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Dietary arachidonic acid improves age-related excessive enhancement of the stress response

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Abstract. – OBJECTIVE: The aim of this study is to understand whether the responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis to stress increases excessively with aging in senescence-accelerated mice-prone 10 (SAMP10) and to investigate the role of arachidonic acid (ARA) in this process.

MATERIALS AND METHODS: The area under the curve of CORT concentration (CORT-AUC), an index of the HPA axis responsiveness to stress, was assessed in SAMP10 subjected to a 30-minute restraint stress up to 120 minutes after the restraint stress onset. Furthermore, the HPA axis responsiveness was evaluated in aged SAMP10 fed 0.4% ARA-containing diet (ARA group) or control diet (CON group) for 4 weeks. Three weeks later, these mice were divided into a group with a 30-minute restraint stress (CON-S or ARA-S group) and a group without restraint stress (CON-NS or ARA-NS group). Hippocampi were collected after stress release and fatty acid and glucocorticoid receptor (GR) protein levels were evaluated in the nucleus and cytosol.

RESULTS: The CORT-AUC of aged SAMP10 was 21% significantly higher than that of young SAMP10. In the ARA group, hippocampal ARA was 0.5% significantly higher than that in the CON group. CORT-AUC in the ARA group was 24% significantly lower than that in the CON group. The ratio of GR protein levels in the nucleus and cytosol in the ARA-S group was 1.72 times significantly higher than that in the ARA-NS group but no difference was observed between the CON-S and CON-NS groups.

CONCLUSIONS: Dietary ARA seems to suppress age-related excessive enhancement of the HPA axis responsiveness via attenuation of age-related decline in hippocampal GR translocation into the nucleus after stress loading, which may contribute to an improvement of mental health.

Key Words:
Arachidonic acid, HPA axis, Aging, Stress.

Abbreviations
ARA = Arachidonic acid; AUC = Area under the curve; CORT = Corticosterone; DHA = Docosahexaenoic acid; EPA = Eicosapentaenoic acid; GR = Glucocorticoid receptor; HDAC6 = Histone deacetylase 6; HPA = Hypothalamic-pituitary-adrenal; HSP90 = Heat shock protein 90; LCPUFA = Long chain polyunsaturated fatty acid; MUFA = Monounsaturated fatty acid; PUFA = Polyunsaturated fatty acid; SAMP10 = Senescence-accelerated mice-prone 10; SFA = Saturated fatty acid.

Introduction

The aging of the population is progressing all over the world and maintaining the health of older people is becoming an important issue. Particularly, the maintenance of mental health is considered an essential factor for people's lives and activities[1]. Stress is an important factor contributing to mental health deterioration, and physiological vulnerability to stress has been demonstrated in humans[2]. The increase in vulnerability to chronic stress with aging has also been reported in rodents[3]. Therefore, the attenuation of enhanced stress vulnerability might help to maintain the mental health of the elderly.

Many studies[3-6] evidence that the enhancement of age-related stress vulnerability is caused by the failure of negative feedback of the hypothalamic-pituitary-adrenal (HPA) axis. In healthy young humans[7-9] and animals[6], after stress loading, cortisol or corticosterone (CORT) transiently rises and immediately returns to the steady-state level due to the negative feedback of the HPA axis. With increasing aging, the negative feedback of the HPA axis diminishes, thus leading to persistently high cortisol or CORT levels[4,6]. High concentrations of CORT have been found to cause neuronal cell injury *in vitro*[10] and *in vivo*[11], leading to mental dysfunction. In fact, interventions that are considered effective in maintaining mental health, such as exercise[12], suppress age-related excessive enhancement of the HPA axis responsiveness. Translocation of glucocorticoid receptor (GR) from the cytosol to the nucleus in the hip-

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pocampus after stress loading contributes to the negative feedback of the HPA axis[5,13,14]. Therefore, the hippocampus plays an important role in stress response.

As the way to improve mental health of elderly, our previous studies have demonstrated that a diet containing docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA) improves mood states[15]. A candidate for the mechanisms of this effect is the suppression of the HPA axis responsiveness by DHA and EPA[16-18]. Although some reports[19-21] indicate that ARA improves hippocampal function, such as memory function, its influence on the HPA axis responsiveness remains to be investigated.

Hippocampal atrophy and function decline are observed in senescence-accelerated mice-prone 10 (SAMP10) at about one year of age, which is earlier than in normal mice[22-24]. As described above, the hippocampus also plays an important role in the negative feedback of the HPA axis; however, the age-related change in the HPA axis responsiveness in SAMP10 remains unclear. We hypothesized that SAMP10 exhibits not only early cognitive decline but also early excessive enhancement of the HPA axis responsiveness because both functions are related to the hippocampus. In this study, we examined whether the responsiveness of the HPA axis enhances excessively with aging in SAMP10 (Experiment 1) and determined the effects of dietary ARA on age-related excessive enhancement of the HPA axis responsiveness (Experiment 2) and hippocampal GR translocation from the cytosol into nucleus.

Table I. Fatty acid composition of the diet in Experiment 1.

Fatty acids	Percentage
16:0 palmitic acid	26.4
18:0 stearic acid	4.4
18:1 (n-9) oleic acid	30.8
18:2 (n-6) linoleic acid	22.5
18:3 (n-3) α-linolenic acid	11.1
20:4 (n-6) arachidonic acid	0.0
Others	4.7
PUFA	34.0
MUFA	31.3
SFA	31.1
n-6/n-3	2.1*

*Numbers in the table are expressed as percentages, except for the n-6/n-3 ratio. PUFA: polyunsaturated fatty acid; MUFA: monounsaturated fatty acid; SFA: saturated fatty acid.

Materials and Methods

Animals, Diets and Experimental Design

All protocols for animal procedures were approved by the Ethics Committee of Animal Experiment in accordance with the Internal Regulations on Animal Experiments at Suntory, which are based on the Law for the Humane Treatment and Management of Animals (Law No. 105, 1 October 1973, as amended 2 June 2017). Male SAMP10 were obtained from Japan SLC, Inc. (Hamamatsu, Shizuoka, Japan). Mice were housed individually in polycarbonate cages with paper bedding, which was changed once a week. The facility was maintained under specific pathogen-free conditions at a temperature of 23 ± 2°C and humidity of 55 ± 10%, with a 12-h light/dark cycle (the light was switched on at 7:00). Before the experiment, the animals were acclimated to the facility for more than 1 week. Mice had *ad libitum* access to water and diet. All the diets were stored at 4°C and changed twice a week to prevent oxidation.

In Experiment 1, we used the diet based on AIN-76 containing 5% fat with modified lipid compositions. The lipids used in Experiment 1 consisted of palm oil, soybean oil, and linseed oil (Showa Kosan Co., Ltd., Minato-ku, Tokyo, Japan). The fatty acid compositions of this diet are shown in Table I. After more than 1-week acclimation period, SAMP10 aged 1 month (n = 20) or 11-12 months (n = 11) received the diet described above for 4 weeks. Subsequently, CORT concentration after stress was evaluated by the modified method as described previously[5]. Briefly, mice were subjected to a 30-minute restraint stress treatment using a breathable stainless cage (Natsume Seisakusho Co., Ltd., Bunkyo-ku, Tokyo, Japan). Blood was collected (~50 μl) from the tail vein before and every 30 minutes after the onset of restraint stress up to 120 minutes by heparinized capillaries (Drummond Scientific Company, Broomall, PA, USA) and centrifuged at 2200 × g for 10 minutes at 4°C. The plasma samples were then obtained and stored at -80°C until measurement of CORT concentration. The area under the curve of CORT concentration (CORT-AUC; an index of HPA axis responsiveness to stress) was calculated. Stress loading and blood collection were performed from 8:00 to 13:00.

In Experiment 2, we also used the diet based on AIN-76 containing 5% fat with modified lipid compositions. The lipids used in Experiment 2 consisted of ARA-enriched triacylglycerol (SUNTGA40S), palm oil, soybean oil, and lin-

seed oil. The fatty acid compositions of this diet are shown in Table II. After more than 1-week acclimation period, SAMP10 aged 11-12 months received the ARA-containing diet (ARA group, n = 14) or the control diet without ARA (CON group, n = 19) for 4 weeks. The responsiveness of the HPA axis was assessed using the approach described in Experiment 1. After that, mice were bred under the same conditions for 3 weeks for recovery. During this time, three mice from the ARA group and four mice from the CON group were excluded from further analysis because of weakness. Then, the mice that were fed the control diet were divided into two groups: CON-S group (n = 8), mice subjected to restraint stress for 30 minutes and CON-NS group (n = 7), mice without restraint stress. Because GR translocation occurred as early as 5 minutes after releasing from stress and decreased after 30 minutes (Supplementary Figure S1D), we collected the hippocampus 5 minutes after releasing stress. In the CON-S group, mice were decapitated (to avoid the effects of anesthesia) 5 minutes after releasing from stress, and the hippocampus was collected to evaluate the phospholipid fatty acid composition and GR protein levels in the nucleus and cytosol. The mice in CON-NS group were also decapitated, and the hippocampus was then collected. The mice fed the ARA-containing diet were also divided into two groups: the ARA-S group (n = 6), mice subjected to restraint stress, and ARA-NS group (n = 5), mice without restraint stress. The hippocampus was collected and used for evaluating the phospholipid fatty acid composition and GR protein levels. All the collected samples were stored at -80°C until use. Stress loading and sample collection were performed from 8:00 to 13:00.

Table II. Fatty acid composition of the diet in Experiment 2.

Fatty acids	CON	ARA
16:0 palmitic acid	26.4	26.6
18:0 stearic acid	4.4	5.3
18:1 (n-9) oleic acid	30.8	27.3
18:2 (n-6) linoleic acid	22.5	12.8
18:3 (n-3) α-linolenic acid	11.1	11.0
20:4 (n-6) arachidonic acid	0.0	8.2
Others	4.7	8.9
PUFA	34.0	34.1
MUFA	31.3	27.8
SFA	31.1	34.3
n-6/n-3	2.1	2.1

Numbers in the table are expressed as percentages, except for the n-6/n-3 ratio. PUFA: polyunsaturated fatty acid; MUFA: monounsaturated fatty acid; SFA: saturated fatty acid.

Fatty Acid Analysis of the Hippocampus

Lipid was extracted and purified from the hippocampus by the method reported by Folch et al[25]. Subsequently, phospholipids, which were obtained using thin-layer chromatography (hexane: diethyl ether = 7: 3), were methylesterified by incubating in methanolic HCl at 50°C for 3 hours. The methylesterified lipids were extracted with hexane, and subjected to capillary gas-liquid chromatography (Agilent 7890B; Agilent Technologies, Santa Clara, CA, USA) using an SP-2330 column (30 m × 0.32 mm × 0.2 μm; Supelco, Bellefonte, PA, USA) with He (at 30 cm/sec) as the carrier. The column temperature was initially 180°C for 2 min and then increased to 220°C at a rate of 2°C/min.

Corticosterone Concentration Measurement

CORT concentration in the plasma collected in Experiments 1 and 2 was measured using DetectX Corticosterone Enzyme Immunoassay Kit (#014, Arbor Assays, Ann Arbor, MI, USA) according to the manufacturer's instructions.

Cell Fractionation

Cell fractionation was performed according to the method described by Mizoguchi et al[5]. The hippocampus was homogenized in Buffer A (10 mM HEPES containing 10 mM KCl, 1.5 mM MgCl2, 0.1 mM EGTA, 0.5 mM DTT, and 1% proteinase inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA, USA) (pH 7.6) in a 1.5 ml tube and then centrifuged at 20,000 × g for 20 min at 4°C. The supernatant was used as the cytosol fraction. The pellet was resuspended in Buffer B (Buffer A containing 0.32 M sucrose) and centrifuged at 1,000 × g for 3 min at 4°C, and the supernatant was discarded. This procedure was repeated twice to wash the pellet. Buffer C (Buffer A containing 0.5 M NaCl and 5% glycerol) was then added to the washed pellet, and the suspension was incubated on ice for 1 h and centrifuged at 20,000 × g for 20 min at 4°C to produce soluble nuclear extracts. The supernatant was used as the nuclear fraction.

Western Blotting

Each fraction collected by the above method was subjected to protein quantification and was mixed with an equal volume of 2 x Laemmli Sample Buffer (#1610737, Bio-Rad, Hercules, CA, USA) containing 5% 2-Mercaptoethanol (#21438-82, Nacalai, Kyoto-shi, Kyoto, Japan). Samples

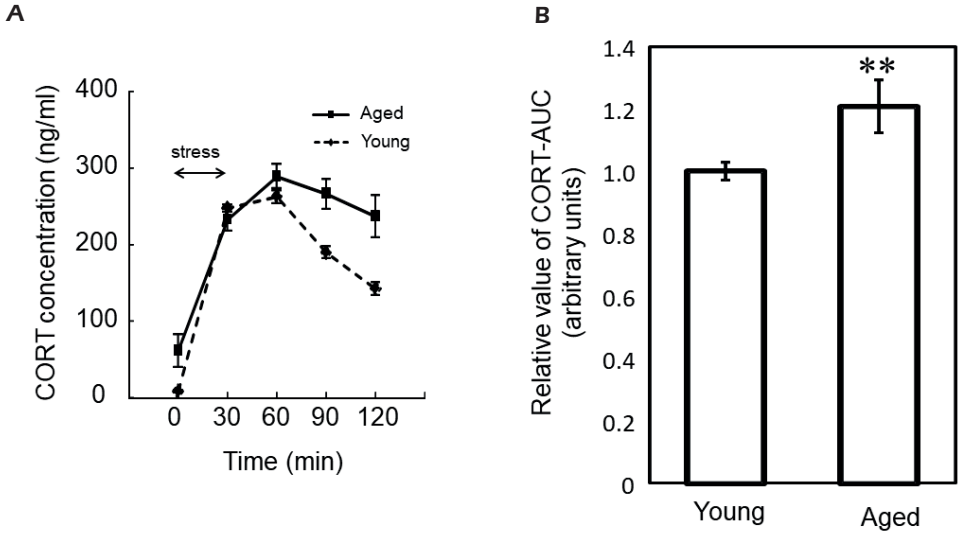

Figure 1. Age-related changes in the HPA axis responsiveness to stress in SAMP10. **A,** Time-dependent changes in the CORT concentration in young and aged SAMP10. **B,** Relative values of CORT-AUC. Data are presented as means ± SEM. $^{**}p < 0.01$ *vs.* the young group by unpaired *t*-test (Young, n = 20; Aged, n = 11). HPA, hypothalamic-pituitary-adrenal; CORT, corticosterone; SAMP10, senescence-accelerated mice-prone 10; AUC, Area under the curve.

(2.0 μg protein/lane) were separated by sodium dodecyl (lauryl) sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were then transferred to polyvinylidene difluoride (PVDF) membranes (#1620261, Bio-Rad, Hercules, CA, USA). After blocking with Blocking One (#03953-95, Nacalai, Kyoto-shi, Kyoto, Japan) at about 20°C for 1 h, the membranes were incubated with primary antibodies diluted in Can Get Signal Immunoreaction Enhancer Solution (#07292-51, Toyobo, Osaka-shi, Osaka, Japan) overnight at 4°C. After washing three times with Tris-Buffered Saline with 0.1% Tween 20 (TBS-T), the membranes were incubated with secondary antibodies at about 20°C for 1 hour. After washing three times with TBS-T, antibody-antigen complexes were detected using an enhanced chemiluminescent system (ECL; GE Healthcare, Chicago, IL, USA). Signal intensities were quantified using the Fusion system (Vilber-Lourmat Sté, Collégien, France). The intensities of GR protein were normalized by the internal standard (p84 for the nucleus and α-tubulin for the cytosol). The ratio of normalized GR protein levels in the nucleus and cytosol (nucleus/cytosol) was also calculated. Anti-NR3C1 antibody (#GTX101120, GeneTeX, Irvine, CA, USA), anti-p84 antibody (#GTX70220, GeneTeX, Irvine, CA, USA), and anti-alpha tubulin antibody (#YL1/2, Santa Cruz Biotechnology, Dallas, TX, USA) were used as primary antibodies. Goat anti-rabbit IgG antibody (#ab97080, Abcam, Cambridge, UK), goat anti-mouse IgG antibody (#ab97040, Abcam), and goat anti-rat IgG antibody (#ab97057, Abcam, Cambridge, UK) were used as secondary antibodies.

Statistical Analysis

Data are presented as means ± SEM. In Experiment 1, CORT-AUC was expressed as a ratio of the young group. In Experiment 2, CORT-AUC was expressed as a ratio of the CON group. GR protein levels were expressed as the ratio in the non-stressed condition in each group, CON-NS or ARA-NS. Data were analyzed by unpaired two-tailed *t*-test. A $p < 0.05$ was considered statistically significant.

Results

Age-Related Changes of CORT-AUC in the HPA Axis in SAMP10

Figure 1A shows time-dependent changes in CORT concentration in young and aged SAMP10 before and after restraint stress in Experiment 1. The concentrations of CORT in young and aged SAMP10 before restraint stress were 8.5 ± 1.5 ng/ml and 62.0 ± 21.7 ng/ml, respectively. Immediately after the restraint stress, CORT concentrations were increased to 248.2 ± 5.0 ng/ml in the

young group and 233.4 ± 14.8 ng/ml in the aged group. CORT concentrations were decreased to 142.5 ± 8.6 ng/ml in young SAMP10 and 237.9 ± 27.3 ng/ml in aged SAMP10 at 120 minutes after the onset of restraint stress. Corticosterone concentration was consistently higher in aged mice than in young mice. The area under the curve of CORT concentration of aged SAMP10 was significantly higher by 21% than that of young SAMP10 (Figure 1B).

Effects of Dietary ARA on Fatty Acid Compositions of the Hippocampus

Table III shows phospholipid fatty acid composition in the hippocampus in Experiment 2. ARA and stearic acid levels were significantly increased in the ARA group compared with those in the CON group. In contrast, DHA level was significantly decreased.

Effects of Dietary ARA on Age-Related Excessive Enhancement of the HPA Axis Responsiveness to Stress

Figure 2A shows time-dependent changes in CORT concentrations in aged SAMP10 in the CON and ARA groups before and after restraint stress. The concentrations of CORT in the CON and ARA groups before restraint stress were 60.0 ± 25.2 ng/ml and 44.7 ± 14.4 ng/ml, respectively. Immediately after the restraint stress, CORT concentrations were increased to 248.0 ± 30.2 ng/ml in the CON group and 185.5 ± 17.9 ng/ml in the ARA group. The CORT concentration at 120 minutes after the onset of restraint stress in the CON group was 253.6 ± 36.7 ng/ml and was higher than that in the ARA group (196.1 ± 17.1 ng/ml). Corticosterone concentration in the ARA group was consistently lower than that in the CON group. In the ARA group, CORT-AUC was significantly lower by 24% than that in the CON group (Figure 2B).

Table III. Phospholipid fatty acid composition in the hippocampus in Experiment 2.

Fatty acids	CON	ARA
16:0 palmitic acid	22.3 ± 0.1	22.2 ± 0.1
18:0 stearic acid	20.9 ± 0.1	21.3 ± 0.0**
18:1 (n-9) oleic acid	15.8 ± 0.2	15.9 ± 0.2
20:4 (n-6) arachidonic acid	11.7 ± 0.1	12.2 ± 0.2*
22:6 (n-3) docosahexaenoic acid	18.3 ± 0.1	17.4 ± 0.3**

Numbers are expressed as percentages. Data are represented as means ± SEM. *$p < 0.05$, **$p < 0.01$ vs. the CON group by unpaired-*t*-test (CON, n = 15; ARA, n = 11).

Effects of Dietary ARA on Hippocampal Glucocorticoid Receptor Translocation into the Nucleus

Supplementary Figure S1A shows the representative results of Western blotting of hippocampal GR, p-84 and α-tubulin in the nucleus and cytosol in young SAMP10 before and after stress loading. The data of quantitative analysis of

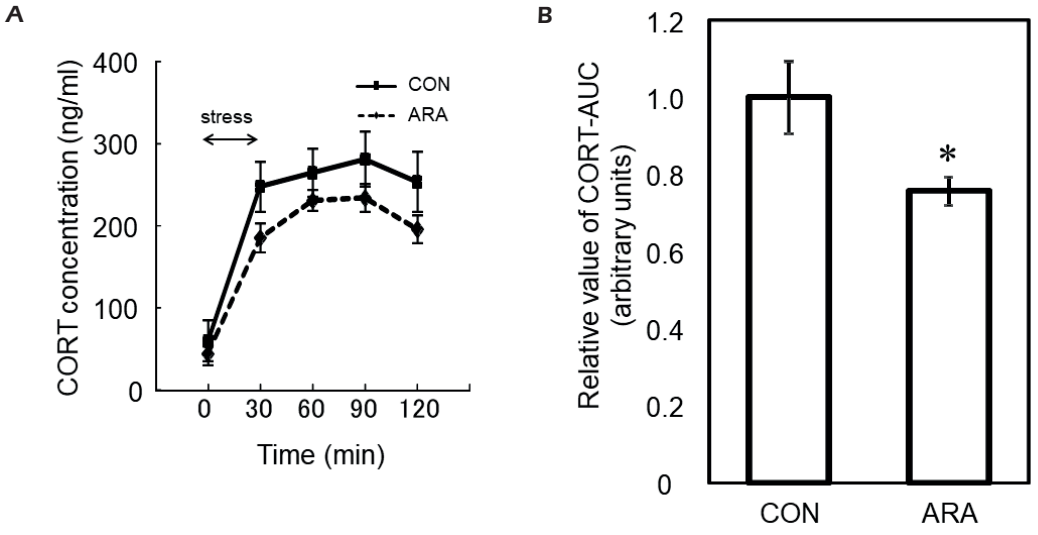

Figure 2. Effects of ARA on the HPA axis responsiveness to stress in SAMP10. **A,** Time-dependent changes in the CORT concentration in the CON and ARA groups. **B,** Relative values of CORT-AUC. Data are presented as means ± SEM. *$p < 0.05$ *vs.* the CON group by unpaired *t*-test (CON, n = 19; ARA, n = 14). CON, control; ARA, arachidonic acid.

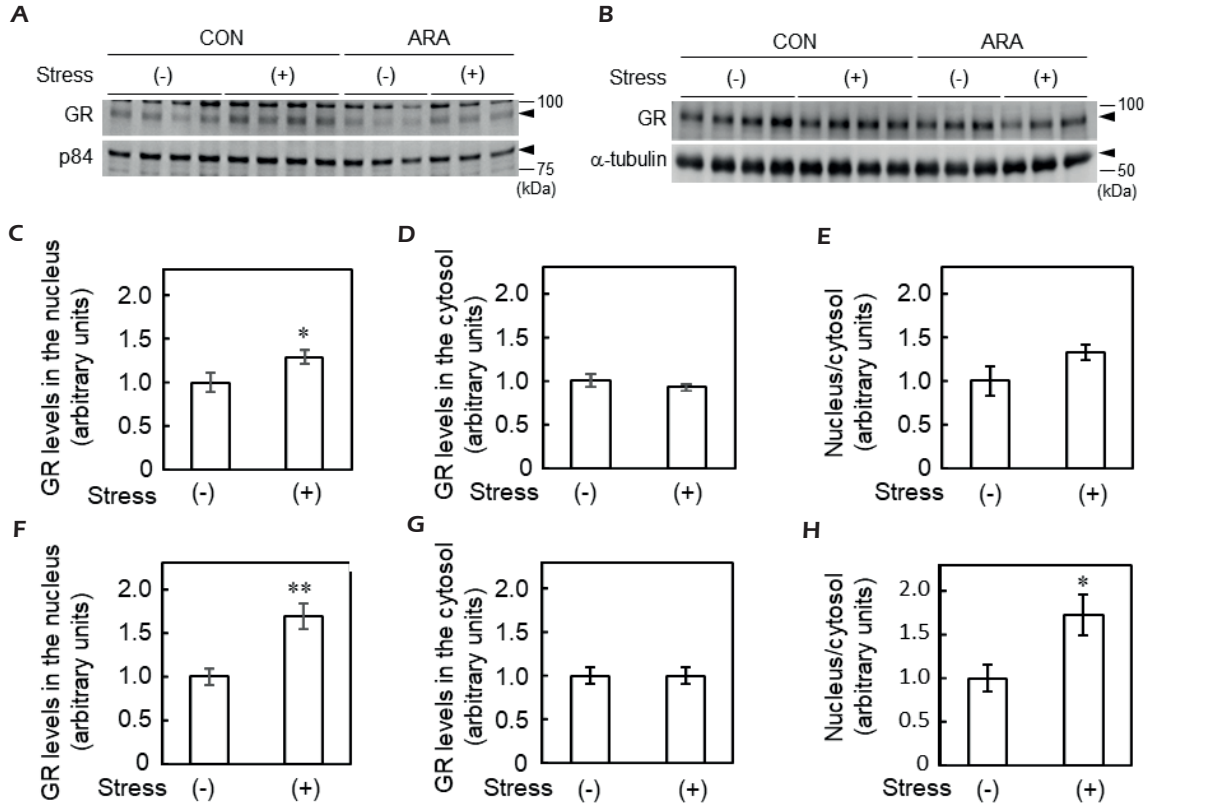

Figure 3. Effects of ARA on hippocampal GR translocation into the nucleus. Representative bands of Western blot analysis of GR and p-84 in the nucleus (**A**) and GR and α-tubulin in the cytosol (**B**). Quantitative data of GR in the nucleus (**C**) and cytosol (**D**) in the CON-NS and CON-S groups and the ratios of GR protein levels in the nucleus and cytosol in the CON-NS and CON-S groups (**E**), quantitative data of GR in the nucleus in the ARA-NS and ARA-S groups (**F**), in the cytosol in the ARA-NS and ARA-S groups (**G**), and the ratios of GR protein levels in the nucleus and cytosol in the ARA-NS and ARA-S groups (**H**). Data are presented as means ± SEM. *$p < 0.05$, **$p < 0.01$ *vs.* the CON-NS or ARA-NS group by unpaired *t*-test (CON-NS, n = 7; CON-S, n = 8; ARA-NS, n = 5; ARA-S, n = 6). The black arrow next to each lane represents the position of the target protein. GR, glucocorticoid receptor.

the normalized GR band intensities in the nucleus (supplementary Figure S1B), cytosol (supplementary Figure S1C) and the ratio of normalized GR protein levels in the nucleus and cytosol (supplementary Figure S1D) were also shown. GR protein levels in the nucleus and the ratio of GR protein levels in the nucleus and cytosol were significantly increased in young SAMP10 about 5 minutes after stress loading (Supplementary Figure S1B, D). Figure 3A-B shows the representative results of Western blotting, and Figure 3C-H shows the quantitative results. Normalized GR protein levels in the nucleus were significantly higher in the CON-S group (1.29 ± 0.08, $p < 0.05$; Figure 3C) than in the CON-NS group. They were also higher in the ARA-S group (1.70 ± 0.15, $p < 0.01$; Figure 3F) than in the ARA-NS group. Normalized GR protein levels in the cytosol were not significantly different between the CON-S group (0.92 ± 0.04, $p = 0.37$; Figure 3D) and the CON-NS group. They were also not significantly different between the ARA-S group (1.00 ± 0.10, $p = 0.98$; Figure 3G) and the ARA-NS group. The ratio of the normalized GR protein levels in the nucleus and cytosol (nucleus/cytosol) was not significantly different between the CON-S (1.33 ± 0.09, $p = 0.10$; Figure 3E) and CON-NS groups. In contrast, this ratio in the ARA-S group (1.72 ± 0.23, $p < 0.05$; Figure 3H) was significantly higher than that in the ARA-NS group.

Discussion

The stress hormone after stress loading is often used as an indicator of the HPA axis responsiveness; the AUC of the stress hormone increases and the negative feedback of the HPA axis is attenuated with aging[4]. For example, the activation of the HPA axis induced by pharmacological chal-

lenges, such as corticotropin-releasing factors[26,27], somatostatin[28], and epinephrine[29], or psychosocial challenges[7,30] is stronger in aged subjects than in young subjects. In addition, cognitively impaired aged rodents have enhanced HPA axis responsiveness compared with healthy young rodents following restraint stress[6]. In Experiment 1, CORT-AUC was significantly increased in SAMP10 aged 12-13 months compared with that in those aged 2 months (Figure 1B), indicating the excessive enhancement of the HPA axis responsiveness in aged mice. Issa et al[6] have shown the excessive enhancement of the HPA axis responsiveness in normal rodents (aged 23-27 months) with cognitive decline, but not in those without cognitive decline. Therefore, SAMP10 can be regarded as an animal model with early decreased negative feedback of the HPA axis, as well as impaired hippocampal function, such as memory dysfunction. To the best our knowledge, this study is the first to investigate the usefulness of SAMP10 for evaluating age-related changes in the HPA axis responsiveness.

Dietary ARA has been demonstrated to positively affect the hippocampal neurogenesis in aged rodents and increase ARA content by about 0.3% in the hippocampus[31]. Experiment 2 showed that dietary ARA supplementation significantly increased ARA content by 0.5% in the hippocampus, suggesting that the increased ARA level in the present study reaches the level that can affect the hippocampal function. In fact, ARA administration reduced CORT-AUC (Figure 2B), which represents the responsiveness of the HPA axis, in aged SAMP10. The reduction in CORT-AUC by dietary ARA was 24%, which is comparable to that of age-related increase, 21%, in SAMP10 mice; therefore, this change is considered physiologically significant. Persistently high CORT levels lead to nerve cell injury and consequent mental disorders due to stress[10,11]. Our previous studies have indicated that the diet containing ARA improves mood states[15], one of the mental health characteristics. It is possible that the attenuation of the negative feedback dysfunction of the HPA axis (i.e., the suppression of the excessive age-related enhancement of the stress response) by ARA found in the present study contributes to the improvement of mental health. However, it remains unclear based on the present research to what extent ARA affects behaviors. Repeated restraint stress at 1 hour/day for 14 days elicits anxiety-like behaviors only in aged rodents[3]. Further investigations including evaluation of the effect of ARA under the condition of repeated stress are needed to clarify whether the attenuation of the negative feedback dysfunction of the HPA axis by ARA affects behavior.

In the hippocampus, GR translocation into the nucleus after stress contributes to the negative feedback of the HPA axis[5,13,14]. Consistently, as shown in supplementary Figure S1, GR protein levels in the nucleus and the ratio of GR protein levels in the nucleus and cytosol were significantly increased in young SAMP10 about 5 minutes after stress loading. However, in aged SAMP10 mice, the ratio of GR protein levels in the nucleus and cytosol, which indicates the activity of GR translocation, did not change significantly after stress loading (Figure 3E), although GR protein levels in the nucleus were slightly increased (Figure 3C). Hippocampal GR translocation into the nucleus is attenuated in aged SAMP10; these findings are similar to those of previous studies, which found that infusion of dexamethasone (DEX), an artificial CORT, into the hippocampus did not affect GR translocation into the nucleus in aged rodents[5]. These findings suggest that the ability to regulate stress responses *via* GR translocation in the hippocampus is attenuated in aged SAMP10, probably leading to an excessive enhancement of the HPA axis responsiveness and persistently high plasma CORT levels. These data reinforce the notion that age-related changes in stress responses, as well as cognitive function, are enhanced in SAMP10.

The present study found that dietary ARA attenuated the age-related reduction of GR translocation into the nucleus in the hippocampus after stress loading. The ratio of GR protein levels in the nucleus and cytosol in aged SAMP10 mice with ARA is comparable with that in young SAMP10 mice (Supplementary Figure S1D). This improvement might contribute to the suppression of excessive enhancement of the HPA axis responsiveness. Regarding the mechanisms that contribute to GR translocation, it has been found that deacetylation of heat shock protein 90 (HSP90) by histone deacetylase 6 (HDAC6) is required for GR translocation[32]. The expression of HDAC6 is lower in SAMP8 than in SAM-resistant mice[33], suggesting that there might be a relationship between HDAC6 and age-related reduction of GR translocation. The effect of ARA on HSP90 or HDAC6 has not yet been determined. Although reports about the influence of ARA on HPA axis are limited, it has been found that ARA promotes DEX-induced Luciferase reporter gene expression in Hela cells more strongly than DHA[34].

Considering that an increase in GR translocation into the nucleus promotes transcriptional activities, our findings are compatible with those in previous studies. However, further studies are needed to clarify how ARA affects GR translocation.

The present work has the following limitation. The experimental conditions for the evaluation of the GR translocation was slightly different from that of the HPA axis; the stress experienced by the animals in the evaluation of GR translocation was the second instance of exposure to stress, whereas the stress experienced in the evaluation of HPA axis was the first instance of exposure. However, duration of three weeks was considered enough to recover from the stress response and for the assessment parameters to return to baseline levels. Thus, the differences between the two conditions were presumed to have minimal effect. Moreover, because both conditions were evaluated in the same experimental animals, they could be strongly suggestive of the relationship between GR translocation and HPA axis.

The present study examined the effect of ARA on age-related excessive enhancement of the HPA axis responsiveness and the attenuation of age-related reduction of GR translocation into the nucleus in the hippocampus. However, a possibility exists that dietary ARA not only influences the hippocampus, but also other brain regions involved in stress response, such as the hypothalamus and pituitary, leading to the attenuation of the negative feedback dysfunction of the HPA axis. Moreover, it remains unclear whether the influences on the HPA axis and GR translocation are specific to ARA or common to long chain polyunsaturated fatty acids (LCPUFA). Further researches are, therefore, needed to clarify the characteristics of the effect of each LCPUFA, including DHA, on the HPA axis and to clarify the site specificity of the effect of each LCPUFA on GR translocation.

Besides excessive enhancement of the HPA axis, dopamine levels in the anterior cortex have been reported to decrease with concomitant deterioration of mental health and cognitive decline in SAMP10, especially under stressful condition. Dopaminergic stimulants-apomorphine and thyrotropin-releasing hormone-ameliorates this deterioration[35]. L'Hirondel et al[36] indicate that ARA promotes the release of dopamine from the striatal synaptosome and inhibits its reuptake. These changes are believed to induce the activation of the dopaminergic neuron. These reports suggest the possibility that ARA ameliorates not only mental health deterioration, but also cognitive decline by improved stimulation of the dopaminergic neuron. Further investigations on the effect of ARA on dopamine in anterior cortex and striatum, and related behavior, such as forced swimming test and water maze, will broaden the understanding of the effects and underlying mechanisms of ARA on brain function.

Conclusions

This study found that dietary ARA suppressed age-related excessive enhancement of the HPA axis responsiveness and attenuated the age-related reduction of GR translocation into the nucleus in the hippocampus after stress loading. The findings suggest that ARA-induced attenuation of age-related decline in the hippocampal GR translocation into the nucleus after stress loading may contribute to the improvement of mental health.

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Authors' Contributions

All authors designed the study. TS, SM and HT performed experiments and analyzed the data. TS drafted the manuscript, and YK, TR and HS reviewed the manuscript. All authors have read and approved the manuscript.

Ethics Approval

All protocols for animal procedures were approved by the Ethics Committee of Animal Experiment in accordance with the Internal Regulations on Animal Experiments at Suntory, which are based on the Law for the Humane Treatment and Management of Animals (Law No. 105, 1 October 1973, as amended on 2 June 2017).

Conflict of Interests

The Authors declare that they have no conflict of interests.

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