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Original Article

Effects of Butter and Cheese on Memory and Learning in Rats

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ABSTRACT

Background and Objectives: Nutrition affects physical status, brain function, memory and learning. High-fat diets can cause memory impairment. The aim of this study was to investigate effects of cheese and butter on the memory and learning of male rats.

Materials and Methods: Totally, 24 Wistar male rats (8-week old) were divided into three equal groups and fed with high-fat chow diets (control group) or two experimental diets of butter and cheese for 12 weeks. Then, spatial memory and passive avoidance learning (PAL) were assessed using Morris water maze test and shuttle-box apparatus, respectively.

Results: In PAL test, step-through latencies in retention (STLr) test and time spent in the dark compartment (TDC) were significantly different between the groups. The step-through latencies in retention scores of the butter group were significantly lower than that of the control and cheese groups. However, dark compartment of the butter group was significantly greater than that of the control and cheese groups. In Morris water maze test, no significant differences were seen in escape latency, travelled distance and mean swimming speed of the various groups. During the probe trial, the butter groups spent significantly less time in the target quadrant than that the control and cheese groups did.

Conclusions: In general, cheese includes further favourable effects on spatial memory and PAL, compared to that butter groups do.

Keywords: Cheese, Butter, Spatial memory, Learning, Memory

Introduction

Learning and memory play fundamental roles in daily life of humans (1, 2). The basis of all training and learning depends on the process of memory. Identification of compounds that strengthen these two behavioural phenomena can help people who suffer from memory problems (3, 4). Studies have shown that diet can affect learning. Appropriate nutrition, in addition to its effects on physical function, includes tremendous effects on mental and brain functions in humans. Nutritional deficiencies may create psychological and mental illnesses (5–7). Consumption of saturated fat-rich foods and refined sugars is increasing in most countries. Excessive dietary intake of such foods can damage ability to learn and memorize (8, 9). These diets negatively affect memory and learning due to the generation of synapse plasticity defects and neurogenesis and hence harming cognitive functioning of the brain (10, 11). Studies have shown that inflammation and various cognitive and memory disorders are associated to excessive intake of refined sugars and saturated fats (8, 10–12). Inflammation is accompanied by increases in serum cytokines such as tumour necrosis factor alpha (TNF-alpha) and interleukin 6 (IL-6) (13, 14). The hippocampus plays an important role in learning and memorizing and high-fat diets (HFD) decrease neurogenesis in the hippocampus (14, 15). A HFD increases plasma free fatty acids (FFA) and induces oxidative stress due to the accumulation of lipid peroxidation in the hippocampus (15). Previous studies have shown

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that obesity and consumption of HFD for nine weeks result in memory impairment while consumption of HFD for 18 weeks results in anxiety-like behaviours (16).

In the last two decades, several studies have been carried out on factors affecting memory. Effects of HFD on memory have been well documented (8, 10, 11, 17). However, effects of these diets depend on other factors. Cheese and butter contain high levels of fat; therefore, it seems that they affect the memory ability (18, 19). Iranians believe that cheese decreases the function of memory and brain cells and dampen people. However, there are other components such as folic acid and folate, vitamins B_9 and B_{12}, B_6, D, K and E, polyphenols, omega-3 fatty acids, iron, zinc, iodine, magnesium and selenium as well as antioxidants in these foods, which may positively affect memory through various mechanisms (4, 6, 7, 20). Moreover, these compounds may not affect memory as HFD may. The aim of this study was to investigate effects of cheese and butter on the memory and learning of rats.

Materials and Methods

Animals and diets

In this study, 24 Wistar male rats (eight8-week old, weighing 262 g ±23) were used. Rats were fed with a standard chow diet for three weeks. Then, animals were randomly divided into three groups of eight rats and fed with control or experimental diets for 12 weeks. Then, effects of a high-fat *ad libitum* diet (control) and two experimental diets were assessed. To prepare the experimental diets, butter (25%) and cheese (50%) were added to a milled high-fat *ad libitum* diet. These were shaped to roll pieces of 4–8 cm, quite similar to commercial chow diets, and dried at room temperature. Fat contents of all diets (Table 1) were approximately similar to each other (nearly 24%). At the end of the 12 weeks of nutritional intervention, behavioural studies were carried out. All procedures of research and animal care were approved by the Veterinary Ethics Committee, Hamadan University of Medical Sciences (VECHUMS), and carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Behavioural studies

Passive avoidance learning (PAL) test

Assessment of the passive avoidance memory was carried out using shuttle-box apparatus with a plexiglass box divided into two light and dark compartments (TDC and TLC, respectively) for use in training and testing. Dimensions of the device included 20 × 20 × 40 cm. The TDC and TLC were separated using 8 × 8 cm guillotine door. Metal bars were installed in the floor of both compartments, connecting to an electric circuit in TDC. A 100-watt lamp was installed 40 cm above TLC. All training and testing steps were carried out between 10:00 and 14:00. During training, each rat was hosted in TLC for 30 s for acclimatization. Then, the guillotine door opened, allowing the rat to go to TDC. After the rat entered TDC, the door closed and the animal was hosted for 30 s. Then, the rat was referred to its cage. The rats that did not enter TDC within 120 s were excluded from the experiment. The entrance latency to TDC (step-through latency, STLa) was recorded. The habituation trial was repeated after 30 min. The first acquisition trial was carried out 30 min later. In this step, the guillotine door was shut after the rat entered TDC and then the animal was foot shocked (50 Hz, 0.8 mA) for 2 s. After 20 s, the rat was removed from the apparatus and transferred to its cage for 2 min. The acquisition trial was repeated and the acquisition of the passive avoidance response was considered successful if the rat did not enter TDC within 120 s. Numbers of electrical shocks and trials were documented (21–24). A retention test was carried out to assess long-term memory 24 h after training (21). Each rat was hosted in TLC, the guillotine door opened after 5 s and the step-through latency in the retention test (STLr) and the time spent in TDC were documented. Test terminated when the animal either entered TDC or stayed in TLC for 300 s, indicating retention of the passive avoidance response. Electric shocks were not delivered while carrying out the retention test (25, 26).

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Morris water maze test

This test was used to assess spatial memory function of the animal (25). For assessment, a black cylindrical pool with a diameter of 180 cm and a height of 60 cm was filled with water (22 °C ±1) to a depth of 25 cm and divided into four equal quadrants. A black circular escape platform (with a diameter of 15 cm) was submerged in the middle of one of the quadrants at a depth of 2 cm below water. This quarter was considered as the target quarter. The pool was located inside a low-light room. As visual indications, signs with various geometric shapes were set on the four surrounding walls. To assess spatial memory, the animals were trained for four consecutive days with three trials per day as follows. The animal was soaked in water randomly from one of the four sides of the pool. The animal was allowed to swim and find the platform within 60 s. The time between entry the water and finding the platform (escape latency) was recorded. If the animal was unable to find the platform within the specified time at any stages, the experimenter guided the animal to the platform. Rats spent 30 s on the platform and were then transferred to their cages for the next step. During four consecutive days of training, animals were subjected to three trials daily with submerged platform in the pool. All movements of the animals were recorded using video camera (Nikon, Melville, NY, USA) connected to a computer. On Day 5, each rat did a 60-s probe trial and a visible platform trial. No platforms were present during the probe trial. The escape latency (the time until reaching the target platform), travel distance, average swimming speed and average time spent in target quarter as an indicator of the spatial memory function of the animal were recorded (22, 27).

Measurement of dietary compounds

Contents of moisture, protein, carbohydrate and fat of each diet were analysed using oven-drying, Kjeldahl, phenol–sulphuric acid and Soxhlet methods, respectively (28).

Fatty acid analysis

After extraction of fats, methyl ester of the fatty acids (FA) was prepared and analysed according to previous studies (29). Varian CP-3800 Gas Chromatography System (Varian Inc., CA, USA) equipped with a flame ionization detector and CP-Sil 88 Columns (length, internal diameter and thickness of 100 m, 0.25 mm and 0.25 µm, respectively) were used for the analysis of FAs. Nitrogen was used as carrier (at 68 PSI) and makeup (25 mL min^{-1}) gases. Air and gas velocities included 30 ml min^{-1}, injector and detector temperatures included 260 °C and the injection volume included 1 µl. The oven temperature was set at 140 °C for 5 min and then increased to 240 °C at a rate of 4 °C min^{-1} and held for 15 min. The analysis time for FAs included 70 min.

Statistical analysis

Data analysis was carried out using SPSS Software v.20.0. The mean and standard deviation (SD) of the data were reported. One-way ANOVA was used to show differences between the groups. Comparison of means of the experimental groups with control group was carried out using Tukey's test. A $P < 0.05$ value was considered as significant level.

Results

Compositions of various diets

Compositions of various diets are shown in Table 1. Quantities of fats in control, butter and cheese groups included 24.17, 24.08 and 24.07%, respectively. In cheese group, quantity of protein (18.28%) was higher than that of two other groups while it included the lowest quantity of carbohydrate (28.43%).

Quantities of saturated and unsaturated fatty acids

Quantities of saturated and unsaturated fatty acids (SFA and UFA, respectively) in diets are shown in Table 2. The highest quantities of SFA (55.62%) and polyunsaturated fatty acids (PUFA) (49.47%) were detected in butter diets and in control groups, respectively.

Table 1. Compositions of diets in control and experimental groups (% w/w)

Diet type	Moisture	Protein	Carbohydrate	Fat
Control (high-fat chew diet)	13.70±2.16	14.67±1.65	39.41±1.65	24.17±1.41
Butter diet	13.70±0.95$^{\#}$	14.38±0.65$^{\#}$	39.67±0.98$^{\#}$	24.08±1.21
Cheese diet	27.33±1.01*	18.28±0.74*	28.43±1.31*	24.07±0.75

Various superscript small letters showed significant differences within a column ($P < 0.05$). * and # indicated statistically significant differences ($P < 0.05$), compared to control and cheese groups, respectively.

Table 2. Fatty acid compositions of diets in control and experimental groups

Fatty acid	Control	Butter	Cheese	*P* value
∑SFA	16.08±0.19	55 .62±1.36$^{*\#}$	40.63±1.16*	<0.001
∑MUFA	26.01±1.03	29.43±0.67$^{*\#}$	37.96±1.09*	<0.001
∑PUFA	49.47±1.78	7.87±0.18$^{*\#}$	16.77±0.48*	<0.001
∑n-9 UFA	25.09±0.94	26.47±0.61$^{*\#}$	33.02±0.95*	<0.001
∑n-6 PUFA	42.7±0.18	7.56±0.17$^{*\#}$	14.81±0.42*	<0.001
∑n-3 PUFA	4.38±0.01	0.31±0.01$^{*\#}$	1.95±0.06*	<0.001
∑ trans	0.58±0.08	2.96±0.07$^{*\#}$	4.94±0.14*	<0.001
∑n-6 PUFA/∑n-3 PUFA	9.72±0.21	25.16±0.5$^{*\#}$	7.73±0.15*	<0.001

* and # indicated statistically significant differences(P<0.05), compared to control and cheese groups, respectively. One-way ANOVA and Duncan's test were used to show differences within the groups. SFA, total saturated fatty acid; total MUFA, total monounsaturated fatty acid; total PUFA, total polyunsaturated fatty acids; P/S, the ratio of polyunsaturated fatty acids to saturated fatty acids

Passive avoidance learning

Results of statistical analysis showed no significant differences in the numbers of trials (Fig. 1A) conducted to achieve learning in passive avoidance test in control, butter and cheese groups (1.53, 1.44 and 1.21, respectively). Step-through latencies in acquisition (STLa) test did not differ within the various groups (Fig. 1B). The retention test, which was carried out 24 h after training, showed a significant difference in step-through latencies in acquisition (STLr) test within the groups (P<0.05). The STLr of the butter group (220.44s±6.32) was significantly lower than that of control (278.96s±10.01) and cheese (248.6s±10.51) groups. A statistically significant difference was seen in the variable of time spent in TDC within the experimental groups. Results showed that TDC of the butter group (152.04s±7.21) was significantly greater than that of control (85.01s±5.92) and cheese (135.2s±6.24) groups (P<0.05).

Morris water maze

In Morris water maze test (Fig. 2A), findings showed no significant differences within escape latency of the various groups (data of Day 4 included control group, 34.15s±3.42; butter group, 37.14s±2.72; and cheese group, 30.9s±2.09). Therefore, it can be concluded that the rats in all groups were able to learn the labour. Moreover, statistical analysis showed no significant differences in travelled distance (Fig. 2B) in Morris water maze test in all groups (data of Day 4 included control group, 701.25±43.20 cm; butter group, 914.12±33.71 cm; and cheese group, 650.55±29.76 cm) and mean swimming speed (control group, 2.82±0.12s; butter group, 2.95±0.09s; and cheese group, 3.09±0.08s). During the probe trial, time spent in the target quadrant (Fig. 2) showed significant differences within various groups. The butter group (18.18s±1.75) spent a significantly lower time in the target quadrant than that cheese (23.13s±1.52) and control (23.65s±1.25) groups did.

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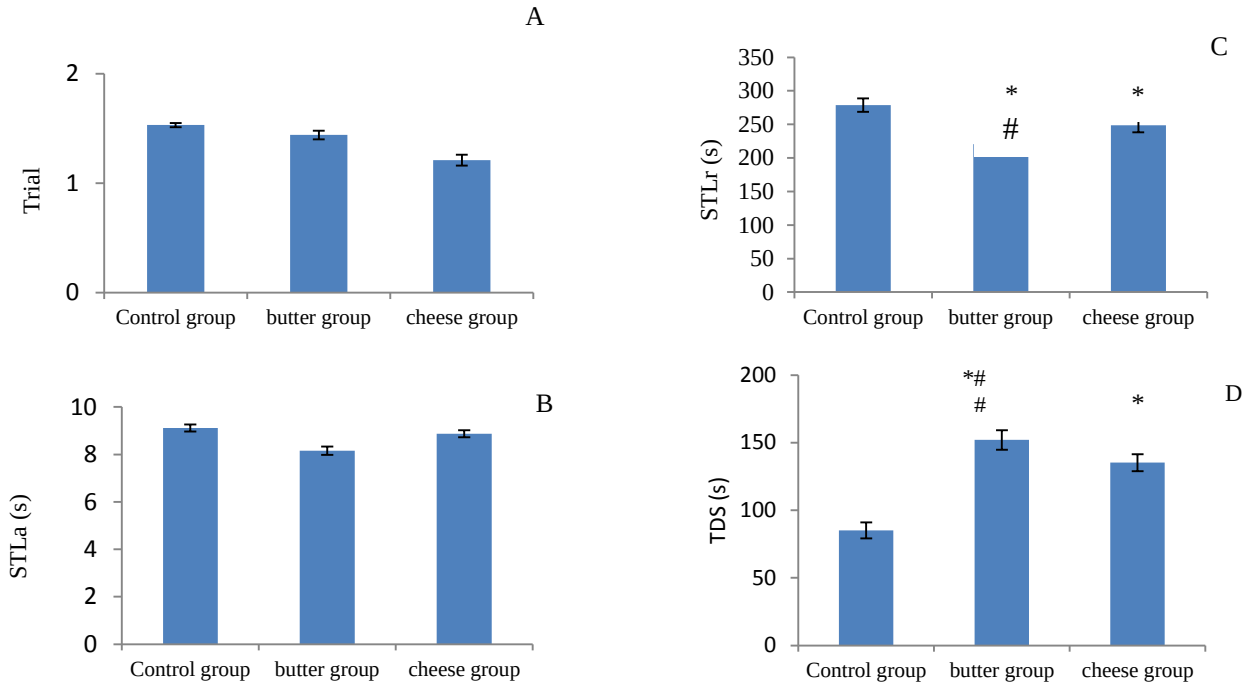


Figure 1. Numbers of trials to achieve learning in passive avoidance test (A), step-through latency in acquisition trial (STLa) (B), step-through latency in retention trial (STLr) (C), and time spent in the dark compartment (TDC) of the passive avoidance learning test (D). Each column and bar represents mean ±SD (standard deviation).
* and # indicated statistically significant differences ($P < 0.05$), compared to control and cheese groups, respectively

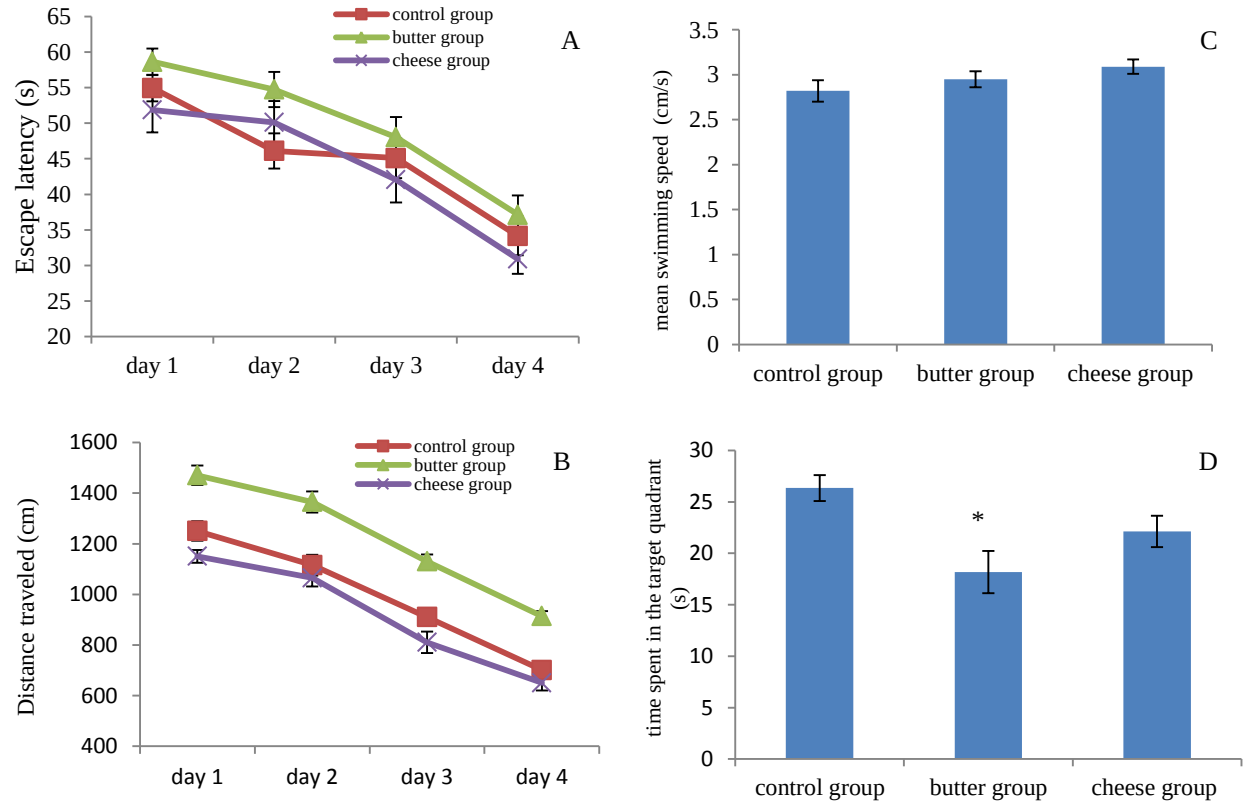


Figure 2. A) Escape latency; B) distance traveled; C) mean swimming speed; and D) time spent in the target quadrant in Morris water maze test. Each column or point and bar represents mean ±SD (standard deviation).
*Statistically significant differences, compared to control group ($P < 0.05$)

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Discussion

Learning ability and memory are human vital characteristics and scientific advancements. Dairy products are important groups in food pyramid and effectively contribute to provide human nutritional requirements (18, 30). Cheese is a highly nutritious dairy product including many varieties. For example, Iranian white cheese included the highest production and consumption rates within various cheeses available in Iran. During cheese ripening, some proteins break down and convert into peptides with antioxidant properties (31). Epidemiological studies have shown that diets with antioxidant compounds can improve memory performance (20, 32). It is believed that cheese affects human memory. However, a little research have been carried out on this issue. The present study was carried out to investigate effects of butter and cheese on spatial and avoidance memory of rats. The shuttle-box apparatus and Morris water maze were used to assess spatial and avoidance memory. Previous studies have shown that SFA and cholesterol damage cognitive memory and hippocampus morphology in rats (33). In the current study, fat contents of various diets acted similarly (approximately 24%); however, types of the FA varied between various diets. Diets containing butter included the highest levels of SFA (55.62%), followed by cheese diets (40.63%). Results of the assessment of learning and memory tests in the present study indicated inverse relationships of the learning and memory with the quantities of dietary SFA. In addition to effects of FA on memory, free radicals have been shown to be generated during metabolism and mitochondrial respiration resulting in oxidative stress and causing abnormal brain function in aging. Oxidative stress during aging causes oxidation of lipids and proteins in central nervous system (CNS); thus, damaging the brain cells (34). Quantities of the free radicals produced in various regions of the brain such as hippocampus, which includes a greater oxygen consumption, are higher in older rats than younger rats (35). Zare et al. (2011) found that oils such as sesame oil with higher UFA included better effects on passive avoidance, compared to that SFA and cholesterol-rich oils such as butter oil did. A possible reason is attributed to effects of the highlighted compounds on the oxidative stress control, changes in UFA of the neuron membranes and changes in cholesterol synthesis pathways (36).

Several nutritional factors play important roles in memory processing. Of the most important nutritional factors are antioxidant compounds such as flavonoids (37). Moreover, nutrients such as iodine, iron, zinc, folate, vitamins B_1 and B_{12}, selenium, and essential fatty acids (EFA) affect learning. Iodine is needed for the formation of thyroxin, which is an essential nutrient for the brain. Iron is necessary for oxygenation and energy production in nervous cells. Zinc plays an important role in cognitive development. Presence of vitamin B_1 is necessary for energy production and utilization of glucose by nervous tissues. Vitamin B_{12} is directly involved in synthesis of neurotransmitters. Selenium contributes to the protection of nervous tissues against aggression by free radicals (7). In general, quantities of minerals are higher in cheese than butter (38). Therefore, another possible reason for the higher memory and learning abilities of the rat groups received cheese it linked to high mineral levels of cheese. In addition, cheese contains several bioactive peptides that may affect body functions, including learning and memory (6).

Conclusion

In this study, effects of butter and cheese on memory and learning of male rats were assessed. In summary, results have shown that cheese includes more favourable effects on spatial memory and avoidance learning of rats, compared to that butter does. However, further comprehensive studies on long-term effects of diets seem necessary. It is suggested to assess effects of these foods on various parts of the CNS. Since the current study was carried out on rats, it is necessary to investigate effects of these diets on humans to achieve further realistic results.

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The authors declare no financial interest.

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