cholesterol levels than in SAMR1 mice.

We next recorded the grading score of SAMR1 and SAMP8 mice to evaluate their aging (Fig. 2). SAMP8 mice was walking slowly and do not get away as compared with SAMR1 mice. The hair of SAMR8 mice was neither smooth nor straight, and became to be severely dried. The passive avoidance test was examined after 8 weeks from starting up Western diet including 7% soy oil. On the second day after given electric shock, SAMR1 and SAMP8 mice spent 133 and 56 seconds respectively on entering to dark box (Fig. 3). Since SAMR1 mice hesitated to enter and checked the entrance of dark box many times, these mice may memorize the electric shock of the first day. Judging from the result that SAMP8 mice entered to dark box early with checking few times, SAMP8 mice were likely to have a poor memory for the electric shock of the first day. These results suggested that SAMP8 mice might be identified as aging mice.

To investigate the effect of olive oil on aging, SAMP8 mice were fed a diet consisting of either 1% olive oil and 6% soy oil (control) or 7% soy oil only, during the 24 weeks. There were no significant differences in body weight and organ weight between control and olive oil group (Table 4). Analysis of blood samples revealed that the level of TG was significantly reduced in the olive oil group (Table 5). Umezawa *et al*. reported that T-CHO and TG level were higher in SAMP8 mice fed on olive oil (rich in the monounsaturated oleic acid) as compared with those fed safflower oil (rich in the omega-6 polyunsaturated linoleic acid) or Fish oil (rich in long chain monounsaturated fatty acid) (27). On the contrary, other research showed that feeding on olive oil, consisting mainly of oleic acid, an omega-9 monounsaturated fatty acids (MUFA) (28, 29), decreased the levels of T-CHO and TG

in Wistar rats (30). Blood fasting and feeding triglyceride levels decreased in C57BL/6J mice with olive oil-added fat diets in comparison to the high-carbohydrate diet (31). Omega-9 MUFA is a natural agonist of the peroxisome proliferator activated receptor (PPAR) (32). The three PPAR isotypes are PPAR alpha, PPAR gamma and PPAR delta/beta and these regulate metabolism (33), inflammation (34), and infection (35), as described previously (36). Thus, olive oil might decrease TG levels by activating the PPARs in SAMP8 mice. Although olive oil tended to decrease T-CHO levels, this difference was not significant. Since mice were fed 1% olive oil in this study, the effect of olive oil on lipid metabolism at a high dosage needs to be examined.

Both the control and olive oil treated mice were given a grade score of 2 for reactivity and passivity (Fig. 4). Grades of glossiness, coarseness, hair loss, and catarrhal change in the periophthalmic lesions occurred at the same degree in two groups. The passive avoidance in SAMP8 mice fed on olive oil for 8 weeks indicated that olive oil group spent 37 seconds entering to dark box with checking the entrance few times on the second day (Fig. 5). This mean that olive oil may not improve forgot a poor memory in SAMP8 mice. As passive avoidance test measures a short-term memory, long-term tests such as a radial arm maze test (37) or Morris water-maze test (38) are needed in further studies.

The use of Olive oil in diet has been associated with a reduction in mortality due to vascular diseases and cancer (1, 39, 40) and mitigates inflammation during experimental sepsis (36). Recently, research has shown that the life span of *C. elegans* was extended by dietary MUFAs, but not PUFAs (16). On the other hand, other studies revealed that dietary oleic acid did not extend wild-type lifespan (41, 42, 43), although they used different oleic acid supplementation protocols. Our results do not indicate an enhanced effect of aging and memory by olive oil, albeit at a concentration of 1%. A range of concentrations needs to be investigated to identify noteworthy trends.

In addition, the type of olive oil and their constituents used in the experiments may also play a role in the outcome. For instance, phenols are not present in refined olive oil (ROO) but virgin olive oil (VOO) and extra virgin olive oil (EVOO) do contain phenols at various concentrations (44, 45). EVOO is known to be rich in phenols, specifically, the catecholic compounds hydroxytyrosol (3,4-dihydroxyphenyl-ethanol, HT) and oleuropein, which act as potent antioxidants, free radical scavengers, and modulators of various oxygen-dependent enzymes. The levels of oxidation markers were not affected by ROO consumption (44), while consumption of EVOO increased the plasma antioxidant capacity in humans (45). HT also improved the antioxidant effect of plasma and prevented DNA damage in LPS stimulated mouse model (46). Similarly, VOO containing phenols potentiates effects related antioxidant stress, which leads to Parkinson's disease and aging. Lack of variation in aging score or memory between control and olive groups may be as a result of the use of VOO in the present study.

Although the grading score for coarseness of skin in control and olive oil groups was not significantly different, the ingestion of olive oil tended to decrease the score, while also preventing hair loss (Fig. 1, 4). The reduction in hair loss in mildly alopecic monkeys, following administration of PUFA, might be due to a decrease in alopecia-related inflammation, associated with the varied levels of the anti-inflammatory cytokine IL-10 and the proinflammatory cytokines TNF-α and IL-1β (47). Anti-inflammatory effects of olive oil with respect to protection against hair fall require further examination.

CONCLUSION

The organ weight, blood markers, movement, and memory indicated severe progression of aging in SAMP8 mice. Although olive oil decreased the levels of TG in blood, the addition of olive oil (1%) to the food did not affect aging phenotypes and memory. However, it is evident that olive oil has the tendency to decrease T-CHO levels and improve hair condition in SAMP8 mice.

CONFLICT OF INTERESTS

None

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