J Phys Fitness Sports Med, 5 (2): 117-122 (2016)
DOI: 10.7600/jpfsm.5.117

JPFSM: ***Review Article***

Prevention of brain aging by green tea components: Role of catechins and theanine

Keiko Unno

Department of Neurophysiology, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan

Received: February 3, 2016 / Accepted: February 15, 2016

Abstract Since aging is the most important risk factor for dementia, measures to slow the onset of brain aging are an important strategy for preventing dementia. Accumulation of oxidative damage is considered to be a major cause of aging. Catechins in green tea (GTCs) have powerful antioxidative activity. Ingestion of GTCs suppressed oxidative damage, brain atrophy and cognitive decline in aged mice. Age-related cognitive decline was significantly suppressed in mice when middle-aged mice started to drink green tea catechins. Middle-aged people are thus expected to be able to suppress brain aging by ingestion of GTCs. In addition, numerous people are stressed under various conditions. Brain aging was accelerated and lifespan shortened in experimental animals that were chronically and psychosocially stressed. Theanine, an amino acid in green tea, suppressed stress-induced aging. However, the anti-stress effect of theanine is blocked by catechins and caffeine that are main components in green tea. Daily drinking of several cups of green tea is considered to suppress brain aging. In addition, theanine-rich green tea or green tea with a lowered level of caffeine is expected to suppress stress and stress-induced aging.

Keywords : brain aging, stress, green tea, catechin, theanine

Introduction

As the elderly rapidly increase in Japan, so does the number of patients with dementia. Aging is related to various diseases such as cancer, life-style related diseases, cardiovascular disease and dementia. Dementia cannot be entirely cured at present, indicating that slowing the brain aging process is an important strategy in the prevention of dementia.

Aging after maturation is termed senescence, and the main cause of senescence is a decrease in biological functions with aging. Although genetic factors are involved in the senescence process, posteriori factors are also heavily involved, for example, the accumulation of damage caused by reactive oxygen species (ROS)[1,2], decreased immune capacity[3], changes in metabolism[4,5], mutation or chemical modification of biopolymers[6], or their deposition in tissues[7], stress due to various environmental factors[8,9], and the lack of exercise[10,11].

Green tea catechins (GTCs) have been demonstrated to prevent brain senescence in animal experiments[12-16]. Scientific evidence of the ability of GTCs to prevent brain senescence is accumulating, and the function of green tea ingestion in maintaining the brain's health gradually being revealed. Theanine, an amino acid abundant in tea leaves but not in most other plants, has been reported to have an anti-stress effect on animals and humans[17-20]. Among green tea components, the effects of catechins and theanine on brain aging are focused on in this article.

Correspondence: unno@u-shizuoka-ken.ac.jp

Oxidative stress and aging model mouse

Accumulation of oxidative stress is thought to be an important senescence-inducing factor[1,2]. Although ROS are generated continuously in the energy producing process, their concentration is kept constant by the presence of antioxidants in the body. While ROS constantly cause damage in proteins and DNA, such are rapidly metabolized, repaired, or removed. However, as the balance is gradually lost with aging between the generation of ROS on the one hand and removal on the other, damage is believed to accumulate[21]. Even though excess ROS induces oxidative damage, it has recently been found that ROS is important in signal transduction[22,23]. When the expression and regulation of ROS is abnormal, biological functions will be altered and result in senescence, neurodegenerative diseases, and lifestyle-related diseases[24].

In many elderly people, brain atrophy in the prefrontal cortex and a decrease in learning and memory abilities have been observed[25]. However, the degree of atrophy is less in elderly people who are active and practice good health, suggesting the ability to suppress brain atrophy.

There are some studies on brain atrophy carried out using animal models. However, since the lifespan of mice and rats, which are frequently used, is as short as 2 to 3 years, brain atrophy cannot be fully observed in these animals.

Senescence-accelerated mice (SAMs), that were developed by a group at Kyoto University in Japan, are inbred mice that exhibit a short-lifespan and various senescence symptoms[26,27]. A clone of the SAMP10 mouse exhibits brain atrophy in the frontal region and cognitive dysfunction with aging[12,13,28,29], showing characteristics similar to human physiological brain aging[30]. The level of superoxide, a form of ROS, was measured in the brain of SAMP10 mice, and compared with the level in SAMR1, a normal mouse[31]. The results showed that ROS generation was higher in the brain of aged mice than in young mice. ROS generation increased with aging in SAMP10 mice. Increased ROS generation is considered to accelerate senescence, suggesting that ingestion of antioxidants may suppress brain aging.

Green tea catechins

Green tea has 20-30 times higher antioxidative activity than that of blueberries and strawberries[32]. Catechin, a tannin and astringent peculiar to green tea, has potent antioxidative activity. The level of catechin in tea leaves ranges between 8 and 20% depending on cultivation and harvest conditions such as season, amount of sunshine, type of tea plant, and geography. A catechin is not a single component, but a family encompassing a variety of molecular forms. Epigallocatechin gallate (EGCG) is the most abundant catechin found in tea leaves with a composition of 5-10%. Other catechins in tea leaves include epigallocatechin (EGC) 1-5%, epicatechin gallate (ECG) 1-2%, and epicatechin (EC) 0.5-1.5% (Fig. 1). The concentration of catechins is about 60 mg/dL when green tea is eluted with hot water. In commercially available bottles of green tea, the concentration is about 50 mg/dL. Some commercial beverages have increased levels of catechins.

Anti-aging effect of GTCs

The learning and memory abilities of SAMP10 mice that had ingested GTCs in drinking water were investigated[12]. Control mice drank water without catechins. Learning ability was examined by exploring how well mice could remember taught behavior. Mice were initially placed into a lighted room. After a while, the entrance to a

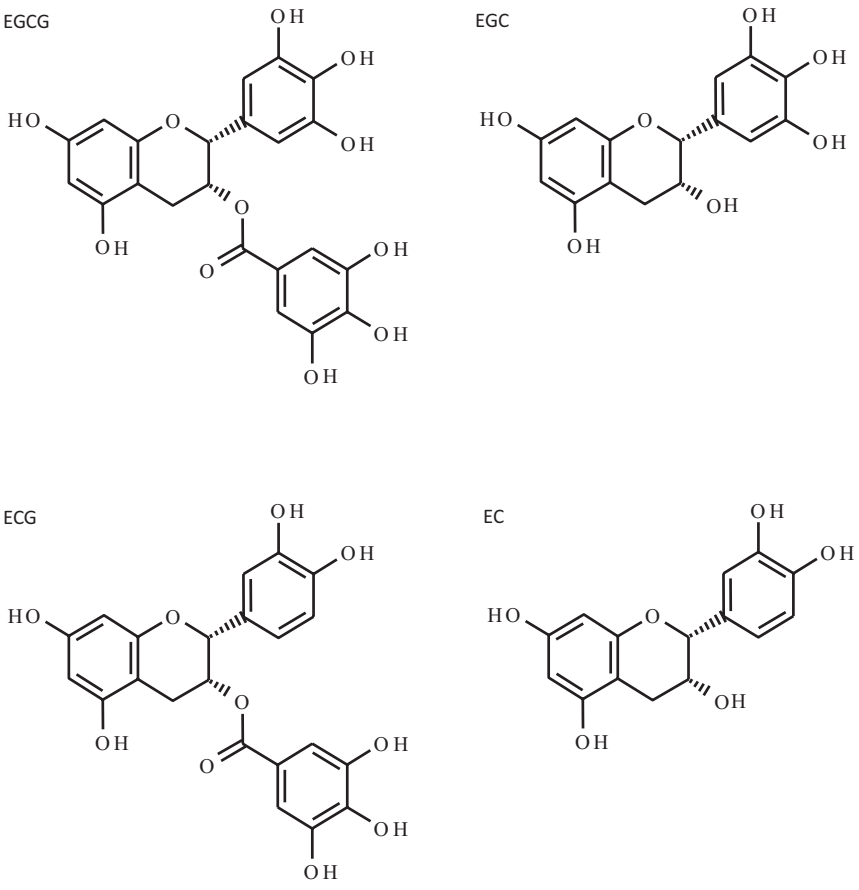

Fig. 1 Structures of GTCs
EGCG: epigallocatechin gallate, EGC: epigallocatechin, ECG: epicatechin gallate, EC: epicatechin.

dark room was opened. The mice immediately entered the dark room. A weak electric shock was delivered to mice to teach them not to enter the dark room. At that point, mice returned to the lighted room. Mice were repeatedly tested (max. 5 times) until they learned not to enter the dark room. The time for learning this task was measured. Longer learning time represented a lower learning ability. One month later, mice were tested as to whether their memory had been retained.

A decrease in learning and memory ability was suppressed in aged mice that had ingested GTCs[12]. Brain atrophy was suppressed and DNA oxidative damage in the brain was lower in mice that had ingested GTCs than in control mice[13]. The increased level of carbonyl proteins, a marker of oxidative damage in proteins, was also significantly reduced in aged mice that had ingested GTCs[33]. One possible beneficial effect of green tea catechins is prevention of a decline in the activity of glutathione peroxidase, an essential enzyme for the reduction of hydrogen and lipid peroxides. A decline in glutathione peroxidase, was prevented in aged mice that had ingested GTCs[33]. These results suggested that brain atrophy and cognitive dysfunction were suppressed by a reduction in oxidative stress as a result of the consumption of GTCs.

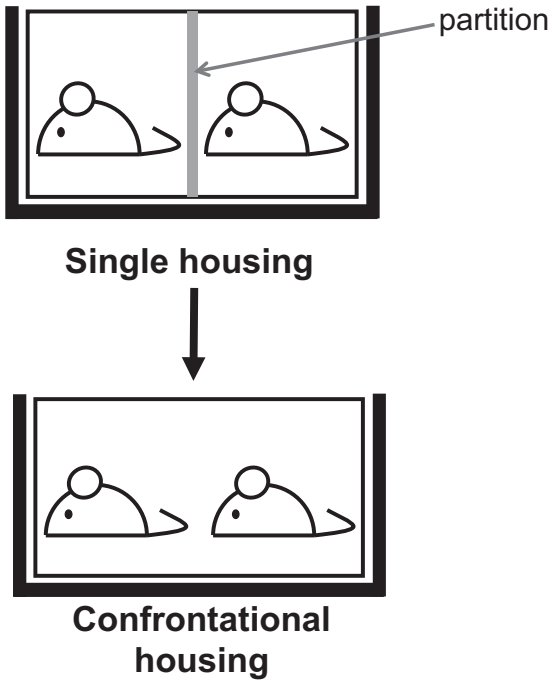

Fig. 2 Confrontational housing and psychosocial stress of mice
After the two mice were housed separately in a cage with a partition, they were housed confrontationally by removing the partition.

Psychosocial stress and aging

Modern life creates stress, and thus many people are stressed. Moderate stress sometimes exerts a positive effect, but long-term stress can trigger depression[34] and cardiovascular diseases[35,36], and also accelerate senescence. Psychosocial stress was applied to mice and the effect examined[19]. Two mice were separately housed in a cage with a partition for establishing territory (Fig. 2). Then, the mice were housed confrontationally in a cage without this partition. Mice were subjected to psychosocial stress caused when an intruding mouse co-existed in its territory. On the other hand, group-housed mice have no territorial disputes because they recognize each other as fellow mice. The results of that study demonstrated that the lifespan of mice housed confrontationally was significantly shorter than that of group-housed mice because brain atrophy and cognitive decline had been accelerated[18]. These results indicate that brain senescence can be accelerated by chronic stress. However, a shortened lifespan and cognitive decline were suppressed in stressed mice that had ingested theanine, an amino acid in tea (Fig. 3). Theanine suppresses aging by reducing psychosocial stress.

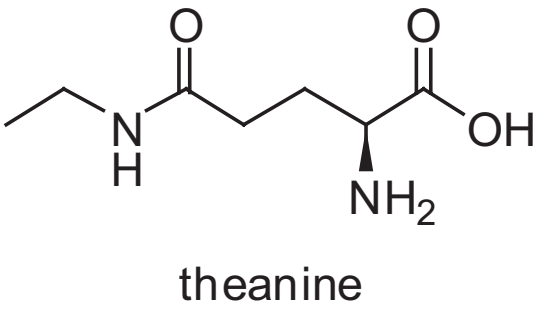

Fig. 3 Structure of theanine

Theanine has been reported to influence the level of glutamic acid (Glu), an excitatory neurotransmitter in the brain, by acting on glutamine (Gln) receptors and inhibiting the incorporation of extracellular Gln into neurons, which suppresses the conversion of Gln to Glu by glutaminase[37,38]. Glu can be decarboxylated into γ-amino butyric acid (GABA). In the hippocampus of mice that ingested theanine in drinking water for 2 weeks, the levels of Glu and pyroglutamic acid were significantly reduced; and, conversely, the level of GABA increased[39], indicating that theanine modulates GABA production from Glu. Glu is the main excitatory neurotransmitter while GABA is the main inhibitory neurotransmitter in the brain. Changes in Glu and GABA metabolism may play important roles in the control of neuronal excitation.

Since the anti-stress effect of theanine is, in part, weakened by catechins and caffeine, major components of green tea[19], the ingestion of theanine-rich or lowered-caffeine green teas would have an effective anti-stress effect. Epidemiologically, consumption of green tea has been reported to be inversely associated with psychological stress[40]. Moreover, the elderly who consumed capsules of theanine-rich green tea powder reportedly showed improved cognitive function[38].

Exercise and green tea

Lack of physical activity is an important cause of most chronic diseases[10]. A physical performance scale showed a significant association with cognitive decline, whereas skeletal muscle mass showed no significant association with cognitive decline[41]. Individuals with sarcopenia had both cognitive and physical impairments, and the effect of sarcopenia on cognition was related to low muscle strength rather than low muscle mass[42]. The combination of exercise and green tea catechin supplementation had a beneficial effect on physical function and muscle mass in women (> 75 years old, n = 128)[43]. Epidemiological studies suggest that exercise and dietary antioxidants are beneficial in reducing age-dependent neurodegenerative disorders[44,45]. EGCG ingestion and voluntary exercise, separately and in combination, were able to attenuate cognitive dysfunction in a transgenic mouse model of Alzheimer's disease[46]. On the other hand, exercise, but not a diet containing EGCG, was reported to enhanced cognition in young and aged BALB/c mice[47,48]. The effects of green tea components on cognitive function and physical activity require further research. Study of sarcopenia, senescence-accelerated (SAMP8) mice, may be useful for investigating the relationship between sarcopenia and cognitive function[49].

Healthy life expectancy, dementia and green tea

The healthy life expectancy of Japanese, i.e., the years that people can live independently without requiring care on a daily basis, was reported in 2013 by the Japanese Ministry of Health, Labour and Welfare to be 71.2 years in men and 74.2 years of age in women. The average life span in Japan for men is 80.2 years and for women is 86.6 years of age (2013, the Ministry of Health, Labour and Welfare). Japan has become a super-aged society. It is important to try and extend the healthy life expectancy to match the average life span. A model for "suppressing aging" would significantly contribute to extension of the healthy life expectancy.

A report from the Japanese Ministry of Health, Labour and Welfare estimated that the number of elderly with dementia worldwide exceeded 460 million people in 2012. In addition, this number is expected to exceed 700 million people by 2025. This number is much higher than previous estimates, and thus a more effective strategy to reduce dementia has become an urgent national health necessity.

The association between green tea consumption and cognitive function was examined in humans[44,50]. The results of those studies showed that a higher consumption of green tea was associated with a lower prevalence of cognitive impairment in humans. Furthermore, a population-based prospective study (Nakajima Project) was carried out on Japanese residents greater than 60 years of age[51]. The multiple-adjusted odds ratio for the incidence of dementia was significantly lower in individuals who consumed green tea every day compared to those who did not consume green tea at all during about 5 years of a follow-up period. Consumption of coffee or black tea showed no association with the incidence of dementia. A pilot study also suggested that green tea consumption is considered to be significantly associated with a reduced risk of cognitive decline[52]. Results demonstrating that green tea consumption is effective in improving cognitive function or reducing the progression of cognitive dysfunction have accumulated. Green tea extract may modulate brain activity in the prefrontal cortex, a key area that mediates working memory processing in the human brain[53]. However, further studies are needed to clarify the specific mechanisms caused by green tea.

Conclusions

The accumulation of oxidative damage is considered to be a major cause of aging. Catechins in green tea have powerful antioxidative activity. Oxidative damage, brain atrophy and cognitive decline were suppressed in aged mice that ingested green tea catechins. In addition, chronic psychosocial stress has been demonstrated to induce a shortened lifespan and accelerated brain aging in experimental animals. However, theanine, an amino acid in green tea, suppresses stress-induced aging. Drinking several cups of green tea daily is considered to suppress brain aging. In addition, theanine-rich or lowered-caffeine green tea is expected to suppress stress and stress-induced aging.

Conflict of interests

The author declares that there is no conflict of interests regarding the publication of this article.

References

1) Oliveira BF, Nogueira-Machado JA and Chaves MM. 2010. The role of oxidative stress in the aging process. *Scientific World Journal* 10: 1121-1128.
2) Dai DF, Chiao YA, Marcinek DJ, Szeto HH and Rabinovitch PS. 2014. Mitochondrial oxidative stress in aging and healthspan. *Longev Healthspan* 3: 6.
3) Simpson RJ, Lowder TW, Spielmann G, Bigley AB, LaVoy EC and Kunz H. 2012. Exercise and the aging immune system. *Ageing Res Rev* 11: 404-420.
4) Finkel T. 2015. The metabolic regulation of aging. *Nat Med* 21: 1416-1423.
5) Kolovou G, Katsiki N, Pavlidis A, Bilianou H, Goumas G and Mikhailidis DP. 2014. Ageing mechanisms and associated lipid changes. *Curr Vasc Pharmacol* 12: 682-689.
6) Rattan SI. 2008. Increased molecular damage and heterogeneity as the basis of aging. *Biol Chem* 389: 267-272.
7) Nowotny K, Jung T, Grune T and Höhn A. 2014. Accumulation of modified proteins and aggregate formation in aging.

Exp Gerontol 57: 122-131.
8) McEwen BS. 2002. Sex, stress and the hippocampus: allostasis, allostatic load and the aging process. *Neurobiol Aging* 23: 921-939.
9) Bauer ME, Jeckel CM and Luz C. 2009. The role of stress factors during aging of the immune system. *Ann NY Acad Sci* 1153: 139-152.
10) Booth FW, Roberts CK and Laye MJ. 2012. Lack of exercise is a major cause of chronic diseases. *Compr Physiol* 2: 1143-1211.
11) Allen J and Morelli V. 2011. Aging and exercise. *Clin Geriatr Med* 27: 661-671.
12) Unno K, Takabayashi F, Kishido T and Oku N. 2004. Suppressive effect of green tea catechins on morphologic and functional regression of the brain in aged mice with accelerated senescence (SAMP10). *Exp Gerontol* 39: 1027-1034.
13) Unno K, Takabayashi F, Yoshida H, Choba D, Fukutomi R, Kikunaga N, Kishido T, Oku N and Hoshino M. 2007. Daily consumption of green tea catechin delays memory regression in aged mice. *Biogerontology* 8: 89-95.
14) Smith A, Giunta B, Bickford PC, Fountain M, Tan J and Shytle RD. 2010. Nanolipidic particles improve the bioavailability and alpha-secretase inducing ability of epigallocatechin-3-gallate (EGCG) for the treatment of Alzheimer's disease. *Int J Pharm* 389: 207-212.
15) Lin SM, Wang SW, Ho SC and Tang YL. 2010. Protective effect of green tea (-)-epigallocatechin-3-gallate against the monoamine oxidase B enzyme activity increase in adult rat brains. *Nutrition* 26: 1195-1200.
16) Rodrigues J, Assunção M, Lukoyanov N, Cardoso A, Carvalho F and Andrade JP. 2013. Protective effects of a catechin-rich extract on the hippocampal formation and spatial memory in aging rats. *Behav Brain Res* 246: 94-102.
17) Kimura K, Ozeki M, Juneja LR and Ohira H. 2007. L-Theanine reduces psychological and physiological stress responses. *Biol Psychol* 74: 39-45.
18) Unno K, Fujitani K, Takamori N, Takabayashi F, Maeda K, Miyazaki H, Tanida N, Iguchi K, Shimoi K and Hoshino M. 2011. Theanine intake improves the shortened lifespan, cognitive dysfunction and behavioural depression that are induced by chronic psychosocial stress in mice. *Free Radic Res* 45: 966-974.
19) Unno K, Iguchi K, Tanida N, Fujitani K, Takamori N, Yamamoto H, Ishii N, Nagano H, Nagashima T, Hara A, Shimoi K and Hoshino M. 2013. Ingestion of theanine, an amino acid in tea, suppresses psychosocial stress in mice. *Exp Physiol* 98: 290-303.
20) Unno K, Tanida N, Ishii N, Yamamoto H, Iguchi K, Hoshino M, Takeda A, Ozawa H, Ohkubo T, Juneja LR and Yamada H. 2013. Anti-stress effect of theanine on students during pharmacy practice: positive correlation among salivary α-amylase activity, trait anxiety and subjective stress. *Pharmacol Biochem Behav* 111: 128-135.
21) Terman A and Brunk UT. 2006. Oxidative stress, accumulation of biological 'garbage', and aging. *Antioxid Redox Signal* 8: 197-204.
22) Demasi M, Simões V and Bonatto D. 2015. Cross-talk between redox regulation and the ubiquitin-proteasome system in mammalian cell differentiation. *Biochim Biophys Acta* 1850: 1594-1606.
23) Vara D and Pula G. 2014. Reactive oxygen species: physiological roles in the regulation of vascular cells. *Curr Mol Med* 14: 1103-1125.
24) Bhat AH, Dar KB, Anees S, Zargar MA, Masood A, Sofi MA and Ganie SA. 2015. Oxidative stress, mitochondrial dysfunction and neurodegenerative diseases; a mechanistic insight. *Biomed Pharmacother* 74: 101-110.
25) Salat DH, Tuch DS, Hevelone ND, Fischl B, Corkin S, Rosas HD and Dale AM. 2005. Age-related changes in prefrontal white matter measured by diffusion tensor imaging. *Ann NY Acad Sci* 1064: 37-49.
26) Takeda T, Hosokawa M, Takeshita S, Irino M, Higuchi K, Matsushita T, Tomita Y, Yasuhira K, Hamamoto H, Shimizu K, Ishii M and Yamamuro T. 1981. A new murine model of accelerated senescence. *Mech Ageing Dev* 17: 183-194.
27) Takeda T, Matsushita T, Kurozumi M, Takemura K, Higuchi K and Hosokawa M. 1997. Pathobiology of the senescence-accelerated mouse (SAM). *Exp Gerontol* 32: 117-127.
28) Shimada A, Keino H, Satoh M, Kishikawa M, Seriu N and Hosokawa M. 2002. Age-related progressive neuronal DNA damage associated with cerebral degeneration in a mouse model of accelerated senescence. *J Gerontol A Biol Sci Med Sci* 57: B415-B421.
29) Shimada A, Keino H, Satoh M, Kishikawa M and Hosokawa M. 2003. Age-related loss of synapses in the frontal cortex of SAMP10 mouse: a model of cerebral degeneration. *Synapse* 48: 198-204.
30) Shimada A and Hasegawa-Ishii S. 2011. Senescence-accelerated mice (SAMs) as a model for brain aging and immunosenescence. *Aging Dis* 2: 414-435.
31) Sasaki T, Unno K, Tahara S, Shimada A, Chiba Y, Hoshino M and Kaneko T. 2008. Age-related increase of superoxide generation in the brains of mammals and birds. *Aging Cell* 7: 459-469.
32) Kimura T, Yamagishi K, Suzuki M and Shinmoto Y. 2002. Relative estimation of the radical scavenging activities of agricultural products. *Nippon Shokuhin Kagaku Kogaku Kaishi* 49: 257-266 (*in* Japanese).
33) Kishido T, Unno K, Yoshida H, Choba D, Fukutomi R, Asahina S, Iguchi K, Oku N and Hoshino M. 2007. Decline in glutathione peroxidase activity is a reason for brain senescence: consumption of green tea catechin prevents the decline in its activity and protein oxidative damage in ageing mouse brain. *Biogerontology* 8: 423-430.
34) Brilman EI and Ormel J. 2001. Life events, difficulties and onset of depressive episodes in later life. *Psychol Med* 31: 859-869.
35) Jenkins CD. 1982. Psychosocial risk factors for coronary heart disease. *Acta Med Scand* 211 Suppl 660: 123-136.
36) Steptoe A and Brydon L. 2009. Emotional triggering of cardiac events. *Neurosci Biobehav Rev* 33: 63-70.
37) Kakuda T, Hinoi E, Abe A, Nozawa A, Ogura M and Yoneda Y. 2008. Theanine, an ingredient of green tea, inhibits [^{3}H] glutamine transport in neurons and astroglia in rat brain. *J Neurosci Res* 86: 1846-1856.
38) Kakuda T. 2011. Neuroprotective effects of theanine and its preventive effects on cognitive dysfunction. *Pharmacol Res* 64: 162-168.
39) Inoue K, Miyazaki Y, Unno K, Min JZ, Todoroki K and Toyo'oka T. 2016. Stable isotope dilution HILIC-MS/MS method for accurate quantification of glutamic acid, glutamine, pyroglutamic acid, GABA and theanine in mouse brain

tissues. *Biomed Chromatogr* 30: 55-61.
40) Hozawa A, Kuriyama S, Nakaya N, Ohmori-Matsuda K, Kakizaki M, Sone T, Nagai M, Sugawara Y, Nitta A, Tomata Y, Niu K and Tsuji I. 2009. Green tea consumption is associated with lower psychological distress in a general population: the Ohsaki Cohort 2006 Study. *Am J Clin Nutr* 90: 1390-1396.
41) Taniguchi Y, Seino S, Fujiwara Y, Nofuji Y, Nishi M, Murayama H, Amano H, Matsuo E and Shinkai S. 2015. Cross-sectional and longitudinal associations of physical performance and skeletal muscle mass with cognition and cognitive decline among community-dwelling older Japanese. *Nihon Ronen Igakkai Zasshi* 52: 269-277.
42) Tolea MI and Galvin JE. 2015. Sarcopenia and impairment in cognitive and physical performance. *Clin Interv Aging* 10: 663-671.
43) Kim H, Suzuki T, Saito K, Yoshida H, Kojima N, Kim M, Sudo M, Yamashiro Y and Tokimitsu I. 2013. Effects of exercise and tea catechins on muscle mass, strength and walking ability in community-dwelling elderly Japanese sarcopenic women: a randomized controlled trial. *Geriatr Gerontol Int* 13: 458-465.
44) Feng L, Li J, Ng TP, Lee TS, Kua EH and Zeng Y. 2012. Tea drinking and cognitive function in oldest-old Chinese. *J Nutr Health Aging* 16: 754-758.
45) Meeusen R. 2014. Exercise, nutrition and the brain. *Sports Med* 44 Suppl 1: S47-S56.
46) Walker JM, Klakotskaia D, Ajit D, Weisman GA, Wood WG, Sun GY, Serfozo P, Simonyi A and Schachtman TR. 2015. Beneficial effects of dietary EGCG and voluntary exercise on behavior in an Alzheimer's disease mouse model. *J Alzheimers Dis* 44: 561-572.
47) Bhattacharya TK, Pence BD, Ossyra JM, Gibbons TE, Perez S, McCusker RH, Kelley KW, Johnson RW, Woods JA and Rhodes JS. 2015. Exercise but not (-)-epigallocatechin-3-gallate or β-alanine enhances physical fitness, brain plasticity, and behavioral performance in mice. *Physiol Behav* 145: 29-37.
48) Gibbons TE, Pence BD, Petr G, Ossyra JM, Mach HC, Bhattacharya TK, Perez S, Martin SA, McCusker RH, Kelley KW, Rhodes JS, Johnson RW and Woods JA. 2014. Voluntary wheel running, but not a diet containing (-)-epigallocatechin-3-gallate and β-alanine, improves learning, memory and hippocampal neurogenesis in aged mice. *Behav Brain Res* 272: 131-140.
49) Guo AY, Leung KS, Siu PM, Qin JH, Chow SK, Qin L, Li CY and Cheung WH. 2015. Muscle mass, structural and functional investigations of senescence-accelerated mouse P8 (SAMP8). *Exp Anim* 64: 425-433.
50) Kuriyama S, Hozawa A, Ohmori K, Shimazu T, Matsui T, Ebihara S, Awata S, Nagatomi R, Arai H and Tsuji I. 2006. Green tea consumption and cognitive function: a cross-sectional study from the Tsurugaya Project 1. *Am J Clin Nutr* 83: 355-361.
51) Noguchi-Shinohara M, Yuki S, Dohmoto C, Ikeda Y, Samuraki M, Iwasa K, Yokogawa M, Asai K, Komai K, Nakamura H and Yamada M. 2014. Consumption of green tea, but not black tea or coffee, is associated with reduced risk of cognitive decline. *PLoS One* 9: e96013.
52) Ide K, Yamada H, Takuma N, Park M, Wakamiya N, Nakase J, Ukawa Y and Sagesaka YM. 2014. Green tea consumption affects cognitive dysfunction in the elderly: a pilot study. *Nutrients* 6: 4032-4042.
53) Borgwardt S, Hammann F, Scheffler K, Kreuter M, Drewe J and Beglinger C. 2012. Neural effects of green tea extract on dorsolateral prefrontal cortex. *Eur J Clin Nutr* 66: 1187-1192.