

## Effect of Butter and Sesame Oils on Avoidance Memory of Diabetic Rats

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### ABSTRACT

**OBJECTIVE:** Diabetes is a metabolic disorder which impairs carbohydrate and lipid metabolism. It is accompanied by cognition impairment in diabetic patients and animal models. Lipids play an important role in nerve membrane composition, structure and function. This study was designed to evaluate the effect of dietary butter and sesame oils on passive avoidance memory of streptozotocin (STZ) diabetic rats.

**MATERIALS AND METHODS:** Thirty six adult male Wistar rats were randomly allocated to four groups: normal control (NC) and diabetic control (DC) with free access to regular rat diet; and diabetic sesame oil (DS) and diabetic butter oil (DB) groups with diet supplemented by 10% butter oil and 10% sesame oil, respectively. Diabetes in DC, DS and DB rats was induced by IV injection of 50 mg/kg (body weight) STZ. Passive avoidance memory was tested six weeks after confirmation of diabetes, and cholesterol and phospholipids were measured in hippocampal tissue.

**RESULTS:** Diabetes, especially in diabetic butter oil group decreased learning and memory. The levels of cholesterol and phospholipids in hippocampus were higher in diabetic control and diabetic butter oil ( $P<0.05$ ) groups in comparison with normal control.

**CONCLUSION:** Consumption of butter may worsen diabetic cognition impairment and the elevation of cholesterol may be a reason for diabetic cognition impairment.

**KEYWORDS:** diabetes, avoidance memory, butter oil, sesame oil, cholesterol, phospholipids, hippocampus

### INTRODUCTION

Diabetes mellitus, a major endocrine disorder and a growing health problem in most countries, is now emerging as a deadly disease. Hyperglycemia leads to long-term complications of diabetes, which are among the major causes of morbidity and mortality in human populations (1). Diabetes has important effects on carbohydrate and lipid metabolism (2). Impairments in learning, memory,

problem solving, mental and motor speed are more common in type 1 diabetic patients than general population (3). Cognitive deficits (4) and poor performance in abstract reasoning and complex psychomotor functioning (5) occur in type 2 diabetes (4). Impaired spatial learning and memory occur in animal models of both types of diabetes (6). In the hippocampus of streptozotocin – induced diabetic rats, long-term potentiation is

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impaired, whereas long-term depression is enhanced, indicating altered hippocampal synaptic plasticity, which is associated with deficits in spatial learning and memory (7). Under certain conditions, dietary fatty acids may induce changes in neurophysiological, cognitive and other behavioral variables (8). Dietary supplementation by a particular proportion of a mixture of n-3/n-6 polyunsaturated fatty acids (PUFAs) exerts many beneficial effects, such as reduced cholesterol level and increased level of PUFA in the neuronal membrane (9). It is suggested that high-fat diet may decrease memory and learning ability by changing the brain fatty acid composition (10). The structural lipids of the mammalian brain are unique and different from other tissues in terms of their high proportion of PUFAs, particularly arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3, DHA). The precursors of these PUFAs, linoleic acid (18:2n-6) and  $\alpha$ -linolenic acid (18:3n-3), can not be synthesized de novo by mammals, and are therefore considered essential fatty acids. Long-term deficiency in  $\alpha$ -linolenic acid leads to changes in brain fatty acid composition, especially to a decrease in DHA, with a loss of learning ability (11). The activity of arachidonic acid is important in controlling the expression of LTP at the hippocampal synapses (12).

The fatty acid composition of milk fat typically comprises 70% saturated fatty acids, 25% monounsaturated fatty acids, and 5% polyunsaturated fatty acids (13). Milk butter is also rich in cholesterol (14). consumption of more abundant saturated milk fatty acids, myristic (14:0), palmitic (16:0), and lauric (12:0) increases concentrations of LDL-c, whereas consumption of more unsaturated fatty acids has the reverse effect (15). Sesame oil is mostly comprised of mono and polyunsaturated fatty acids (43.3% and 41.1%, respectively), in which oleic (39.09%) and linoleic (40.39) are the most abundant with high content of phytosterols (16).

Since cholesterol uptake is blocked by blood brain barrier, de novo synthesis is responsible for the cholesterol content of brain (17).

Although there are lots of studies that have searched the effects of cholesterol on learning and memory, there is so many controversies in this area, focused on beneficial (18, 19) or adverse (20, 21, 22) effects of cholesterol. We did not find any report about the effect of oils, especially butter and sesame oils on diabetic animal cognition and cholesterol and phospholipids content of hippocampus, thus this study was conducted to compare the effect of butter oil and sesame oils on avoidance memory, cholesterol and phospholipids content of hippocampus in normal and diabetic rats.

## MATERIALS AND METHODS

### Animals

Male Wistar rats (180–200g), from laboratory animal breeding council of Jundishapur University, were used in this study. The animals were kept in a controlled environment under standard conditions of temperature at  $23\pm 1^\circ\text{C}$ , alternating light/dark repeated every 12h with food and water freely available throughout the study. Diabetes induced by IV injection of 50 mg/kg streptozotocin (STZ), dissolved in 0.1 M citrate buffer (PH=4.5). Non-diabetic animals were injected by the same volume of citrate buffer, as diabetic rats. One week later blood glucose of all rats was measured by glucometer (Bionime), and those with plasma glucose greater than 300 mg/kg considered as diabetic.

Forty rats were assigned to four experimental groups. Normal (N) and diabetic (D) groups had unlimited access to a normal diet, prepared manually according to the rat energy, protein, vitamins and minerals requirements (Table 1). Ten percent sesame oil and butter oil (prepared by heating butter and removal of

**Table1- Composition of diet**

|                | Normal diet | Sesame oil diet | Butter oil diet |
|----------------|-------------|-----------------|-----------------|
| Energy (ME/Kg) | 3010        | 3653            | 3552            |
| Protein (%)    | 17.70       | 19.50           | 19.50           |
| Fat (%)        | 5           | 14.05           | 14.05           |
| Fiber (%)      | 5.6         | 5.2             | 5.2             |

the basal water) were added to the diet of diabetic butter (DB) and diabetic sesame (DS) oil groups, respectively. protein, vitamins and minerals of the diets were balanced according to the energy changes (Table 1). Six weeks later avoidance memory was tested. All rats sacrificed at seventh week, their brains were removed immediately and put at  $-20^{\circ}\text{C}$  until assay of cholesterol, phospholipids and protein in hippocampus.

### Avoidance memory

The experimental device was a  $30\text{cm}\times 30\text{cm}$  electronic avoidance-response chamber with 50 cm height, made of plexiglass. The chamber had a bottom of parallel 2 mm stainless steel bars spaced 0.5 cm apart. A platform (height: 5 cm, diameter of its top surface: 7 cm) was fixed at center on the bottom of the chamber, providing rats a shelter from the electronic attack. Before normal test, rats were continually trained in a one trial step-down inhibitory avoidance task for 4 times (once/day, conducted between 10:00 and 12:00 am), and tested for their memory retention of the escape platform from electronic attack at the same time 72 h after training. Rats were placed on the platform, and their latency to step-down, first placing their four paws on the grids, was measured. In training sessions, immediately upon stepping down, the rats received a 0.5 mA, 2s foot shock. No foot shock was given in test sessions (23). Step-down latencies and errors (during 2 min) were taken as a measure of memory retention.

### Biochemical parameters

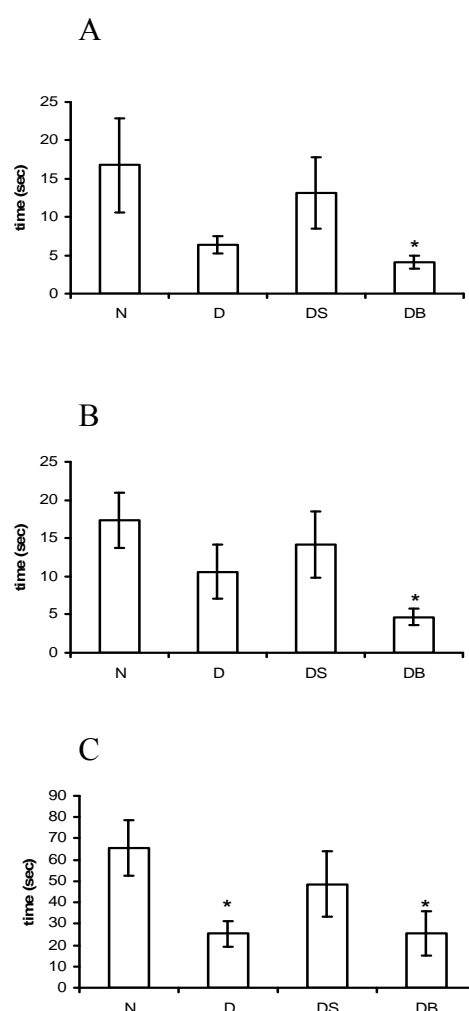
Right and left hippocampi were carefully separated from the brain and their lipid was extracted by the method of Folch (24) to measure phospholipids and cholesterol. Total protein of the hippocampus was measured in non-lipid phase. Phospholipids, Cholesterol and protein were measured by the method of Stewart (25), Loeffler and Mc Dougald (26) and Bradford (27), respectively.

### Statistics

The data was analyzed by SPSS 16, showed as mean  $\pm$  SEM and compared by one way ANOVA, post hoc test LSD and P values of less than 0.05 were considered to be significant.

### RESULTS

There was not any significant difference between step-down latencies of any groups at the first and second training sessions. At the third and fourth training sessions (figure 1A and 1B) step-down latencies in N group ( $16.78\pm 6.12$  and  $17.33\pm 3.66$ ) was greater



**Figure 1-Effects of butter oil and sesame oil on step down latencies of normal (N), diabetic (D), diabetic+butter oil (DB) and diabetic+sesame oil (DS) groups. A: Third training session; B: fourth training session; C: test session (N=9 rats). Asterisks (\*) indicate significant differences with normal group ( $p < 0.05$ ).**

**Table 2- Effects of butter and sesame oils supplemented diet on hippocampal level of cholesterol (mg/g protein) and phospholipids (mg/g protein)**

| Group       | phospholipid   | cholesterol     |
|-------------|----------------|-----------------|
| N<br>(n=5)  | 10.0 ± 2.10 ** | 22.0 ± 2.48 *** |
| D<br>(n=5)  | 16.0 ± 2.70    | 33.0 ± 6.24 *   |
| DS<br>(n=5) | 13.6 ± 1.39 *  | 27.1 ± 1.93 **  |
| DB<br>(n=5) | 21.8 ± 2.90    | 64.2 ± 10.09    |

Asterisks: indicate significant differences with DB group.

\*: P<0.05, \*\*: P<0.01 and \*\*\*: P<0.001

than DB groups ( $4.11 \pm 0.81$  and  $4.67 \pm 1.10$ ) ( $p < 0.05$ ), but the step-down latencies of D ( $6.36 \pm 1.14$  and  $10.6 \pm 3.57$ ) and DS ( $13.11 \pm 4.69$  and  $14.22 \pm 4.34$ ) groups were not significantly different from other groups. At the test session (figure 1C) step-down latency of group N ( $65.56 \pm 13.19$ ) was greater than groups DB ( $25.33 \pm 10.44$ ) and D ( $25.44 \pm 5.96$ ), ( $P < 0.05$ ) but the analysis failed to show a significant difference with DS groups ( $48.55 \pm 15.38$ ). Table 2 shows that the level of hippocampal cholesterol increased significantly in DB group in comparison with N ( $P < 0.001$ ), DS ( $P < 0.01$ ) and D groups ( $P < 0.05$ ). The hippocampal content of phospholipids was increased as well in DB group in comparison with N ( $P < 0.01$ ) and DS ( $P < 0.05$ ) groups.

## DISCUSSION

As showed in figure 1 (A – C) diabetes has decreased memory retention, and consumption of butter oil not only worsened it but also decreased learning in diabetic animals. However, supplementation of the diet of diabetic groups with 10% sesame oil moderately prevented memory reduction in diabetic groups.

The type of fatty acids in the diet determines the type of fatty acids available for the composition of membrane phospholipids. A phospholipid made of saturated fatty acids has a different structure and is less fluid than a phospholipid including unsaturated and essential fatty acids (28, 29). The more

membrane fluidity the more increase in memory (30). In the present study some detrimental effects of diabetes on memory may have been prevented by increasing membrane fluidity in DS group.

Oxidative stress is involved in the pathogenesis of many central nervous system disorders (e.g. neurodegenerative diseases) or in the physiological process of aging (31). According to the literature, brain is very vulnerable to oxidative stress due to its high polyunsaturated fatty acids (PUFAs) content which are particularly susceptible to reactive oxygen species (ROS) damage (32, 33). Sesame oil, in comparison to other dietary oils such as peanut and sunflower, offers better protection against increased blood pressure, hyperlipidemia and lipid peroxidation by increasing enzymatic and non-enzymatic antioxidants (34). It is strongly believed that the protective effect of sesame oil is due to the presence of lignans (sesamin, sesamol and sesamolol) and vitamin E in it (35, 36). Diabetes is associated with an increased production of reactive oxygen species (ROS), enhanced oxidative stress and changes in the antioxidant capacity (22). Increased TBARS (lipid peroxidation productions) of frontal cortex and hippocampus may lead to apoptosis of nerve cells, which eventually affects the function of learning and memory, because lipid peroxidation induces changes in the structure and function of biological membranes resulting in a change of the structural arrangement of membrane lipids (37). Impairment of learning and memory of D and DB groups may be related to the increased oxidative stress in diabetic animals brain, but sesame oil as an antioxidant may reduce the oxidative stress, and lead to the better behavioral activity of the animals in DS group. Cholesterol is ubiquitous in the central nervous system (CNS) and vital for such brain functions as signaling, synaptic plasticity, learning and memory. Cholesterol is so important for brain function that it is generated independently of cholesterol metabolism in the rest of the body and is sequestered from the body by the blood brain barrier (BBB). A large

number of studies confirmed that systemic cholesterol levels do not influence cholesterol in the CNS (38, 39). However, there are many reports focused on the opposite effects of cholesterol on learning and memory. Diet high in cholesterol and fat for eight weeks resulted in deficits in working memory in the water maze (40). Rats, mice and rabbits given calcium channel blockers that reduce the esterification of cholesterol and increase the hydrolysis of existing cholesterol esters (41, 42), demonstrate improvements in a number of learning and memory paradigms including passive avoidance (43).

Clinical and experimental studies suggest that hyperglycemia and/or insulin-deficiency itself may be responsible for impaired cognitive function in type 1 diabetes (44). It is more susceptible to demonstrations of dyslipidemia in diabetic animals than in normal controls by replacing normal diets with high-fat diets (45). However, whether a high-cholesterol diet plays a major role in diabetic brain remains unknown. There are some conflicting data on the relationship between the level of cholesterol circulating in the plasma and the development of disorders of the central nervous system (CNS) such as Alzheimer's disease. Some studies suggest that there is a relationship between them (46), while there was no correlation in other reports (47). In the present study diabetes especially in the DB group which its diet was supplemented by butter oil decreased memory and increased cholesterol in the hippocampus. Hyperglycemia increases permeability of blood-brain barrier (48), then the increased blood LDL-c may cause cholesterol depositing in brain though it is reported that plasma cholesterol cannot affect the level of cholesterol in brain (49). Nerve cell

dysfunction would be induced and abilities of learning and memory would be impaired due to the enhanced toxicity of hyperglycemia and hypercholesterolemia (22). On the other hand, high-fat diets, which promote the development of insulin resistance and glucose intolerance, may be associated with memory deficits (50). PL plays an important role in brain but the elevated PL levels may cause the accumulation of cholesterol because PL has a relatively strong affinity for cholesterol and have been suggested to be the cause of the high concentration of cholesterol at the cell surface (51). Increased cholesterol in brain is closely correlated with dementia (52).

## CONCLUSION

According to our data different type of oils besides to their fatty acid composition, cholesterol content and anti-oxidative properties have different effects on cognition in diabetic animals. We suggest oils with more unsaturated fatty acids, less cholesterol and anti-oxidative function (e.g. sesame oil) which may improve behavior, whereas oils with more saturated fatty acids and cholesterol (e.g. butter oil) may have detrimental effects on cognition of diabetic animals. These differences may be related to their potency in altering oxidative balance, changing fatty acid content of neuron membrane and stimulating pathways leading to cholesterol and phospholipids synthesis.

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