A comparative study on the effect of high cholesterol diet on the hippocampal CA1 area of adult and aged rats

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Abstract: Dementia is one of the most important problems nowadays. Aging is associated with learning and memory impairments. Diet rich in cholesterol has been shown to be detrimental to cognitive performance. This work was carried out to compare the effect of high cholesterol diet on the hippocampus of adult and aged male albino rats. Twenty adult and twenty aged male rats were used in this study. According to age, the rats were randomly subdivided into balanced and high cholesterol diet fed groups. The diet was 15 g/rat/day for adult rats and 20 g/rat/day for aged rats for eight weeks. Serial coronal sections of hippocampus and blood samples were taken from each rat. For diet effect evaluation, Clinical, biochemical, histological, immunohistochemical, and morphometric assessments were done. In compare to a balanced diet fed rat, examination of Cornu Ammonis 1 (CA 1) area in the hippocampus of the high cholesterol diet adult rats showed degeneration, a significant decrease of the pyramidal cells, attenuation and/or thickening of small blood vessels, apparent increase of astrocytes and apparent decrease of Nissl's granules content. Moreover, the high cholesterol diet aged rats showed aggravation of senility changes of the hippocampus together with Alzheimer like pathological changes. In conclusion, the high cholesterol diet has a significant detrimental effect on the hippocampus and aging might pronounce this effect. So, we should direct our attention to limit cholesterol intake in our food to maintain a healthy lifestyle for a successful aging.

Key words: Cholesterol, Aging, Cornu Ammonis, Hippocampus, Pyramidal cells

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Introduction

Progressive disorders affecting behavior, thinking, memory and daily activities is known as dementia syndrome. The most common type of dementia is Alzheimer's disease [1]. A person who has dementia is always developing agitation and other behavioral symptoms, making it much harder to care for him [2]. In many vertebrates hippocampus forms a major component of the brains. The consolidation of information from short-term memory to long-term memory is a role of hippocampus [3]. Cornu Ammonis 1 (CA1) is the zone that is most sensitive to various insults [4]. Aging is associated with impairment in certain aspects of cognition, especially learning and memory. The hippocampus formation is one of the most brain areas affected by aging in both humans and other mammalian species [5]. Several studies demonstrated that greater intake of cholesterol increased atherosclerotic picture in target organs [6]. Thus, the present study was performed to investigate the effect of high cholesterol diet on the structure of hippocampus in the adult and aged rats.
Materials and Methods

The animals

Twenty adult (three-month-old) and twenty elderly aged (18-24 month-old) male albino rats (Wistar strain) obtained from the National Center of Researches (Cairo, Egypt) were used in this study. The rats were kept in the laboratory under constant conditions of temperature (24±2°C) for, at least, one week before and through the experimental work.

Diet

Two different types of diet (balanced and high cholesterol diets) were used in this study. They were prepared in the National Center of Researches, Nutrition Department (Table 1) [8, 9]. Cholic acid (0.25 g/100 g) was added to high cholesterol diet to increase the absorption of cholesterol [7]. Diet was given to rats as 15 g/rat/day for adult rats and 20 g/rat/day for aged rats for eight weeks [8]. The rats were put individually in separate cages to allow for the careful control of food intake for each animal. The cages were provided with white paper bedding to allow complete collection of any remaining food. The rats received food in heavy pot containers and received water ad-libitum. The food was introduced daily and any remaining food was removed, weighed and recorded.

The experimental protocol

According to age and type of diet the animals were classified into adult balanced group; adult high cholesterol diet group; aged balanced group and aged high cholesterol diet group. At the end of the experiment, all rats were fasted for 12 hours, anaesthetized and blood samples were collected from retro orbital venous plexus. The specimens of the brain were obtained after an injection of the heart by 10% formalin.

Clinical assessment

All rats were observed for activity, bowel habits, food intake and changes in body weight that were calculated by using the following formula:

\[
\text{Weight change} = \frac{(W2−W1)}{W1} \times 100 \quad [10]
\]

W1=Weight at the beginning of the experiment.
W2=Weight at the end of the experiment.

Biochemical assessment

The collected blood samples were used to detect serum levels of low density lipoprotein (LDL) [11], high density lipoprotein (HDL), cholesterol and triglycerides [12].

Histological assessment

Each brain was cut into two halves, one was fixed in 10% formalin and the other was fixed in Golgi Cox fixative and staining solution for two months. Each cerebral hemisphere was cut coronally into two halves to reach the site of hippocampus and then the specimens were dehydrated, cleared, and embedded in paraffin blocks. Serial coronal sections were cut into 5–7 μm thick and stained with haematoxylin and eosin (H&E), toluidine blue [13], and Golgi Cox [14] stains.

Immunohistochemical assessment

The paraffin embedded tissues were used for immunohistochemical localization of glial fibrillary acidic protein (GFAP), a marker of astrocytes (1:50–1:100, no: M076101, Dako, Glostrup, Denmark). Formalin fixed paraffin embedded tissue sections were deparaffinized and endogenous peroxidase was blocked with H2O in methanol. The sections were heated in 0.01 mol/l citrate buffer in a microwave pressure cooker for 20 minutes. The slides were allowed to cool to the room temperature, and nonspecific binding was blocked with normal horse serum for 20 minutes at the room temperature. The MIB-1 monoclonal antibody was used for detection of nuclear GFAP. Counterstaining was done using Mayer’s hematoxylin (Cat. No.94585, BioGenex, Antony, France) [15].

Morphometric assessment

Five different H&E-stained sections from five different rats were examined in each group to 1) count the number of pyramidal cells in H&E sections and the number of

| Composition (g/100 g) | Balanced diet | High cholesterol diet |
|----------------------|---------------|-----------------------|
| Protein              | 10            | 10                    |
| Unsaturated fat (corn oil) | 10            | 10                    |
| Cholesterol          | 0             | 2                     |
| Carbohydrates        | 74.4          | 72.4                  |
| Mineral mix.         | 3.5           | 3.5                   |
| Vitamin mix.         | 1             | 1                     |
| Methionine           | 0.1           | 0.1                   |
| Cellulose            | 1             | 1                     |

Table 1. The composition of both balanced and high cholesterol diet [8, 9]
astrocytes in GFAP-immunostained sections [8]. 2) Measure the thickness of hippocampus [16]. The data were obtained by using computerized image analyzer (Lecia Imaging System Ltd., Cambridge, England). Hippocampus sections were randomly selected for morphometric measurements. Ten readings were obtained in each specimen and the mean values were obtained.

The scoring system for toluidine blue was tested according to the following semi-quantitative scale: (−), negative; (+), very weakly positive; (++), weakly positive; (+++), moderately positive; (++++) strongly positive; (+++++), very strongly positive.

Statistical analysis
All statistical analyses were performed with SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Measurement data were expressed as mean±SD. Mann-Whitney and Wilcoxon analysis of variance were used to compare continuous variables among groups. P<0.05 was considered statistically significant.

**Results**

**General appearance and body weight of the animals**
All the rats in the adult and aged groups showed no change in their physical activity or in the bowel habits all over the period of the experiment. However, there was an increase in food intake observed from the second week till the end of the experiment in high cholesterol rats as compared to balanced rats. All the animals showed a significant increase in the body weight at the end of the experiment as compared to the beginning of the experiment (P<0.05). However, the percentage (%) of increase in the body weight was higher in the rats that received high cholesterol diet (adult 36.6% and aged 28.9%) than in those that received balanced diet (adult 6.9% and aged 5.6%) (Fig. 1).

**Biochemical results**
In both adult and aged groups, the serum of cholesterol fed animals showed a significant decrease in the level of HDL (P<0.01) and increase in the levels of LDL (P<0.01), total cholesterol (P≤0.001), and triglycerides (P<0.01), when compared with the balanced groups. However, these changes were more significant in the aged than in the adult groups (P≤0.01) (Table 2).

**Histological and immunohistochemical results**
Light microscopic results of H&E-stained sections in adult balanced diet fed rat revealed that the hippocampus is composed of four areas; CA1-CA4 (Fig. 2A). Each area appeared in three layers; polymorphic, pyramidal, and molecular. Capillaries and different glial cells scattered inside molecular and polymorphic layers (Fig. 3A). Microglia, oligodendroglia and astrocytes constituted the glial cell types (Fig. 4A). The pyramidal nerve cells appeared as large triangular cells with large vesicular nuclei and prominent processes (Fig. 5A). Comparing the hippocampus of adult balanced diet fed rats with aged balanced diet fed rats, it could be noted that the aged rats showed a significant (P<0.01)

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**Table 2.** Mean±SD of the serum levels (in mg/dl) lipid profile in experimental groups

|                  | LDL | HDL | Cholesterol | Triglyceride |
|------------------|-----|-----|-------------|--------------|
|                  | Adult |     |              |              |
| Balanced group   | 66.5±0.7 | 79.9±1.1 | 22.4±0.8 | 17.3±0.7 |
| Cholesterol group| 79.9±1.1 | 77.9±1.1 | 22.4±0.8 | 17.3±0.7 |
|                  | Aged |     |              |              |
| Balanced group   | 70.5±0.7 | 89.6±1.4 | 16.9±0.7 | 11.9±0.9 |
| Cholesterol group| 89.6±1.4 | 77.9±1.1 | 16.9±0.7 | 11.9±0.9 |

SD, standered deviation; LDL, low density lipoprotein; HDL, high density lipoprotein. *Significant difference (P<0.05).

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![Fig. 1. Mean body weight of the experimental groups (in grams) at the start and the end of the experiment. *Significant difference (P<0.05).](http://dx.doi.org/10.5115/acb.2014.47.2.117)
A decrease in the hippocampus thickness (Table 3, Fig. 2B), slight disorganization of the pyramidal cell layer (Fig. 3B), few degeneration of pyramidal cells and slight spongiosis (Fig. 4B). Also, there were senile neuritic plaques, intracellular Hirano’s bodies, and lipofuscin pigmentation (Fig. 5B, C).

Comparing the adult balanced diet fed rats with the adult
cholesterol diet fed rats, hippocampus showed, no significant change in thickness ($P \geq 0.05$), numerous blood capillaries and peripheral degeneration (Table 3, Fig. 2C). Dispersed small vacuolations, disorganization and shrinkage of the pyramidal cells (Fig. 3C). Also, there was hyalinized pyramidal cells with eccentric nuclei, presence of either thickened and attenuated capillaries (Fig. 4C) and many glial cells. Some pyramidal cell showed disintegrated axon (Fig. 5D).
Comparing the aged balanced diet fed rats with the aged cholesterol diet fed, the hippocampus of the latter showed multiple vacuolations specially in CA1 area, meningeal fibrosis and peripheral degeneration with no significant change in hippocampus thickness ($P \geq 0.05$) (Fig. 2D). Also, there was many degenerated pyramidal cells, vacuolations and degeneration in the molecular layer (Fig. 3D) with massive neurogliosis (Figs. 3, 4D) and many thickened and attenuated capillaries (Fig. 4D). In addition, extra neural Hirano bodies, intracellular tangles and granulovacuolar degeneration, Lewy bodies and apoptotic oligodendroglia appeared (Fig. 5E).

Comparison between adult and aged groups showed that there was a significant decrease in the mean number of pyramidal cells ($P < 0.001$) in all aged groups than in all adult groups. In both adult and aged animals, the number of pyramidal cells showed a significant decrease in the cholesterol groups, when compared with the balanced groups. This decrease was more significant in the aged ($P < 0.001$) than in the adult rats ($P < 0.01$) (Table 3).

Golgi Cox-stained sections recorded the shape of pyramidal cells in adult balanced group (Fig. 6A) in comparison to others groups. They revealed that, the pyramidal cell in aged group showed sclerotic appearance, body deformity, short thickened hollow axon and few non-branched, hollow, spineless dendrites (inset). (C) Adult cholesterol diet fed rats shows, with ballooned non-dendritic body and disintegrated axon. (D) Aged cholesterol diet fed rats shows, with either clumped or ballooned non-dendritic body and severely disintegrated axon (A–D, Golgi Cox, ×1,000).

Light microscopic results of toluidine blue stained sections recorded the strongest pyramidal cell activity (Nissl's granules density) in the adult balanced group (++++) (Fig. 7A) in comparison with other experimental groups. The activity became decreased in aged balanced group (Fig. 7B) and adult cholesterol group (+) (Fig. 7C) and very decreased in aged cholesterol group (+) (Fig. 7D).

Immunohistochemical results for GFAP-stained sections revealed that CA1 area had a cytoplasmic brown reaction in few small dispersed non-branched astrocytes in adult balanced diet fed rats (Fig. 8A), small branched astrocytes in aged balanced diet fed rats (Fig. 8B), and large branched

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Table 3. Mean± SD of both thickness of hippocampus and pyramidal cells number

|                  | Mean thickness of hippocampus (µm) | Mean No. of pyramidal cells | Mean No. of astrocytes |
|------------------|------------------------------------|-----------------------------|------------------------|
|                  | Balanced | Cholesterol | Balanced | Cholesterol | Balanced | Cholesterol |
| Adult            | 63.1±9.1 | 59.7±5.9    | 61.7±7.1 | 50.7±3.9*   | 21.00±3.34 | 28.20±3.64* |
| Aged             | 48.6±5.1 | 47.3±4.3¹   | 45.2±5.8¹| 25.7±2.3³   | 30.20±1.63| 40.73±1.53³ |

SD, standards deviation. *Significant in comparison with balanced group of the same age. †Significant difference from corresponding adult group.

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Fig. 6. CA1 area pyramidal cells. (A) Adult balanced diet fed rats shows with triangle cell body with nodded axon and multiple branched dendrites with spines (inset) (B). (B) Aged balanced diet fed rats with sclerotic cell membrane, short hollow axon and few non branched, hollow, spineless dendrites (inset). (C) Adult cholesterol diet fed rats shows, with ballooned non-dendritic body and disintegrated axon. (D) Aged cholesterol diet fed rats shows, with either clumped or ballooned non-dendritic body and severely disintegrated axon (A–D, Golgi Cox, ×1,000).
astrocytes in both adult and aged cholesterol rats (Fig. 8C, D). The reaction became very strong in the latter. Comparison between adult and aged groups showed that there was a significant increase in the number of astrocytes ($P<0.0001$) in all aged groups than in all adult groups. In both adult and aged animals, the number of astrocytes showed a significant increase in the cholesterol groups, when compared with the balanced groups ($P<0.0001$) (Table 3).
Discussion

In the present study, the possibility that aging process decreases the memory function might be proved through the decrease in the amount of Nissl’s granules and the increase in number and size of astrocytes [8]. Moreover, through the disarrangement and shrinkage of pyramidal cells [17], the decrease in dendritic arborisation and spine numbers, the latter was assured by a notable correlation between age-related reductions in spine densities and age-dependent declines in learning and memory [18]. Also, the synchronization of lipofuscin pigment neuritic plaques, granulovacuolar degeneration, and Hirano bodies recorded in this study might be associated with memory decline, as they had been observed in the hippocampus of elderly as well as in Alzheimer’s disease [4]. The pathogenesis of hippocampus aging was proven to occur through several mechanisms; the blood vessel pathology detected in the present study supported the reduction in plasticity of the brain microvasculature as a causing mechanism [19]. In the present study, the histological and morphometric results of high cholesterol diet fed adult rats were observed by other researchers [20-22]. In the present study, high cholesterol diet fed to aged rats resulted in aggravation of senility changes of the hippocampus together with marked pathological changes that accompanied cholesterol administration. Several studies have been made to know how high cholesterol diet can produce these changes in the hippocampus. The exact mechanism may be due to neurodegeneration that could be attributed to the increase in the expression of inflammatory mediators [23] or to the atherosclerosis that accompanies hypercholesterolemia [24]. The present study doubt on the possibility of occurrence of Alzheimer’s disease in old age when high cholesterol diet is taken for a long time. This could be proven through; the pathological changes observed in the microvessels of the hippocampus as [20] who found this similar to the vascular pathology seen in Alzheimer’s disease. Also, through the increase in numbers of neuritic plaques when associated with the increase in the numbers of tangles and both triggering the degree of cognitive decline [25]. Also, Lewy’s bodies noticed in this work might trigger dementia through blocking the effects of dopamine and acetylcholine [26]. Moreover, Lewy’s bodies often co-occur with Alzheimer’s disease if accompanied with senile plaques and granulovacuolar degeneration [27]. The free extracellular Hirano bodies as well as neurofibrillary tangles (NFTs) or ‘ghost tangles’ might be the remnant of the dead neurons [28]. The increase in tangles’ number correlated with the incidence of Alzheimer’s disease [25].

NFT and senile plaques are the main pathological features of Alzheimer’s disease. They are formed by accumulation of abnormally hyperphosphorylated tau and proteolytic processing of amyloid precursor protein (APP) into β-amyloid peptide (Aβ) respectively [29]. Hypercholesterolemia strongly accelerated Aβ accumulation and tau pathology that accompanied by microglia activation and aggravation of memory impairment in mouse models [30] and rabbits [31]. A diet-induced hypercholesterolemia increased Aβ levels and extracellular deposition in the brain in transgenic mouse model of Alzheimer’s disease [32-34]. A disturbed cholesterol homeostasis within lipid rafts might influence APP processing [35, 36]. Also, cholesterol disturbance in diabetic rat increases tau phosphorylation that might be due to either increase JNK activity or activation of the tau kinase, glycogen synthase kinase 3β [37, 38].

It could be concluded that, aging process has degenerative changes in the hippocampus. High cholesterol diet is a risk factor for the hippocampus of both adult and aged but it is more serious for the latter as it can produce Alzheimer’s disease like pathological changes. So, it is recommended that a life style depending on a low cholesterol diet should be maintained.

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