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Increased Levels of 27-Hydroxycholesterol Induced by Dietary Cholesterol in Brain Contribute to Learning and Memory Impairment in Rats

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Scope: Dietary cholesterol has been shown to play a role in the development of Alzheimer's disease (AD). It is proposed that oxysterol especially 27-hydroxycholesterol (27-OHC) may play a potential role in β-amyloid peptides (Aβ) production and accumulation during AD progression.
Methods and results: To investigate the mechanisms of dietary cholesterol and 27-OHC on learning and memory impairment, male Sprague-Dawley rats are fed with cholesterol diet with or without 27-OHC synthetase inhibitor (anastrozole) injection. The levels of cholesterol, 27-OHC, 24-hydroxycholesterol (24S-OHC), 7α-hydroxycholesterol, and 7β-hydroxycholesterol in plasma are determined; apolipoprotein A (ApoA), apolipoprotein B (ApoB), HDL-cholesterol (HDL-C), and LDL-cholesterol (LDL-C) in plasma or brain; CYP27A1 and CYP7A1 in liver and CYP46A1 and CYP7B1 in brain; cathepsin B, cathepsin D, and acid phosphatase in lysosome; and Aβ1-40 and Aβ1-42 in brain. Results show increased levels of 27-OHC ($p < 0.01$), LDL-C ($p < 0.01$), and ApoB ($p < 0.01$), and decreased level of HDL-C ($p < 0.05$) in plasma, upregulated CYP27A1 ($p < 0.01$) and CYP7A1 ($p < 0.01$) expression in liver, altered lysosomal function, and increased level of Aβ in brain ($p < 0.05$).
Conclusions: This study indicates that the mechanisms of dietary cholesterol on learning and memory impairment may be involved in cholesterol metabolism and lysosome function with the increase of plasma 27-OHC, thus resulting in Aβ formation and accumulation.

1. Introduction

Dietary cholesterol is an important source of cholesterol in the human body that plays a role in Alzheimer's disease (AD) pathological progresses. A cholesterol-rich diet has been reported to influence central steps of brain amyloid pathogenesis in animals.[1,2] Cholesterol in the human brain makes up ≈25% of the cholesterol in the entire body, involving in maintaining neural development, synaptic plasticity, and brain function. Cholesterol metabolism disorders in brain have been involved in neurodegenerative diseases such as AD.[3] Scientific evidence has shown that the homeostasis of cholesterol in the brain is closely related to the metabolism of β-amyloid peptides (Aβ),[4,5] while the role of dietary cholesterol within the pathogenetic cascade of excessive Aβ deposition in the brain of AD needs to be further identified.

Oxysterols, the cholesterol metabolites, contribute to eliminating excessive cholesterol in brain by diffusing across the blood–brain barrier into systemic circulation. Recent evidences have indicated that oxysterols which act as a link between cholesterol metabolism and hypercholesterolemia play important roles in the development of AD.[6–8] The oxysterols that are likely to contribute to pathogenesis of AD are 24-hydroxychosterol (24S-OHC) and 27-hydroxycholesterol (27-OHC), which can freely cross the blood–brain barrier.[9] The high level of 27-OHC in brain and increased level of neurodegeneration have been observed with a high-cholesterol diet (CD) feeding rabbits.[10] In the early stages of AD, the elevated level of 27-OHC was observed in brains of AD patients with increased Aβ production and accumulation.[8] Similarly, multiple evidences demonstrated that 27-OHC can increase the production of Aβ and were related to the AD-like pathology.[10–12] Furthermore, 27-OHC has been verified that it mediates the negative effects of dietary cholesterol on cognition by using CYP27$^{-/-}$ mice which is lacking 27-OHC.[13] What is more, our previous studies showed a disruptive effect of 27-OHC on learning and memory by inducing Aβ deposition in brain of rat.[14] Interestingly, disturbed functions of neuronal lysosomes were in connection with pathological features of AD including brain deposition of Aβ.[15,16] It has revealed that lysosomal enzyme cathepsin B and cathepsin D participated in the degradation of β-amyloid (Aβ) in brain lysosome.[17]

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In addition, recent studies in our group manifested dietary cholesterol indeed promoted production of oxysterols especially the 27-OHC in plasma and brain, but the potential mechanism of dietary cholesterol on cognition is still unknown. It is noteworthy that dietary cholesterol may affect learning and memory ability in the form of 27-OHC. Thus, we proposed that 27-OHC as a mediator which plays a vital role in the learning and memory impairment induced by high CD. Anastrozole as an inhibitor of CYP27A1 was used in this study to inhibit the synthesis of 27-OHC.[18] The aim of this study was to investigate the effects and mechanisms of dietary cholesterol and 27-OHC on learning and memory ability in rats.

2. Experimental Section

2.1. Animals and Models

Ten-month-old male specific pathogen-free Sprague–Dawley rats (weighing of 450–600 g) were provided by the Academy of Military Medical Science. The animals were housed in the room with 12 h light/dark cycle, one per cage, temperature 22–25 °C, 40% humidity, and free access of water. Animal experiments have been approved by the ethics committee of Capital Medical University (Ethics: AEEI-2014-047). Forty-eight rats were randomly assigned to four groups, 12 rats in each group, according to initial plasma levels of cholesterol and body weight after adaptive feeding for 1 week. The four groups are as follows: control group was given a standard rodent diet and subcutaneously injected with 1.4 mg saline; CDgroup was given 0.5% CD and subcutaneously injected with 1.4 mg saline; CD and 27-OHC synthetase inhibitor group (CD + ANS group) was given 0.5% CD and subcutaneously injected with 1.4 mg anastrozole; and 27-OHC synthetase inhibitor group (ANS group) was given a standard rodent diet and subcutaneously injected with 1.4 mg anastrozole. 27-OHC, anastrozole, or saline was injected everyday continuously for 4 weeks. After 4 weeks' intervention, behavioral tests have been conducted for 1 week and each group was given a standard rodent diet during the tests. After the behavioral tests, rats were anesthetized and blood samples were collected. Then, animals were euthanized and fresh tissues (brain, liver, heart, kidney, and spleen) were collected, weighed, and subsequently frozen at −80 °C until use.

2.2. Morris Water Maze

The spatial learning and memory of rats were evaluated using Morris water maze.[19] Briefly, a pool was filled with water and opaque dyes; the body of rat was made prominent using ink to improve the ability of the tracking software to detect the rats. Animals were released for swimming at random positions facing the wall, and the time taken to reach the escape platform and the swimming distance were measured in each trial. Four groups of rats were allowed to swim for 120 s, four times per day with 30 s interval time for four days. Animals were trained from four entry points in different quadrants facing the wall and the time of escape latency, swimming distance, and average speed were recorded. Rats would be guided to the platform for 15 s if they do not find it in 2 min. After four days of training, the probe trails were performed without the platform in the fifth day. The animals were released from the third quadrant and allowed to swim freely for 120 s, and the retention time in the original target quadrant as well as the frequency of crossing platform were recorded.

Table 1. The primer sequences for CYP27A1, CYP46A1, CYP7A1, and CYP7B1.

Primer	Forward sequence (5′–3′)	Reverse sequence (5′–3′)
CYP27A1	AACAAGGACTTTGCCCACATG	GCGCAGGGTCTCCTTAATCA
CYP46A1	CAGCTTCCTTCTGGGACATCTC	GAGCACACGGCCACAAG
CYP7A1	CCTGCTTTGAACTGGAGTTTG TG	GCCCGGGACTGGTCTAGAG
CYP7B1	TAGGACTAAACCACAGTCGC	TGCAGCCTTATTCCGCTA
GAPDH	AGACAGCCGCATCTTCTTGT	CCGATACGGCCAAATCCGTT

2.3. Light–Dark Box

The learning and memory impairment of rats were detected with light–dark box after 4 week intervention.[14] Rats were put into the bright room and back to the entrance of darkroom, where they would suffer from electric shocks. The number of errors into the darkroom within 5 min and the first time as escape dark latency were recorded automatically.

2.4. HPLC–MS

The plasma samples were centrifuged at 3500 rpm for 8 min at 4 °C and stored at −80 °C until measurement. HPLC–MS was used to measure the amounts of oxysterols (27-OHC, 24S-OHC, 7α-hydroxycholesterol (7α-OHC), and 7β-hydroxycholesterol) as previously reported.[20] Stock solutions containing oxysterols (0.2 mg mL^{-1} in methanol) were prepared. 19-hydroxycholesterol (19–OHC) as internal standard with the concentration of 100 ng mL^{-1} was added to the calibration standards for oxysterol (the concentrations were 50, 100, 200, 400, and 800 ng mL^{-1}, respectively).

2.5. Real-Time PCR

Total mRNA was extracted from brains and livers of rats using the SV Total RNA Isolation System (Promega Company, USA). Three microgram of total RNA was added to the reverse transcription using the Reverse Transcription System (Promega Company, USA). mRNA expression levels of CYP46A1 and CYP7B1 in brains, and mRNA expression levels of CYP27A1 and CYP7A1 in the livers were analyzed with real-time PCR. Primers were designed specifically by searching the NCBI database. The forward and reverse primer sequences used in this study are shown in **Table 1**. Real-time PCR reactions were performed following the protocols using a CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories, Germany). The procedures were as

Table 2. Body weight of rats pre- and post-intervention ($n = 12$).

Groups	Control	CD	CD+ ANS	ANS	*F*	*p*-Value
Pre-intervention						
Body weight (g)	571.92 ± 15.79	582.00 ± 17.36	587.17 ± 16.11	584.00 ± 7.77	2.001	0.128
Post-intervention						
Body weight (g)	534.42 ± 12.70	538.50 ± 22.80	547.75 ± 13.95	540.50 ± 12.02	0.969	0.416

All the data are shown as mean ± SE.

follows: enzyme activation at 95 °C for 3 min, denaturation at 95 °C for 3 s, annealing/extension at 59 °C for 30 s, and extension at 72 °C for 30 s, including a total of 40 cycles. The gene GAPDH served as an internal reference for normalization.

2.6. Western Blot

Approximately 100 mg of brain or liver tissues was lysed in RIPA buffer containing protease inhibitor (1 mM PMSF) and centrifuged at 15 000 rpm for 20 min at 4 °C. The supernatant was reserved and the protein concentrations were determined by bicinchoninic acid method. Equal amount of proteins were added into SDS-PAGE and transferred to PVDF membranes according to the previous reports.[21] The antibodies (USA Abcam company) used were as follows: CYP27A1 1:1000, CYP46A1 1:1000, CYP7B1 1:1000, CYP7A1 1:1000, Cathepsin B 1:1000, Cathepsin D 1:1000, and Goat anti-rabbit IgG 1:5000. Fluorchem FC 2 software was used to analyze the image and the relative expression of target proteins was calculated with the following formula: relative coefficient = target protein/GAPDH.

2.7. ELISA Assay

The rat Aβ1-40 ELISA Kit and the rat Aβ1-42 ELISA Kit (Life technology) were used for Aβ determination. The ELISA Kit (rat) for LDL-cholesterol (LDL-C) and apolipoprotein B (ApoB) were used to determine the concentrations of LDL-C and ApoB in brains and livers. Plasma and tissue samples were prepared the same as the previous steps. The general processes were the same as other previous reports.[22] The Aβ1-40, Aβ1-42, LDL-C, and ApoB concentrations of the samples were then determined following the manufacturer's protocol. Optical densities were read at 450 nm by spectrophotometer (USA Biorad company), and the concentrations of Aβ1-40, Aβ1-42, LDL-C, and ApoB were determined by comparison with the corresponding standard curves. All readings were in the linear range of the assay.

2.8. Immunofluorescence

Cathepsin B Activity Assay Kit (USA Abcam company), Cathepsin D Activity Assay Kit (USA Abcam company), and Acid Phosphatase Activity Assay Kit (Beijing fangchen company) were used to detect the activity of lysosome function related proteins in brain.[23] The measurements were conducted according to manufacturer's instructions strictly.

2.9. Statistical Analysis

SPSS 18.0 was used for statistical analysis. All data were expressed with mean and standard error (mean ± SE). One-way ANOVA (analysis of variance) and least significance difference post-hoc test were used to evaluate the significant differences between groups. Two-way ANOVA analysis was used for repeated measurement data in water maze test. Values of $p < 0.05$ were considered to be statistically significant.

3. Results

3.1. Body Weight and Organ Coefficient of Rats

Body weights of rats pre- and post-intervention are shown in **Table 2**. Although the body weights for each group decreased after intervention, there is no significant difference in body weight between the CD group and anastrozole intervention group ($p > 0.05$). The organs (brain, liver, heart, kidney, and spleen) of rats were removed and weighed at the end of experiments, and then calculated the coefficient (organ weight/body weight). As shown in **Table 3**, there were no significant differences in organ coefficient among four treated groups ($p > 0.05$).

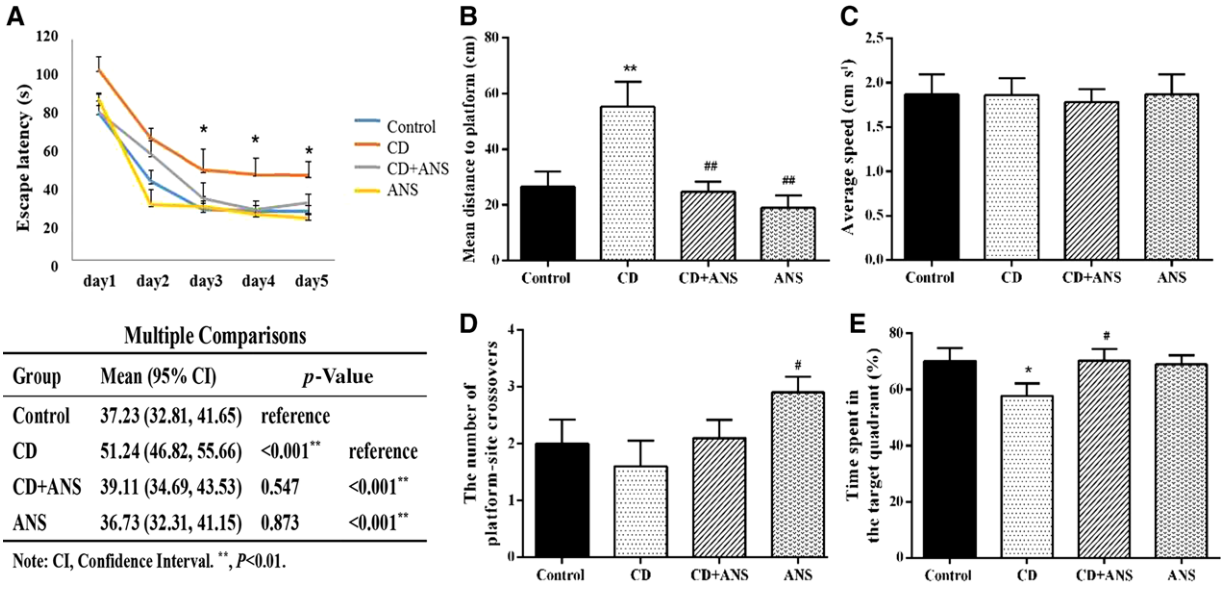
3.2. Morris Water Maze

The test was used to assess the effect of dietary cholesterol on spatial learning and memory of rats. Results are shown in **Figure 1**. Repeated-measures two-way ANOVA identified a significant reduction in escape latency with training period ($F = 133.98$, $p < 0.001$). There were significant differences in escape latency of rats among treated groups ($F = 9.88$, $p < 0.001$); Figure 1a). Compared with control group, the escape latency was obviously increased in CD group ($p < 0.001$) but not in CD + ANS group ($p > 0.05$) and ANS group ($p > 0.05$). A significant reduction in escape latency was observed in CD + ANS group ($p < 0.001$) and ANS group ($p < 0.001$) compared with CD group, and there were no interaction effects between factors of intervention time and group ($F = 1.22$, $p > 0.05$). The mean distances to platform of rat were significantly different among four groups as well (Figure 1b). Compared with control group, the mean distance to platform of rats in CD group increased

Table 3. The organ coefficient of rats at the end of the experiments (n = 12).

Groups	Control	CD	CD+ ANS	ANS	*F*	*p*-Value
Brain (%)	0.31 ± 0.007	0.30 ± 0.016	0.30 ± 0.007	0.29 ± 0.008	1.025	0.391
Heart (%)	0.31 ± 0.006	0.31 ± 0.020	0.32 ± 0.009	0.29 ± 0.010	0.783	0.51
Liver (%)	2.34 ± 0.200	2.44 ± 0.116	2.49 ± 0.061	1.98 ± 0.067	2.413	0.079
Kidney (%)	0.16 ± 0.015	0.14 ± 0.007	0.14 ± 0.006	0.17 ± 0.005	1.846	0.153
Spleen (%)	0.59 ± 0.013	0.59 ± 0.036	0.62 ± 0.016	0.58 ± 0.017	0.942	0.428

All the data were showed as mean ± SE.



Multiple Comparisons

Group	Mean (95% CI)	*p*-Value	
Control	37.23 (32.81, 41.65)	reference	
CD	51.24 (46.82, 55.66)	<0.001**	reference
CD+ANS	39.11 (34.69, 43.53)	0.547	<0.001**
ANS	36.73 (32.31, 41.15)	0.873	<0.001**

Note: CI, Confidence Interval. **, *P*<0.01.

Figure 1. Spatial learning and memory ability in rats measured by Morris water maze. a) Escape latency; b) mean distance to platform; c) average speed; d) the number of platform-site crossovers; e) time spent in the target quadrant. All the data are shown as mean ± SE. *$p < 0.05$ compared with control group; **$p < 0.01$ compared with control group; #$p < 0.05$ compared with CD group; and ##$p < 0.01$ compared with CD group.

significantly ($p < 0.05$). Comparing with CD group, the mean distance to platform decreased significantly in CD + ANS group ($p < 0.01$) and ANS group ($p < 0.05$). However, there was no difference in average speed ($p > 0.05$; Figure 1c).

After the 5 days' training, the number of platform-site crossovers and the time spent in the target quadrant were recorded to evaluate the spatial learning and memory ability of rats. As shown in Figure 1d, the number of platform-site crossovers for ANS group was remarkably increased as compared to CD group ($p < 0.05$). No significant differences were observed in control group and CD + ANS group. The time spent in the target quadrant in CD group was significantly less than that in control group, while rats in CD + ANS group spent much more time in target quadrant than those in CD group (Figure 1e).

3.3. Light–Dark Box

The test was used to detect the ability of passive avoidance learning. As shown in **Figure 2**a and b, the time before entering into the dark area decreased in CD group and the number of errors entering into the dark increased significantly in CD group and CD + ANS group compared with control group. Whereas, longer time and less error number were observed obviously in CD + ANS group and ANS group in comparison with CD group.

3.4. The Levels of Cholesterol and Oxysterols in Plasma

As shown in **Figure 3**, there were significant differences among four groups in the levels of plasma cholesterol ($p < 0.05$) and 27-OHC ($p < 0.01$) but not in the levels of 24S-OHC ($p > 0.05$), 7α-OHC ($p > 0.05$), and 7β-hydroxycholesterol ($p > 0.05$). The levels of plasma cholesterol increased obviously in both CD group ($p < 0.05$) and CD + ANS group ($p < 0.01$) compared with control group. What is more, the plasma level of 27-OHC in CD group is significantly higher ($p < 0.01$) than that in control group. However, it was significantly lower in CD + ANS group ($p < 0.01$) and ANS group ($p < 0.01$) compared with that in CD group. Although there were no significant differences in plasma level of 7α-OHC among four groups, CD group exhibited higher plasma 7α-OHC than other groups, which was statistically significant ($p < 0.05$).

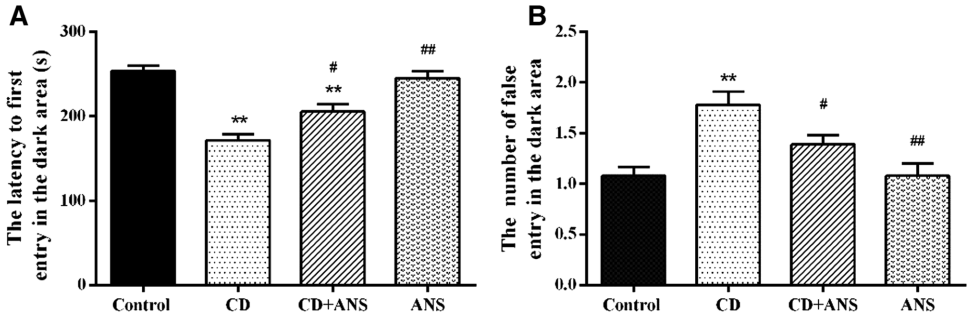


Figure 2. Passive learning and memory ability in rats tested by light–dark exploration. a) The latency to first entry in the dark area; b) the number of false entry in the dark area. All the data are shown as mean ± SE. *$p < 0.05$ compared with control group; **$p < 0.01$ compared with control group; #$p < 0.05$ compared with CD group; and ##$p < 0.01$ compared with CD group.

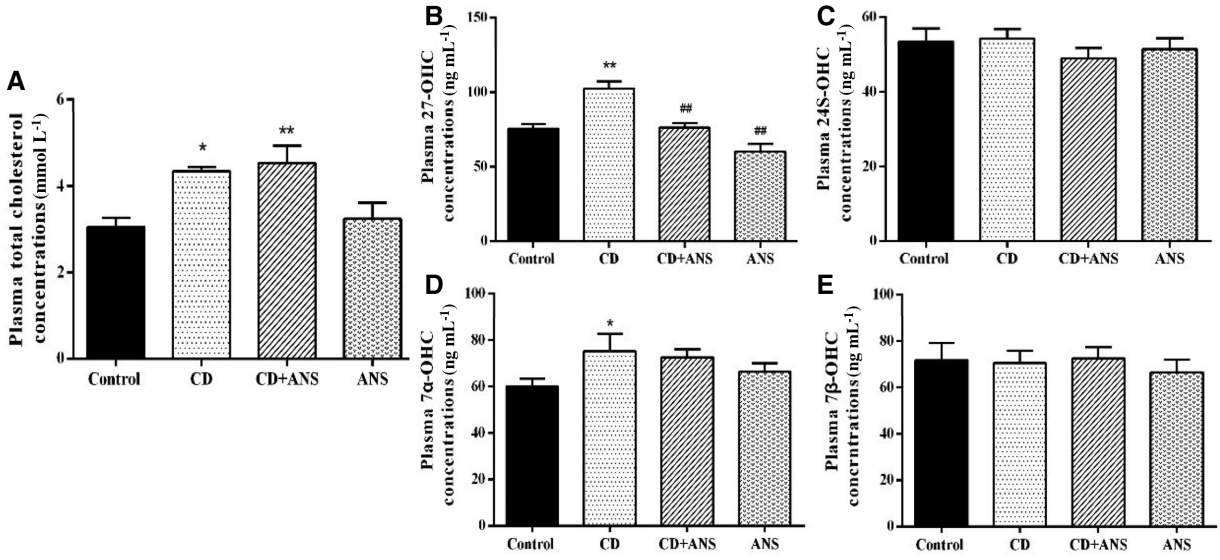


Figure 3. The levels of total cholesterol and oxysterols in plasma detected by HPLC–MS. a) Total cholesterol; b) 27-OHC; c) 24S-OHC; d) 7α-OHC; and e) 7β-hydroxycholesterol. All the data are shown as mean ± SE. *$p < 0.05$ compared with control group; **$p < 0.01$ compared with control group; #$p < 0.05$ compared with CD group; and ##$p < 0.01$ compared with CD group.

3.5. The Expressions of CYP27A1 and CYP7A1 in Livers and CYP46A1 and CYP7B1 in Brains

Gene and protein expressions of four enzymes (CYP27A1, CYP7A1, CYP46A1, and CYP7B1) related to cholesterol metabolism in livers and brains were detected by real-time PCR and western blot. Results are shown in **Figure 4**. There were significant differences in the gene expression levels of CYP27A1 ($p < 0.01$) and CYP7A1 ($p < 0.01$) in livers, which were upregulated in CD group as compared with control group, while the mRNA levels of CYP27A1 ($p < 0.01$) and CYP7A1 ($p < 0.01$) were downregulated in CD + ANS group and ANS group in comparison with that in CD group. Compared with control group, the protein expression of CYP27A1 ($p < 0.01$) and CYP7A1 ($p < 0.01$) was upregulated in CD group, while the protein expression of CYP27A1 and CYP7A1 was downregulated in CD + ANS group and ANS group in comparison with CD group ($p < 0.01$). Similarly, the protein expression of CYP27A1 were significantly downregulated in CD + ANS group ($p < 0.01$) and ANS group ($p < 0.01$) as compared with control group. And the protein expression of CYP7A1 was also downregulated in CD + ANS group ($p < 0.05$) and ANS group ($p < 0.01$) comparing with control group.

Furthermore, downregulation of CYP46A1 mRNA in rat brain was only found in ANS group ($p < 0.05$), while the downregulation of CYP46A1 protein was found in CD + ANS group ($p < 0.05$) and ANS group ($P < 0.01$) compared with CD group. There were no significant differences in CYP7B1 mRNA expression among four groups. Nevertheless, a significant downregulation of CYP7B1 protein expression in ANS group has been observed ($p < 0.05$).

3.6. The Levels of LDL-C and ApoB in Plasma and Brains and HDL-C and ApoA in Plasma

As shown in **Figure 5**, the levels of plasma LDL-C (5a) and brain LDL-C (5e) significantly increased along with CD feeding (CD group) ($p < 0.01$). Whereas, the levels of plasma LDL-C and brain

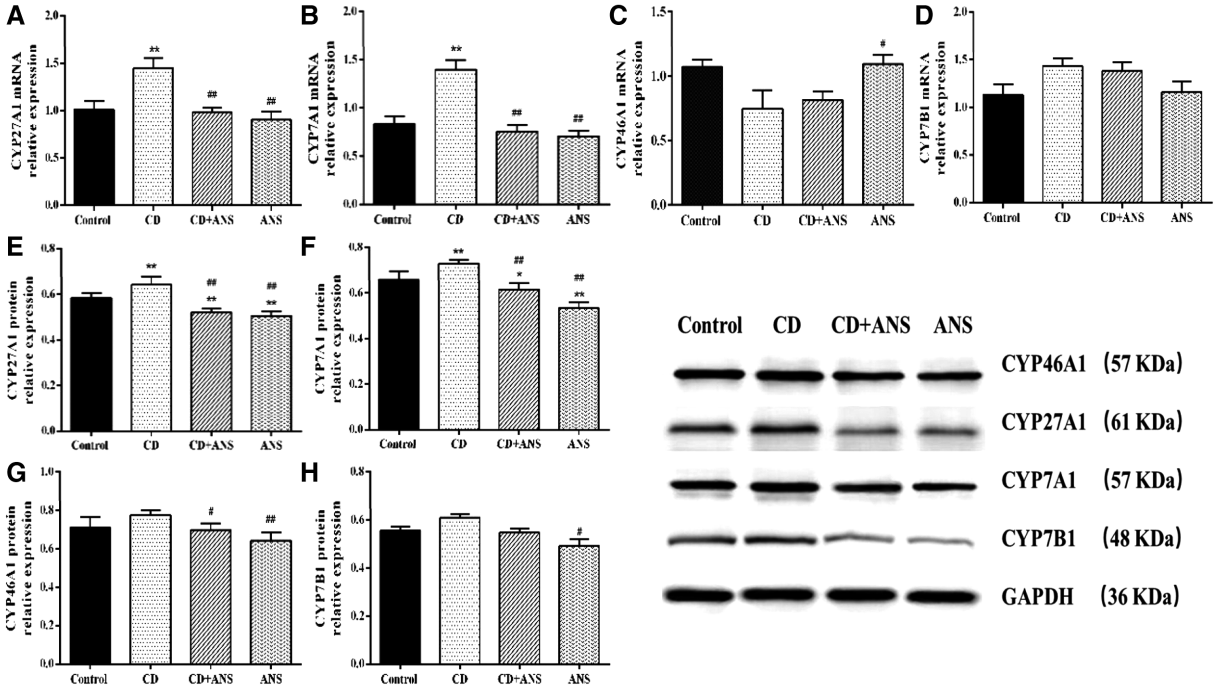


Figure 4. The gene and protein expression of CYP27A1 and CYP7A1 in livers and CYP46A1 and CYP7B1 in brains. a) CYP27A1 mRNA; b) CYP7A1 mRNA; c) CYP46A1 mRNA; d) CYP7B1 mRNA; e) CYP27A1 protein; f) CYP7A1 protein; g) CYP46A1 protein; and h) CYP7B1 protein. All the data are shown as mean ± SE. $*p < 0.05$ compared with control group; $**p < 0.01$ compared with control group; $\#p < 0.05$ compared with CD group; and $\#\#p < 0.01$ compared with CD group.

LDL-C decreased significantly in CD + ANS group ($p < 0.05$) and ANS group ($p < 0.01$). Meanwhile, as shown in Figure 5D and F, the levels of plasma ApoB increased significantly in CD group ($p < 0.01$) and the levels of brain ApoB increased slightly with no significance ($p > 0.05$). However, the level of ApoB in brains decreased in ANS group in comparison with CD group ($p < 0.05$). In addition, the levels of plasma HDL-cholesterol (HDL-C) (5b) and apolipoprotein A (ApoA) (5c) were also determined in this study and the results manifested that dietary cholesterol induced the decrease in plasma level of HDL-C in plasma ($p < 0.05$), but no differences of ApoA were observed ($p > 0.05$). Nevertheless, anastrozole had no effects on both levels of HDL-C and ApoA in plasma among four different groups.

3.7. The Activity and Protein Expression of Cathepsin B, Cathepsin D, and Acid Phosphatase

To determine the effect of dietary cholesterol on lysosome functions in brains, cathepsin B, cathepsin D, and acid phosphatase were detected on the enzyme activity and protein expression by immunofluorescent assay. As shown in **Figure 6**, acid phosphatase with higher enzyme activity ($p < 0.05$) and lower protein expression ($p < 0.05$) was detected in ANS group compared with CD group. There were no statistical variances in cathepsin B specific activity and protein expression. Despite no difference in cathepsin D specific activity was observed among four groups, protein expression of cathepsin D was upregulated in CD group ($p < 0.05$) and downregulated in ANS group ($p < 0.01$).

3.8. The Levels of Aβ1-40 and Aβ1-42 in Brains

There were significant differences among four groups in the levels of Aβ1-40 ($p < 0.01$) and Aβ1-42 ($p < 0.05$) in brains respectively (**Figure 7**). The brain levels of Aβ1-40 and Aβ1-42 in CD group were significantly higher than that in control group ($p < 0.05$). Whereas, the levels of Aβ1-40 and Aβ1-42 in ANS group were significantly lower than that in CD group ($p < 0.01$).

4. Discussion

Although dietary cholesterol is no longer a concern for its overconsumption in healthy human, high intake of dietary cholesterol has been reported to correlate with elevated serum total cholesterol. And plasma level of cholesterol increased by high CD has become a recognized risk factor for AD. Peripheral cholesterol cannot enter into the brain due to the impermeability of the blood–brain barrier (BBB) of lipoproteins that carry cholesterol. While cholesterol oxidized products (oxysterols, 24S-OHC and 27-OHC) are able to cross the BBB freely with the influx and efflux. A meta-analysis indicated that 24S-OHC and 27-OHC are elevated in AD and mild cognitive impairment subjects compared to controls.[24] Meanwhile, increased levels of 27-OHC induced by a high-CD have been reported to modify the neurodegeneration, and the level of 27-OHC in AD brains increased significantly during the disease progression.[8] Additionally, our previous study has demonstrated that 27-OHC may regulate the cholesterol synthesis in C6 glioma cells.[25] Maura et al. revealed that 27-OHC

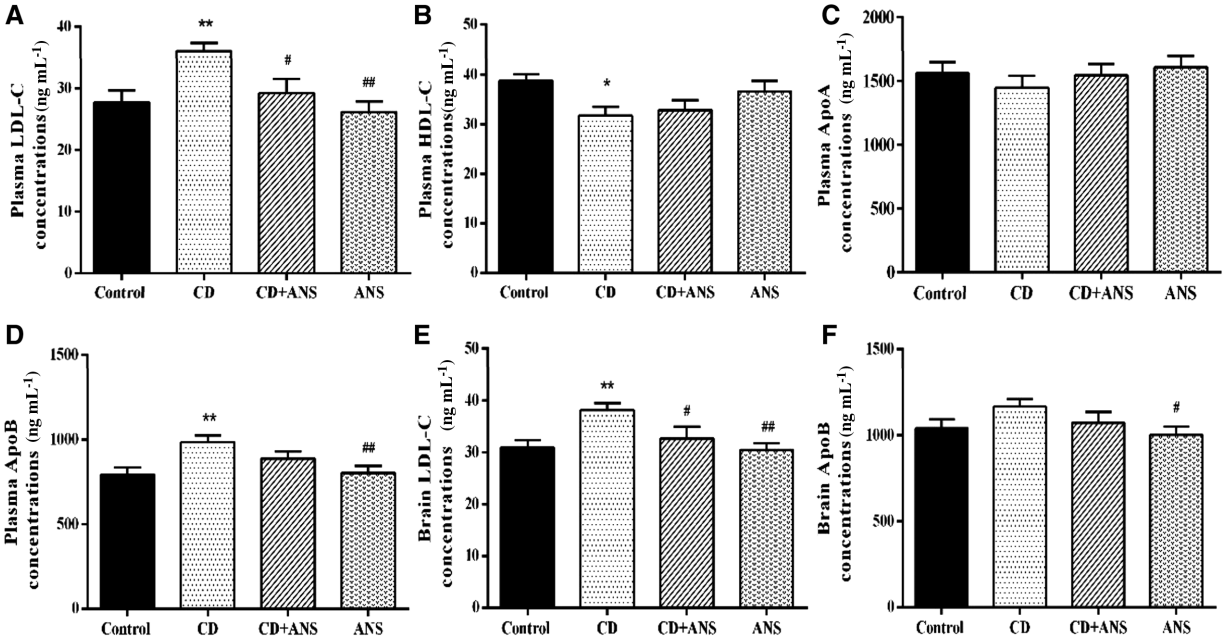


Figure 5. The levels of LDL-C and ApoB in plasma and brains and HDL-C and ApoA in plasma. a) Plasma LDL-C; b) plasma HDL-C; c) plasma ApoA; d) plasma ApoB; e) brain LDL-C; and f) brain ApoB. All the data are shown as mean ± SE.*$p < 0.05$ compared with control group; **$p < 0.01$ compared with control group; #$p < 0.05$ compared with CD group; and ##$p < 0.01$ compared with CD group.

can mediate the negative effects of dietary cholesterol on cognition in mice by using the CYP27$^{-/-}$ mice model.[13] In the present study, we hypothesized that 27-OHC might be the mediator in regulating the cholesterol metabolism related with cognitive impairment.

In this study, we demonstrated that CD was closely related to the learning and memory impairment in rats probably by increasing the level of plasma oxysterol especially 27-OHC, which may result in disorders of cholesterol metabolism, lysosomal dysfunction, and increased Aβ formation and accumulation. These findings provide further insights into the involvement of dietary cholesterol in AD or mild cognitive impairment and the potentially significant role that 27-OHC plays in the pathogenesis of AD.

Accumulating epidemiological and experimental studies have confirmed that high-CDs have affected the cognitive performance in animal models.[26–28] In the present study, we found that CD feeding for 4 weeks has no effect on the body weight and organ coefficient of rats, which are consistent with the results of Li[29] and Ya.[27] What is more, the spatial and passive learning and memory impairment of rats have been observed after CD feeding. However, other researches have indicated that rats fed with high CD for 2 months learned a water-maze task more quickly than rats fed with a regular diet and CD rats had better performance in learning and memory.[27,28] These differences may be attributed to differences in the age of rats, which were 10 months old for our study and one month old for these studies. Importantly, despite the fact that cholesterol supplementation facilitates the acquisition of learning, the effect of dietary cholesterol on memory consolidation may depend on the age of animal as well.[28]

To elucidate the specific role of 27-OHC in the learning and memory abilities of rats, we assessed the effect of dietary cholesterol on plasma cholesterol and oxysterols. The present study showed that high CD in rats was associated with increased level of 27-OHC in plasma and total cholesterol. These results were in accordance with the research that higher levels of 27-OHC in the brain as well as increased levels of neurodegeneration in the hippocampus were observed with rabbits feeding a high-CD.[10] Besides, elevated level of 7α-OH in plasma was also observed in CD group.

Peripheral 27-OHC was catalyzed by enzyme CYP27A1 in liver and 27-OHC in the brain can be transferred into 7α-hydroxy-3-oxo-4-cholestenoic acid (7-HOCA) by enzyme CYP7B1, and then reached the liver for final elimination. In addition, cholesterol can be converted into 24-OHC which can cross the BBB by another enzyme CYP46A1. Then 24-OHC can be further cleaved by the enzyme CYP7A1. Our findings in this study manifested that high CD has a correlation with upregulation of CYP27A1 and CYP7A1 in livers, indicating that dietary cholesterol affected peripheral cholesterol metabolism principally by increasing the level of plasma 27-OHC with CYP27A1 upregulation. Anastrozole has been reported to downregulate the levels of 27-OHC synthetase CYP27A1,[18] which was further confirmed in our study. The decreased level of 27-OHC has also been observed by using the inhibitor anastrozole, which can be attributed to the downregulation of CYP27A1. Interestingly, anastrozole is an inhibitor of aromatase CYP19A1 as well. The CYP19A1 has been reported to be responsible for the final step in the biosynthesis of estrogens.[30] What is more, 27-OHC is an endogenous selective estrogen receptor modulator.[18] Therefore, the downregulation of CYP27A1 and CYP19A1 may both contribute to the decreased level of 27-OHC.

Generally, altered cholesterol homeostasis and elevated plasma LDL-C or reduced plasma HDL-C continued to represent

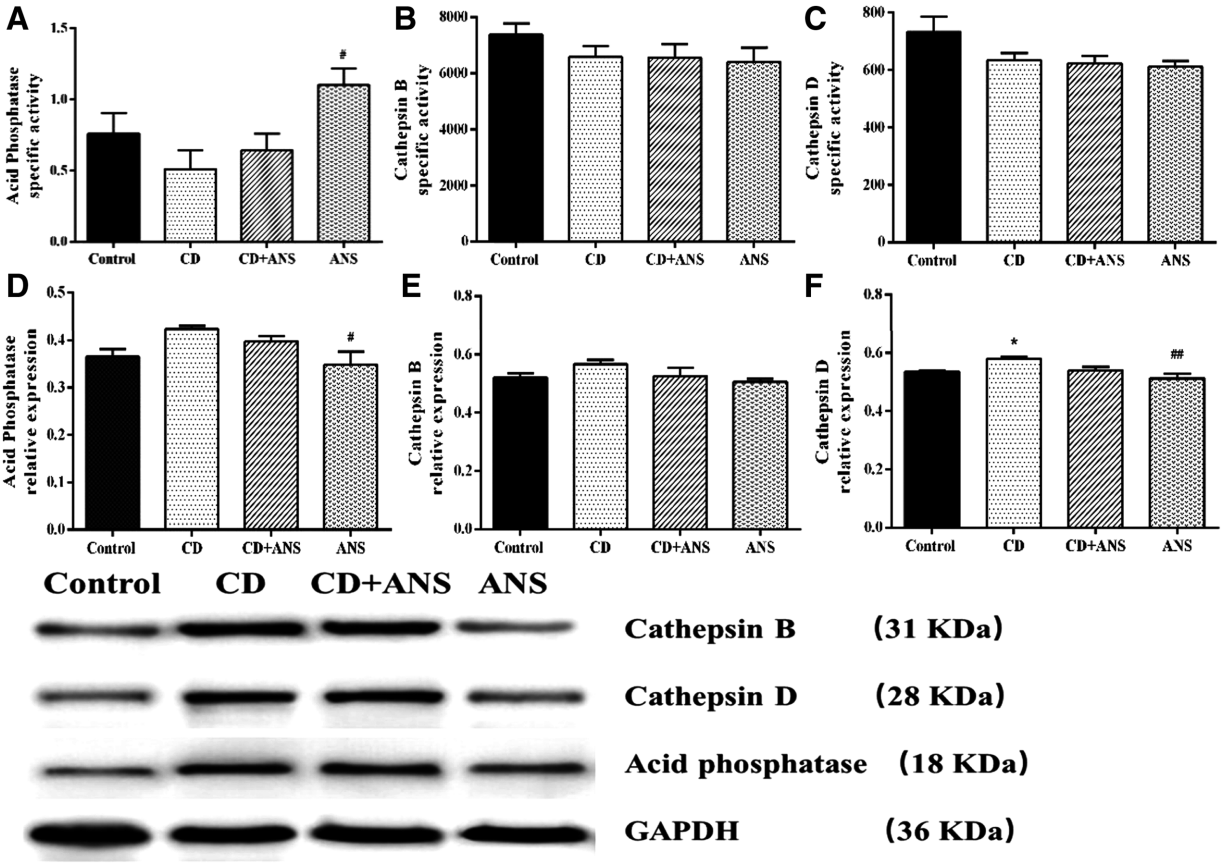


Figure 6. The activity and protein expression of cathepsin B, cathepsin D, and acid phosphatase in brains. a) acid phosphatase activity; b) cathepsin B activity; c) cathepsin D activity; d acid phosphatase protein; e cathepsin B protein; and f cathepsin D protein. All the data are shown as mean ± SE.*$p < 0.05$ compared with control group; **$p < 0.01$ compared with control group; #$p < 0.05$ compared with CD group; and ##$p < 0.01$ compared with CD group.

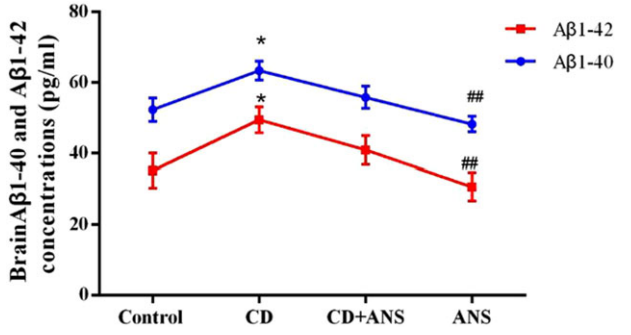


Figure 7. The levels of Aβ1-40 and Aβ1-42 from brain tissue in different groups. All the data are shown as mean ± SE. *$p < 0.05$ compared with control group; **$p < 0.01$ compared with control group; #$p < 0.05$ compared with CD group; and ##$p < 0.01$ compared with CD group.

robust risk factors of sporadic AD.[31] Apoprotein B was the exclusive apolipoprotein of LDL-C that is normally found only in the periphery. It has been reported that total cholesterol level has strong correlations with LDL-C and ApoB levels.[32,33] Previous research have shown that rabbits fed a cholesterol-enriched diet exhibited increased level of Aβ in brain as well as lysosomal dysfunction in neurons.[34] Therefore, we determined the levels of LDL-C and ApoB in plasma and in brain, founding that elevated levels of LDL-C appeared both in peripheral and brain and ApoB increased in peripheral significantly. Although there was a slightly upward trend of ApoB in brain, there were no statistically significant differences existed in four groups, which may be due to the individual differences. Besides, increased levels of LDL-C and ApoB were associated with abnormal lysosome function, mainly the upregulation of cathepsin D, which was related to degradation of Aβ. Although there were no significant variations in the activity of cathepsin B, cathepsin D, and acid phosphatase with high CD, a slightly downward trend existed in CD group. As such, the expression of cathepsin B and acid phosphatase presented a weak rising trend. Whereas, the expression of cathepsin B and acid phosphatase of group treated with 27-OHC synthetase inhibitor (anastrozole) exhibited the inverse trend. These results provided evidence for 27-OHC mediating the levels of LDL-C and ApoB, and probably by regulating the activity and expression of related enzymes in lysosome. However, the role of 27-OHC in maintaining lysosome function needs to be verified in the further studies.

The β-amyloid hypothesis has been recognized in AD pathology. A recent meta-analysis stated that increased Aβ level was

associated with cognitive impairment and decline in cognitively normal older adults.[35] What is more, elevated dietary cholesterol has been reported to increase Aβ accumulation or deposition in numerous animal models.[36–39] We have verified that dietary cholesterol increased Aβ production and accumulation in brain, which resulted in learning and memory impairment significantly. Since cholesterol levels influence the production and deposition of the pathogenic Aβ, cholesterol transport and homeostasis are thus closely linked to multiple aspects of Aβ biology.[40–42] 27-OHC has been reported to exhibit neurotoxic effects on neurons by increasing Aβ production and accumulation dose-dependently.[33] Our results manifested that excessive 27-OHC crossing BBB into brain contributes to Aβ deposition, and consequently impairs learning and memory function, which was similar to the previous reports.

In addition, Aβ in neurons can be degraded by lysosome and lysosome dysfunctions were related to higher levels of Aβ in brain. Studies by Chen have shown that cholesterol-enriched diet for rabbits induced endolysosome dysfunction including decreased activities of three lysosome enzymes.[34] However, this correlation is not obtained in rats with CD in our study except for a small tendency to reduce these enzyme activities without statistical difference. One explanation for the different results is that higher CD (2%) and longer feeding time in rabbits have more significant effects on lysosome dysfunctions than that in this study. Another explanation is the fact that rabbits are more sensitive to CD than rats. Due to diverse animals and controversial results, further research need to be conducted for verifying this point.

5. Conclusion

The present results indicate that elevated levels of 27-OHC induced by high CD may provide negative effects on the learning and memory of rats and β-amyloid peptides production and deposition in the brain of rats. The potential mechanism may be the involvement of 27-OHC in the cholesterol metabolism and lysosome function. These findings suggest that 27-OHC may be a mediator for cholesterol metabolism and cognitive function. However, 27-OHC plays a role in the maintenance of lysosomal function and β-amyloid peptides production and deposition, which needs to be verified in the further studies.

Abbreviations

24S-OHC, 24-hydroxycholesterol; 27-OHC, 27-hydroxycholesterol; 7α-OHC, 7α-hydroxycholesterol; 7β-OHC, 7β-hydroxycholesterol; AD, Alzheimer's disease; ANS, anastrozole; ApoA, apolipoprotein A; ApoB, apolipoprotein B; Aβ, β-amyloid peptides; CD, cholesterol diet; HDL-C, high-density-lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol

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Keywords

27-hydroxycholesterol, cholesterol metabolism, cognitive impairment, dietary cholesterol, oxysterols, β-amyloid peptides

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