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Original Research Article

Germinated Sang-Yod Rice Improves Learning Ability and Memory of Obese Mice

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ABSTRACT

Obesity is prevalent worldwide. This might be a driving force for development of weight control recipes. In this study, effects of germinated Sang-Yod rice as food supplement on the learning ability and memory of obese mice were investigated according to Episodic-like memory task. Three groups of male C57/BL/6J mice (n = 6) were independently fed with a diet for 12 weeks as follows: CONTROL (a group of mice fed with normal diet); HFD (a group of mice fed with a high-fat diet); and HFD-GR (a group of mice fed with a high fat diet plus 1% germinated Sang-Yod rice/kg/day). The feed intake amount was recorded daily. Experiments involving the behavioral task started on the 10th week. All mice showed increased body weight over time. The body weight changes among the animal groups were not significant and the changes were not proportional to the amount of fat consumed. In comparison with HFD mice, those of CONTROL and HFD-GR groups were well in recognition of the previously explored objects and proficient in remembering the previous locations of objects and discriminating the existence of objects at different time points. Germinated Sang-Yod rice might be beneficial to obese people, if consumed daily as a supplement, by improving memory and learning ability.

Keywords: Obesity, Obese mouse model, Germinated rice, Sang-Yod rice, Episodic-like.

Introduction

Rice is mostly consumed by population of Asian countries.[1] Brown rice is obtained by removing the outermost husk of rice seed. Its colour is determined by pigments that are present in the pericarp layer.[2] Germinated rice is prepared by soaking the intact rice grains in water for 12 h and leaving in moist environment with good ventilation for approximately 24 h.[3] Several beneficial ingredients are concomitantly produced, including GABA (γ-aminobutyric acid) and amino acids such as glutamic acid, glycine, lysine, and leucine.[4] Thus, germinated rice is alternately called GABA rice. GABA is an inhibitory neurotransmitter of mammals and has a number of effects on central as well as peripheral nervous systems. Studies have demonstrated that impaired GABA signaling can affect sleep quality and abilities to relieve stress or to do planned actions. Interestingly, these worse symptoms have been improved by consuming GABA-supplemented foods.[5-7] Another study has indicated a preventive potential of GABA rice on Alzheimer's disease.[8]
With modern-day lifestyles, obesity is prevailing. Increased cell mass of adipocytes due to obesity brings about chronic/low-grad inflammation. This triggers macrophages, monocytes, lymphocytes, and mast cells to release inflammatory cytokines into blood circulation and ultimately leads to insulin resistance/glucose intolerance of cells of adipose tissues.[9,10] In turn, increased free fatty acid load occurs in all organs including in blood circulation (also called hyperlipidemia). Therefore, overall metabolisms are affected. Complications associated with metabolic defects include diabetes, cardiovascular disease, cognitive disability, sleep disorder, and joint complication, depending on the obese state of individuals.[11] Additionally, reduction of blood supply to the brain due to hyperlipidemia results in brain ischemia, followed by cognitive impairment and/or memory loss.[12] Presently, strategies for treating obesity ranges from physical activity improvement to using conventional medication, traditional medicine, and functional remedies.[1,13] In this study, impacts of germinated Sang-Yod rice as a food supplement on learning ability and memory were investigated using obese mouse model based on the Episodic-like memory task theory.[14]

Materials and Methods

Preparation of germinated Sang-Yod rice
Sang-Yod rice is a red indigenous rice variety of Thailand. Rice seeds cropped by less than 4 months were de-hulled. The intact rice acquired was gently cleaned and soaked in tap water for 12 hours in the dark. After the soaked water was drained out, the rice was wrapped by using cotton canvas and placed on a mash-basket for 24 hours to obtain 2-3 mm sprouted root. The germinated rice was dried in a hot air oven at 45^{o}C until moisture content was reduced to ~8% and ground to a fine powder by using multi-function disintegrator (WF-20B). The rice powder was kept at 4^{o}C until use.

Animal preparation
The use of animals was ethically approved by the Faculty of Medicine, University Kebangsaan Malaysia, Kuala Lumpur, Malaysia (The ethic approval number: FISIO/PP/2018/JAYAKUMAR/26-SEPT./953-OCT.-2018-DEC. -2018). Eight-teen C57/BL/6J mice, aged 6 weeks-old, were purchased from Monash University, Kuala Lumpur, Malaysia. They were acclimated 4 per cage (73 x 23 x 14 cm) in controlled conditions, e.g., 22±2^{o}C and a 12-h light/dark cycle, for 2 weeks during which they were accessible to standard chow and tap water. Each mouse was weighed and arranged in a group for a certain weight equivalent among three groups at start (n = 6), including

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CONTROL (a group of mice fed with normal diet), HFD (a group of mice fed with a high fat diet), and HFD-GR (a group of mice fed with the high-fat diet plus 1% germinated Sang-Yod rice/kg/day. The feeding programs were continued for 12 weeks. Animal diets, either normal or high fat diet, were purchased from Altromin Spezialfutter GmbH & Co. KG (Germany), and the diets' compositions were detailed in Table 1. Changes of the body weight and the amount of diet consumed were recorded daily for the entire 12-weeks period.

Behavioural investigation
The Episodic-like memory task [14] was applied to determine effects of geminated Sang-Yod rice supplement on learning ability and memory in obese mouse model as follows.

Preparation of special apparatuses
An open field: It was an acrylic open box, measuring 40 cm x 40 cm x 50 cm for wide x long x high, respectively. Six-teen similar quadrants were drawn onto the box's floor. The box was set up in a sound-proof room with homogenous illumination surrounding and placed in the room's center. The field was cleaned by using 70%v/v ethanol after each trial. A video camera was fixed to the room's ceiling exactly above the field for recording animals' movements.
Objects: Two types of objects with sufficient weight were used. The differences in terms of color, shape and surface texture, were taken into consideration. Four copies of each type were required. A type previously used in sample trials might not be used in both discrimination and test trials. Used objects were cleaned with 70% v/v ethanol after each trial.

Protocols of habituation
- A mouse was habituated to the field (no objects) for 7 consecutive days. The animal was allowed to explore the field for 1 min following its release at the field's center and returned to its home cage.
- The animal was habituated to the field (no objects) for 5 min, once per day. This task was repeated for 3 consecutive days.
- The mouse was habituated for 10 min to the field where two objects were placed at different corners. This operation was carried out three times per day with 20 min interval.

Assessment of the Episodic-like memory
For a mouse, two sample trials (called "Sample 1" and "Sample 2") and a trial either "discrimination" or "test" were performed.
In "Sample 1" trial, four copies of a type of objects were placed in triangular pattern on the field (see Figure 1). A mouse released at the field's center was allowed to explore the objects for 10 min and returned in its home cage. After 50 min, the "Sample 2" trial was started.
In "Sample 2" trial, four copies of another type of objects were used and placed on different corners of the field. The mouse released at the field's center was allowed to explore the objects for 10 min. After homing for 50 min, the discrimination trial was started.
In the discrimination trial, two copies of objects from the "Sample 1" trial (called "old familiar" objects) were placed at NE and SW corners. Other two objects from the "Sample 2" trial (named "recent familiar" objects) were located at NW and SE corners. The mouse released at the field's center was allowed to explore objects for 10 min before left it out. Animals' movements were recorded as digital files by the video camera fixed to the ceiling.

Data processing
Time durations spent by a mouse for exploring objects were analyzed manually from video files. Exploration was accepted when the mouse approached objects with distances of less than 2 cm by vibrissae, snout or forepaws.

Statistical analysis
Data were analyzed by using SPSS 19.0 software package and expressed as means ± standard errors. In cases when significant interaction effect was apparent, repeated measures and one-way between group ANOVA were applied and followed by Tukey's honest significant difference (HSD) test for post-hoc analysis. Statistical significance was accepted at $p < 0.05$.

Results and Discussion

The prevalence of obesity is increasing worldwide. This would be a driving force for researchers to search new recipes for weight control. By feeding mice with diets differed in % fat and % energy acquired from fat, including normal diet (4% fat, 10% energy; CONTROL group), high fat diet (35% fat, 60% energy; HFD group), or high fat diet supplemented with 1% germinated Sang-Yod rice/kg/day (HFD-GR group), for 12 weeks, it was found that their body weight increased by time. Higher weight gained was observed for mice of HFD and HFD-GR groups, in compared to CONTROL group (Figure 2). The magnitude of weight increased were HFD > HFD-GR > CONTROL, although insignificantly different among these groups of mice. Therefore, an association between high fat intakes and gaining body weight was strengthened, while supplement of 1% germinated Sang-Yod rice/kg/day to high fat diet was beneficial for weight management (Figure 2). This study is the first report on the usefulness of germinated Sang-Yod rice in weight control. Consisting with a previous literature, daily consumption of brown rice has been proposed for body weight management.[2] Molecular mechanisms underlining this weight-controlling effect are being carried out, focusing on Leptin/Akt/eNOS signaling pathway.[15]
Direct association between obesity and impaired recognition has been demonstrated in clinical practice.[16] For years, increased GABA concentrations in the brain has been the focus of drug therapies for epileptic seizures.[3] Recently, dietary supplements that contain GABA are recommended for people to help relieve anxiety and increase sleep quality.[6] GABA of brown rice can be stimulatory produced by germination.[4] In analysis of GABA content in germinated Sang-Yod rice, the level of 10 mg/100g rice was detected (unpublished data). This quantity conforms to the standard for GABA rice of Thailand (in Thai version, not cited). Consequently, the basis of Episodic-like memory task[14] was applied to determine effects of 1% germinated Sang-Yod rice as a supplement on learning ability and memory of obesity-induced mice. Mainly, different versions of objects were used and placed at different corners on an open field at various time periods. Test animals released on the field's center would explore such objects. Data of times spent by the animals were analyzed to discriminate their recognition memory, location memory, as well as temporal order memory. In Figure 3 and 4, results of the past consciousness and recognition exhibited by CONTROL mice were prior considered, indicating that the "what", "where" and "when" hypothesis of the test's assumption was fulfilled and realized. For example, these mice spent longer times for exploring the "old familiar" objects than for the "recent familiar" objects ($p < 0.02$) and were more preferable to explore the displaced "old familiar" objects than the stationary "old familiar" objects ($p < 0.037$). In the discrimination trial, animals would explore the "old familiar" objects and the displaced "old familiar" objects. It was found that HFD-GR mice explored these different objects with time durations longer than HFD mice did. In addition, HFD-GR mice spent more time to explore two "old familiar" objects than that used by HFD mice. In sum, mice of HFD group spent the shortest time period for exploring both the "old familiar" objects and the "recent familiar" objects, and the time durations of these tasks were insignificantly different. Notably, HFD-GR mice spent longer time for exploring the "old familiar" objects than for the "recent familiar" objects. There was significant difference in the times spent on these tasks by HFD-GR mice in compared to that of CONTROL and HFD counterparts. Additionally, the investigation concerning objects' preference of mice was carried out in that a couple of the stationary "old familiar" objects and the displaced "old familiar" objects were utilized. Results showed that mice of CONTROL and HFD-GR groups spent longer times for exploring the displaced "old familiar" objects than for the stationary "old familiar" objects.

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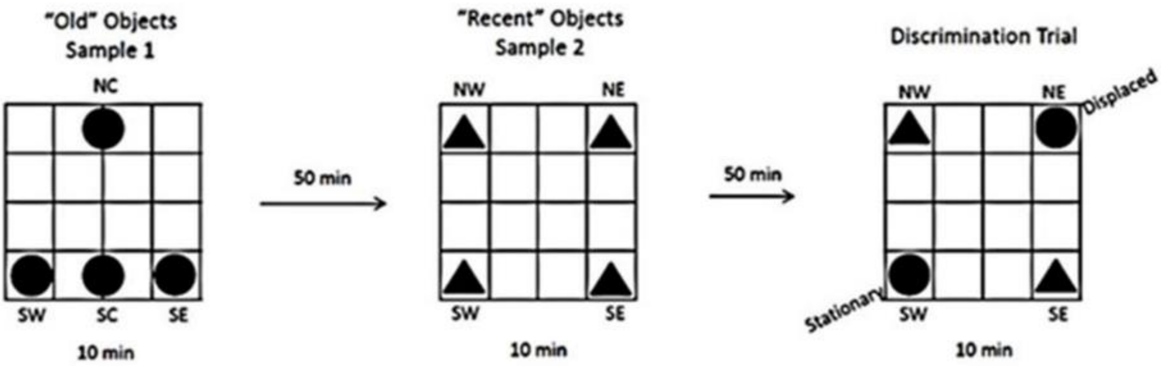

Figure 1: Schematic drawing of the "what", "where", and "when" object exploration based on the Episodic-like memory theory

Table 1: Compositions of normal diet and high fat diet (HFD) for feeding animals

On a caloric basis (per 100 g)	Normal diet (C1090–10) (per 10% energy from fat)	HFD (C1090–60) (per 60% energy from fat)
Moisture	7.9%	2.9%
Crude Ash	4.3%	3.2%
Crude Fiber	3.1%	4.7%
Crude Fat	4.0%	35.0%
Crude Protein	20.7%	21.0%
Nitrogen free extractives	60.0%	33.2%
Total calories (kcal)	351	523

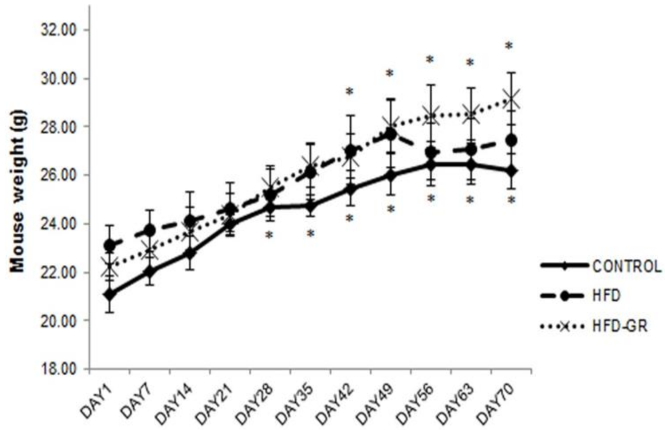

Figure 2: Changes of mice body weight in CONTROL, HFD, and HFD-GR groups; Data were mean ± SEM; *$p < 0.05$ compared within a group at the day designated

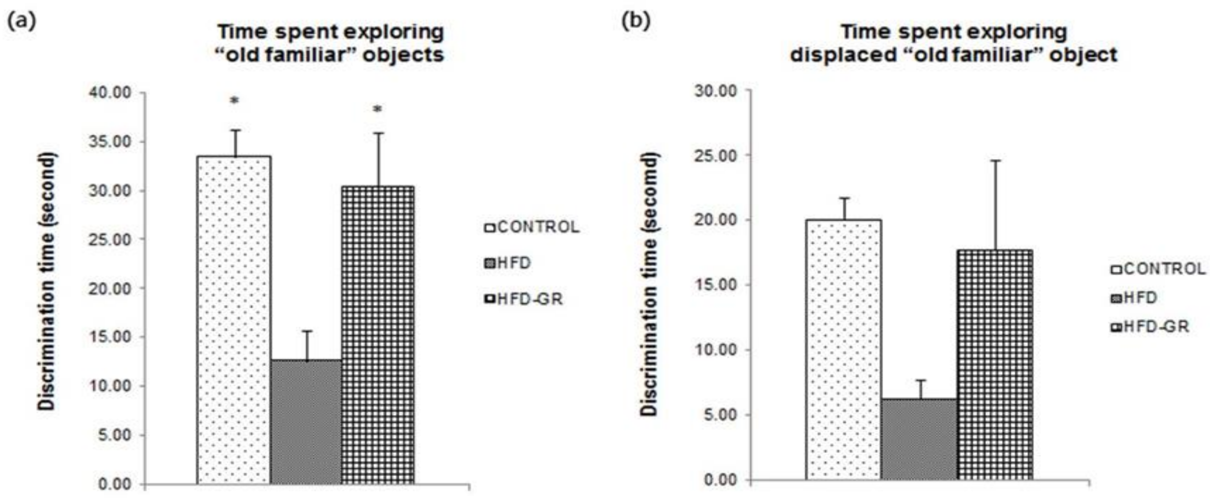

Figure 3: Data were mean ± SEM of times spent to explore the "old familiar" objects **(a)** and the displaced "old familiar" objects **(b)** of mice in CONTROL, HFD, and HFD-GR groups; *$p < 0.05$ compared to HFD group

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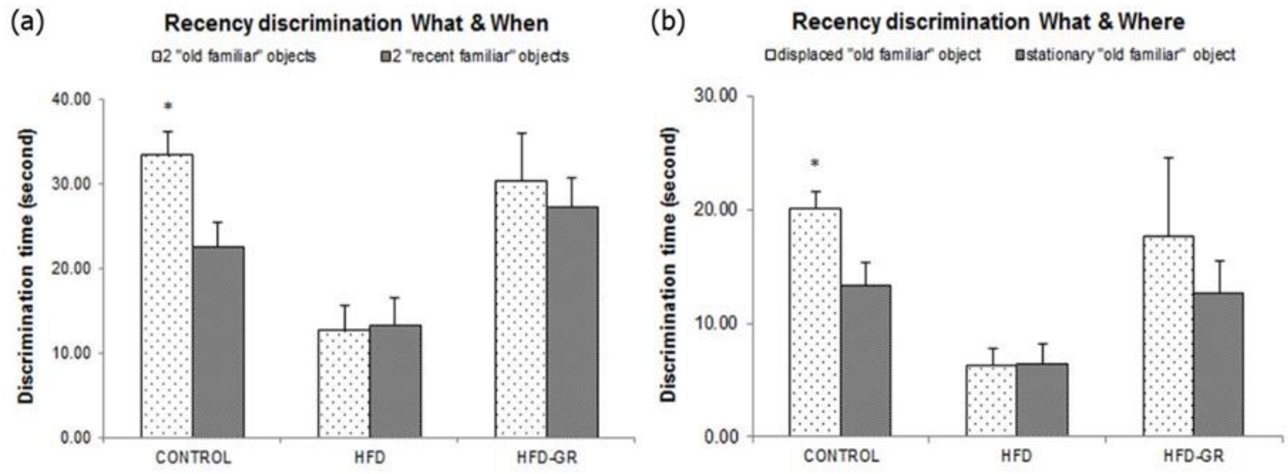

Figure 4: Data were mean ± SEM of times spent to discriminate the "what and when" and the "what and where" objects of mice in CONTROL, HFD, and HFD-GR groups; $*p < 0.05$ by one-tailed t-test for dependent groups.

Instead, HFD mice spent similar time durations for exploring both the displaced "old familiar" objects and the stationary "old familiar" objects, and these time periods were significantly shorter than that of CONTROL and HFD-GR mice. Associating these recent outcomes, it was possible for obese people to improve abilities in identification and ordering of events by germinated Sang-Yod rice supplement. However, translation of this knowledge can be restricted because GABA transporters in the blood-brain-barrier (BBB) of mice have been reported to exhibit GABA efflux rate of approximately 17 times higher than the influx rate,[17] while information regarding GABA permeability through human BBB has been unavailable at present. In fact, increased GABA concentration of human brains has been achieved by certain probiotic strains in the gut, such as *Lactobacillus* and *Bifidobacterium*, which effectively convert glutamate in glutamate-rich foods into GABA. Subsequently, the absorbed GABA is transported to the brain passing the BBB through GABA receptors of the enteric nervous system (ENS).[18] Besides GABA, glutamate has been enormously supplied by germinated Sang-Yod rice.[4] It might be beneficial to people who aim to control their weight, to increase GABA concentration in their brain, and to improve learning ability and memory by consuming GABA-rich rice as daily carbohydrate source.

Conclusion

Regarding the Episodic-like memory theory, HFD-GR mice responded well to all tasks assigned. It was implied that learning ability and memory of obese mice consuming high fat diet supplemented by 1% germinated Sang-Yod rice was improved. HFD mice had less competency in discriminating the "what and when" as well as the "what and where" past events, suggesting obesity mediated inadequate brain function. In compared to HFD mice, HFD-GR ones were competent in recognizing the previously explored objects, remembering where particular objects previously presented, and discriminating when different objects formerly shown. Such limited abilities of obesity-induced mice were mitigated by daily feeding of 1% germinated Sang-Yod rice.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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