Short-term high-fat diet favors the appearances of apoptosis and gliosis by activation of ERK1/2/p38MAPK pathways in brain

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ABSTRACT

High-fat diet (HFD) has been associated with neuroinflammation and apoptosis in distinct brain regions. To explore the effect of short-term (7, 14 and 21 days) high-fat overfeeding on apoptosis, inflammatory signaling proteins, APP changes and glial cell activities in cerebral cortex and cerebellum. Mice were fed with HFD for different lengths (up to 21 days) and after each time body weights of mice was tested, then the apoptotic proteins, IL-1β, APP, BACE1and MAPKs, Akt and NF-κB signaling activity were evaluated by western blots. Results demonstrate that short period of high-fat overnutrition significantly promotes apoptosis, APP expression at day 21 of cerebral cortex and at day 7 of cerebellum compared to chow diet. In addition, increased GFAP+astrocytes, Iba-1+microglia and IL-1β 30 were observed in cerebral cortex after 21 days HFD, but no changes for 7 days overfeeding of cerebellum. Serendipitously, ERK1/2 pathway was activated both in cerebral cortex and cerebellum for different time course of HFD. Furthermore, increased phospho-p38 MAPK level was observed in cerebellum only. In consistent with in vivo results, SH-SY5Y cells treatment with cholesterol (50 μM, 100 μM) for 48 h culture in vitro demonstrated that pro-apoptotic proteins were enhanced as well. In brief, short-term HFD consumption increases sensitivity to apoptosis, APP and IL-1β production as well as gliosis in cerebral cortex and cerebellum, which may be related to enhancement of ERK1/2 and p38 MAPK activation.

INTRODUCTION

The worldwide prevalence of obesity is increasing worldwide and the main cause of obesity is intake of a high-fat diet (HFD), also known as the western diet [1]. Long-term consumption of HFD (≥30% of energy from fat) is associated with a pattern of chronic inflammation characterized by releasing pro-inflammatory cytokine and cytotoxic mediators in central nervous system (CNS) [2–4]. In addition, as major immune cell types in CNS, microglia and astrocytes are critical in regulating neuroinflammation and liberating inflammatory mediators [5]. Recent studies have suggested that HFD-associated neuroinflammation can become an important risk factor for the development of neurodegenerative disorders, such as Alzheimer’s disease (AD), and lead to cognitive deficits. Furthermore, a larger body of literature demonstrate that prolonged HFD-induced cognitive impairment exhibit increased apoptosis in the hippocampal neurons [6–10]. These studies have suggested that HFD intake increases susceptibility of hippocampus to excessive proinflammatory responses.
Interestingly, even as little as 3–10 days exposure, short-term HFD consumption has demonstrated that production of proinflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα), apoptosis and memory impairments in humans and rodents in long before obesity symptoms appearance [11–15]. Nevertheless, a literature suggests that short-term HFD overnutrition does not induce NF-κB inflammatory signaling of subcutaneous white adipose tissue in humans [16].

On the other hand, mounting evidence has indicated that mitogen-activated protein kinase (MAPK) pathway is involved in the cellular responses to the metabolic stresses like HFD [17–20]. MAPK pathways, consisting of ERK1/2, ERK5, JNK, and p38/SAPKs, play complex space– and time–dependent patterns and participate in cell survival and apoptosis [21]. For example, enhanced ERK signaling contributes to cellular survival in short time, but the delayed activation of this kinase can lead to cell death [22]. In addition, while p38/MAPK signaling has been implicated in initiating apoptosis, p38 MAPK can also sometimes prevent cell death under some conditions [23]. However, less abundant evidence is available showing whether short-term HFD intake could affect apoptosis, APP expression, inflammatory biomarkers and gliosis in cerebral cortex and cerebellum mediated by MAPKs signaling transduction. Therefore, the current study aimed to explore the potential effects of short-term HFD consumption (7, 14 and 21 days) on apoptosis proteins, IL-1β, APP (amyloid-beta precursor protein) production and activation of glial cell in different brain regions. We also investigate whether stress response pathways, such as MAPK signaling, are involved these process.

**MATERIALS AND METHODS**

**Animals, diet compositions and treatments**

Adult male C57BL/6J wild type mice (8-weeks of age) were purchased from Gem Pharma tech Co., Ltd (Licensed production number: SCXK-(SU)-2018-0008; Nanjing, China). Low fat and no sugar Chow diet (TP23100) and High fat diet (HFD, TP23103) were purchased from Trophic Animal Feed High-tech Co., Ltd (Nantong, China). The Diet compositions were displayed in Table 1. After one week adaptation, mice were randomly divided into three groups (n = 5 mice/group). Control group mice were treated with chow diet. In addition, 7 days group, 14 days group and 21 days groups were fed with HFD for 7 days, 14 days and 21 days, respectively, Mice were subsequently humaney sacrificed. Brain specimens were collected and preserved at ~80°C until further use. All animal experimental procedures were reviewed and approved by the Committee Guide of Wenzhou Medical University (ethical number 2019-75; Wenzhou, China.). All mice were maintained on a 12 hours light-dark cycle and temperature 24°C–25°C with free access to water and food. All surgery was conducted under sodium pentobarbital anesthesia and efforts were made to reduce animal suffering.

**SH-SY5Y cell line culture and cholesterol treatment**

SH-SY5Y Cells (ATCC) was was maintained in Dulbecco’s modified Eagle’s medium134 (HyClone, SH30022.01B) supplemented with 4 mM L-Glutamine (Sigma) and 10% fetal bovine serum (Gibco®). Cell lines was cultured at 37°C and 5% CO2. Cells were treated with 50 μM and 100 μM cholesterol for 48 h, respectively. Methods of protein extraction and immunoblotting analysis are showed in Immunoblotting analysis.

**Immunofluorescence (IF) staining**

The immunofluorescent assay were carried out according to procedures performed by us [24, 25]. Briefly, brains were placed in fresh 30% sucrose solution for 24 h, then embedded in OCT compound (Sakura Finetek, Torrance, CA, USA) and cut at a thickness of 40 μm on a cryostat (CM1950; Leica, Mannheim, Germany). Brain sections were then incubated with following antibody for 1 h in room temperature: glial fibrillary acidic protein (GFAP; 1:400), a marker of astrocytes and ionized calcium binding adaptor molecule 1 (Iba-1; 1:400), a marker of microglia/macrophage-specific calcium-binding protein. After washing 30 min with PBS, then sections were incubated with Dy Light 488-conjugated goat anti-rabbit and goat anti-mouse (1:400; Jackson Immuno Research Labs) antibody for 1 h in room temperature, and washed three times with PBS. Stained sections were captured under a confocal laser scanning microscopy (Nikon Corporation, Tokyo, Japan). Optical density (OD) of immunoreactive structures were measured using ImageJ software (developed at the National Institutes of Health), similar method as previously have described by us [24].

**Immunoblotting analysis**

The western blotting methods were performed according to guidelines previously established by our group [24, 26, 27]. In brief, mice were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg, i.p.) at day 7, day 14 and day 21 after high fat and chow diet feeding. Mouse were then decapitated following brain extraction (cerebral cortex and cerebellum). The brain tissue were cut into smaller pieces and incubated in RIPA buffer (Beyotime
Table 1. Compositions of chow diet and high fat diet nutritional facts.

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Biotechnology; P0013B) through ultrasonic cell disruptor. After centrifugation, the supernatants were obtained. Proteins (10~15 μg) from lysates were separated on 5~10% Tris–glycine SDS–PAGE gels and transferred onto nitrocellulose membranes. Membranes were incubated at room temperature for 1 h in 5% (w/v) nonfat dry milk in TBST (Tris-buffered saline, 0.1% Tween 20), washed in TBST and incubated with primary antibodies overnight at 4°C. The primary antibodies were listed in Table 2. After incubation at 4°C, membranes were rinsed for 3 times (10 min each time) at room temperature with agitation in TBST, incubated with the species appropriate secondary antibody for 60 min at room temperature, and rinsed the membrane for a further 3 times (10 min each time) in TBST at room temperature before ECL detections. ImageJ software (National Institutes of Health) was used for quantification on densitometry of protein bands.

SH-SY5Y Cells were rinsed twice with PBS and lysed in RIPA buffer for 30 min on ice. Protein collecting form SH-SY5Y Cells and western blotting also used the aforementioned procedure.

Statistical analysis

Experiments were repeated at triple or four times independently. All data were reported as Mean ± SD (standard deviation). All statistical analyses were performed using GraphPad software (GraphPad Prism version 8.00, San Diego, CA, USA). The body weight data of the mouse were analyzed by two-way ANOVA followed by Sidak’s multiple comparisons test to compare differences between both groups. The additional data with three or more groups were analyzed with a one-way ANOVA, followed by post hoc testing with Tukey LSD. Differences between two groups were tested by unpaired two-tailed Student’s t-test. The significance level were set when *P < 0.05, highly as significant when **P < 0.01.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

RESULTS

Effects of short-term HFD on total body weight, expression of amyloid precursor protein (APP) and BACE1 in a mouse model at two months of age

To identify whether short-term intake of HFD could significantly raise body mass, mice from the two diet groups were weighed on the day of diet beginning (day 0) and tested every seven days (Figure 1A). As illustrated in Figure 1B, the body weight of HFD-fed mice were significantly raised compared to that of chow diet mice on day 0 (22.06 ± 0.33 g vs. 21.12 ± 1.10 g; p < 0.01; n = 5 per group), on day 7 (22.60 ±0.34 g vs. 25.06 ± 0.65 g; p < 0.01; n = 5 per group), on day 14 (22.8 ± 0.29 g vs. 27.08 ± 1.11 g; p < 0.001; n = 5 per group), on day 21 (22.54 ± 0.22 g vs. 29.36 ± 0.75 g; p < 0.001; n = 5 per group).
Table 2. Key resource table.

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By western blotting, we next analyzed APP production and expression of BACE1 (a key enzyme involved in APP metabolism) at different time-point (Figure 1C). Short-term HFD intake elevated the level of APP after 7 days, 14 days and 21 days, compared to chow diet groups (p < 0.05; p < 0.01) (Figure 1D). On the other hand, BACE1 expression were significantly up-regulated at day 14 after HFD exposure, but this protein reduced to normal level compared to chow diet groups after 21 days HFD (p < 0.01) (Figure 1E). Thus, our results demonstrates that increased protein expression of APP is achieved by responding to short-term HFD. In addition, fluctuations of BACE1 proteins during short-term HFD demonstrate APP metabolism mediated by BACE1 is complex in vivo.
Effects of short-term HFD on apoptosis proteins and aging marker p21 in cerebral cortex

As demonstrated in Figure 2A, the PARP protein level was significantly enhanced at day 21 compared to chow diet groups ($p < 0.01$) (Figure 2B). In contrast to PARP expression, the anti-apoptotic BCL-2 protein showed opposite trend and significantly reduced after 21 days HFD compared to chow diet mice ($p < 0.05$) (Figure 2C). The pro-apoptotic Bax was raised at day 7 and 21 and reduced at day 14 compared to chow diet groups ($p < 0.05; p < 0.01$) (Figure 2D). Intriguingly, no significant difference changes were found for pro-apoptotic Bad at different time-points (Figure 2E). Furthermore, cerebral cortex senescence was significantly induced at day 7 and 21 compared to chow diet ($p < 0.05; p < 0.01$) (Figure 2F) and confirmed by p21, which invokes cell cycle arrest and senescence [28]. On the other hand, caspase-3 protein exhibited similar results as the p21 protein expression ($p < 0.01$) (Figure 2G). Taken together, these findings indicate that short-term consumption of a HFD not only accelerates cell death through apoptosis but also advances senescence in cerebral cortex.

Changes of APP, BACE1, apoptosis proteins in cerebellum after 7 days HFD

To evaluate how 7 days of HFD feeding affects the activities of APP, BACE1 and cell death, cerebellum proteins were analyzed by western blotting. Similarly, the results indicated that level of APP was also significantly elevated at day 7 compared to chow diet ($p < 0.01$) (Figure 3A–3B), however, BACE1 did not demonstrate obvious changes (Figure 3C).

In the next step, we investigated whether 7 days HFD consumption also change the apoptotic signal in cerebellum, western blots were used to assess the changes of p53, cleaved-caspase 8, caspase 3, PARP, cytochrome C, Bax, Bad and BCL-2 (Figure 3). Statistical analysis shows that the amounts of p53 (Figure 3D, 3E) and cleaved-caspase 8 (Figure 3D, 3F) were evidently enhanced after consumption of HFD for 7 days ($p < 0.01$), however, this short period of HFD did not bring about significant difference in cerebellum caspase 3 and PARP activity as illustrated in Figure 3G and 3H.

Figure 1. A timeline exhibiting expression changes of APP and BACE1 after short-term HFD exposure for mice cerebral cortex. (A) Experimental scheme for testing the effect of short period HFD on brain of adult mice. (B) Body weight curve of the two treatment groups at different time courses. (C) Western blotting and (D–E) densitometry analysis for APP and BACE1 from cerebral cortex tissue lysates obtained from mice with chow diet and HFD ($n = 3$ to $4$ per group). GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Diet compositions are shown in Table 1. Data shown as means ± SD. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. chow diet group, by two-way ANOVA followed by Bonferroni’s post hoc test for body weight analysis and one-way ANOVA by Tukey’s test for densitometry assay.
Similar results were also found for apoptotic proteins released from the mitochondria. As depicted in Figure 3I, statistical analysis demonstrated that pro-apoptotic proteins cytochrome C, Bax, Bad were increased significantly ($p < 0.01; p < 0.05$) (Figure 3I–3L), however, in this time point (7 days) a significant reduction in in cerebellum anti-apoptotic BCL-2 activity could be detected ($p < 0.05$) (Figure 3M). Together, the results demonstrate that intake of 7 days HFD elevate the level of APP and accelerate the occurrence of apoptosis in cerebellum.

**Effects of short-term HFD on IL-1β production, glial cell activation markers in cerebral cortex and cerebellum**

To investigate whether short-term HFD could activate pro-inflammatory actions and glial activation, western blotting and IF staining were conducted with IL-1β (a key player in the regulation of inflammatory processes) [29], Iba-1 (ionized calcium binding adapter protein 1; a hallmark of microglial activity) [30] as well as GFAP and vimentin antibody. As illustrated in Figure 4A, the expression of IL-1β 30 were increased considerably at day 30 compared to chow diet ($p < 0.001$) (Figure 4A–4B) in cerebral cortex. In the meantime, IL-1β 17 remained unchanged after 21 days HFD and showed a decrease at day 14 in these regions ($p < 0.001$) (Figure 4C). On the other hand, the level of GFAP and Iba-1 exhibited evidently higher than chow diet ($p < 0.05; p < 0.01$) (Figure 4D–4E). However, vimentin did not display obviously difference at various time course (Figure 4D–4F). Moreover, the IF staining results in Figure 4G also demonstrated hypertrophic astrocytes [31] and hypertrophic microglia [32] delineated by red boxes at day 21 compared to chow diet groups. In addition, consistent with the results from western blot analysis, Quantitation optical density of GFAP+ astrocytes (Supplementary Figure 1A) and Iba1+ microglia (Supplementary Figure 1B) staining was significantly enhanced at 21 days HFD compared with chow diet group ($p < 0.01$). Therefore, the results indicates that short-term HFD can promote neuroinflammation and glial cell activities in cerebral cortex. Moreover, no significant difference was found in IL-1β 30 and IL-1β 17 between chow diet and HFD mice in cerebellum (Figure 4H–4J). Contrary to cerebral cortex findings at day 21, no changes were observed in GFAP, vimentin and Iba-1 in 7 days HFD-fed mice compared to chow diet mice in cerebellum (Figure 4K–4M). These observations demonstrate that cerebellum may be resistant apoptosis occurrence within 7 days of eating HFD.

![Figure 2. Association of apoptosis and HFD in mice cerebral cortex at separate times.](image-url) (A) Western blot analysis of cerebral cortex tissues of mice with HFD and chow diet controls ($n = 3$ to $4$ per group). (B–G) Quantification of PARP (B), Bcl-2 (C), Bax (D), Bad (E), p21 (F), caspase3 (G). GAPDH as a loading control. Values are presented as means ± SD. *$p < 0.05$ and **$p < 0.01$ versus chow diet; one-way ANOVA by Tukey’s test.
Effects of short-term HFD on Akt/MAPK/NF-κB p65 pathways in cerebral cortex and cerebellum

On the 21st day after HFD in mice cerebral cortex, phosphorylation of ERK1/2 was significantly enhanced compared to chow diet (p < 0.05) (Figure 5A–5B). However, JNK and NF-κB p65 phosphorylation demonstrated significant lower than chow diet groups (p < 0.05) (Figure 5C, 5F). In addition, no significant differences in phospho-p38 MAPK and phospho-Akt were observed between chow diet and HFD mice (Figure 5D, 5E).

As illustrated in Figure 5G, western blot indicated that activities of phospho-ERK1/2, phospho-p38 MAPK and MEKK1 were increased significantly 1 weeks after HFD-fed mice in cerebellum (p < 0.05; p < 0.01) (Figure 5H, 5J, 5L). However, no significant results were found in phospho-JNK and phospho-Akt between chow diet and HFD mice (Figure 5I, 5K).

Cholesterol (a component of HFD) promotes apoptosis of SH-SY5Y Cells after 48h culture and downregulates Akt/MAPK/NF-κB p65 signaling

As shown in Figure 6A–6E, the stimulation of SH-SY5Y cells with 50 μM and 100 μM cholesterol [33] significantly decreased the level Bcl-2 in associated with evident increased Bax and Bad, compared with control group (0 μM) (p < 0.01; p < 0.001) (Figure 6B–6D). Interestingly, phospho-ERK1/2, phospho-JNK phospho-p38 MAPK and phospho-Akt were obviously

![Figure 3](image-url)
reduced compared to control group (0 μM) (Figure 6E). Altogether, while cholesterol-treated cell models in vitro can partially simulate apoptosis in cortex and cerebellum of HFD-fed mice, these results do not represent the changes of stress signaling in vivo.

Schematic explanation suggests that activation of ERK1/2 pathways was occurred in mice cortex after 21 days HFD feeding. Meanwhile, ERK1/2, p38 MAPK and MEKK1 signaling were elevated simultaneously in cerebellum at day 7 of HFD-fed mice (Figure 6F).

Figure 4. Effect of HFD on activation of astrocytes and microglia in the mice cortex and cerebellum. (A) Western blots and (B–F) densitometric analysis for IL-1β 31 kDa (B), IL-1β 17 kDa (C), GFAP (D), Iba-1 (E), vimentin (F) after HFD in mice cerebral cortex at day 7, day 14 and day 21 (n = 3 to 4 per group). (G) Immunohistochemistry of GFAP+ astrocytes and Iba-1+ microglia in cerebral cortex tissue sections. High magnification images of astrocytes and microglia in a larger box outlined by red color indicated by arrows. Magnification, ×40. Scale bars, 10 μm. (H) Western blot analysis of cerebellum tissues of mice with HFD and chow diet controls (n = 3 per group). (I–M) Quantification of IL-1β 31 kDa (I), IL-1β 17 kDa (J), Iba-1 (K), GFAP (L) and vimentin (M). Vinculin and β-actin as a loading control. Values are presented as means ± SD. *P < 0.05 and **P < 0.01 versus chow diet; one-way ANOVA by Tukey’s test for cerebral cortex. Two-tailed Student’s t test for cerebellum.
DISCUSSION

In this study, we investigated the effects of short-term HFD on APP expression, apoptosis, IL-1β, glial cell activities as well as MAPK, Akt, NF-κB p65 pathways in the mouse cerebral cortex and cerebellum. The results in the C57BL/6J mice demonstrated that intake of HFD for only 7 days resulted in increased APP level in cortex and cerebellum. Furthermore, HFD consumption significantly contributed to apoptosis appearance, IL-1β elevation and gliosis at different time courses from 7 days to 21 days in cerebral cortex region. Meanwhile, HFD intake after 7 days triggered apoptosis increase in cerebellum. Nevertheless, no alterations of gliosis and IL-1β were brought about for cerebellum. Unexpectedly, increased activation of ERK1/2 level was observed both in cerebral cortex and cerebellum in HFD. In addition, in vivo studies showed that cholesterol advanced apoptosis of SH-SY5Y cells accompanying reduction activities of Akt/MAPK/NF-κBp65 signaling. Our data show a possible mechanism in the pathophysiology of apoptosis induction and glial

![Figure 5. Effect of HFD on signaling pathways in the mice cortex at 21 days and cerebellum at 7 days.](image)

(A) Western blots and (B–F) Quantitation for phospho-p44/42 MAPK (Erk1/2) (B), phospho-SAPK/JNK (C), phospho-p38 MAPK(D), phospho-Akt (E), phospho-NF-κB p65 (F) in the cortex of mice after 21 days HFD (n = 3 per group). (G) Western blots and (H–L) Quantitation for phospho-p44/42 MAPK (Erk1/2) (H), phospho-SAPK/JNK (I), phospho-p38 MAPK (J), phospho-Akt (K), MEKK1 (L) in the cerebellum of mice after 7 days HFD (n = 3 per group). Vinculin and β-actin as a loading control. Values are presented as means ± SD. *P < 0.05 and **P < 0.01 versus chow diet; Two-tailed Student’s t test.
cell activities associated with cerebral cortex and cerebellum dysfunctions under HFD conditions. Taken together, we demonstrated that short-term HFD consumption differentially contributes to apoptosis, neuroinflammation and APP expression in brain accompanying increased activity of ERK1/2 and p38 MAPK, which appears to be related to brain region-dependent and time-dependent changes.

Interestingly, some reports indicate that dietary administration of HFD in APP transgenic mice do not alter the production of APP [34, 35], while other finding demonstrates that HFD promotes APP expression [36]. On the other hand, other reports claim that APP production was increased after HFD for 4 weeks in normal adult rats [37] and no changes for APP mRNA level after short-term HFD in 4-weeks-old ordinary mice [13]. In the present study, however, our results showed that increased APP production continued to exist from day 7 to day 21 after transitory HFD. Similar to studies in APP after, whether HFD affect the level of BACE1 expression remains controversial. Miren

![Figure 6](image_url)

**Figure 6.** Changes of apoptosis and signaling pathways induced by cholesterol in SH-SY5Y cells. SH-SY5Y Cells were treated with cholesterol at indicated concentration (0 μM, 50 μM, and 100 μM) for 48 h (n = 3 per group). (A) Western blots and (B-D) Quantitation for Bcl-2 (B), Bax (C), and Bad (D) in the supernatants of SH-SY5Y Cells. (E) Western blots of phospho-p44/42 MAPK (Erk1/2), phospho-SAPK/JNK, phospho-p38 MAPK, phospho-Akt, phospho-NF-κB p65 from supernatants of SH-SY5Y Cells after 48 h culture. The levels of phosphorylated signaling-related proteins after treatment with 50 μM, and 100 μM cholesterol were normalized to the levels of the 0μM treatment group. (F) Diagram showing the mechanism of how short-term HFD aggravates apoptosis, glial cell activation and APP production in cerebral cortex and cerebellum. Vinculin as a loading control. Values are presented as means ± SD. *P < 0.05 and **P < 0.01 versus chow diet; one-way ANOVA by Tukey’s test. Red arrow indicates up-regulation and Black arrow indicates down-regulation.
Etchetoa et al. reported that BACE1 protein level was not influenced by HFD in APP/PS1 mice [36]. On the contrary, high fat/cholesterol induced significantly increased expression of BACE1 in C57BL/6 mice [9]. Our results suggested that fluctuating changes of BACE1 were seen in cerebral cortex, and these changes were significant in day 7 when compared to chow diet group. But there was no changes in BACE1 level in cerebellum. On the other hand, APP is cleaved by BACE1 (β-site amyloid precursor protein cleaving enzyme-1), contributing to Aβ aggregation. However, we did not detect Aβ (β-Amyloid) formation in the supernatants by western blots (data not shown). Thus, we speculate that temporary HFD leading to increased APP may be related to enhanced apoptotic process [38, 39], but not Alzheimer’s development. Together, although the exact reason for the discrepancy between their results and ours remain unknown, we assume that it may be due to the changes in detection methods or experimental design.

In line with previous research, HFD administration can result in an increase of microglia, astrocytes and neuroinflammation in brain [2, 10, 12, 40]. We found that GFAP (Gliaal fibrillary acidic protein; markers for the activation of astrocytes) was increased significantly at day 14 and day 21 after HFD. However, vimentin level (type III intermediate filament protein; a marker for astrocytes) remained unchanged during short-term HFD. It is possible that vimentin is also present in other cell types of brain [41, 42]. In addition, Iba-1 level also was raised after 21 days HFD. Furthermore, IL-1β 30 and IL-1β 17 expression increased significantly at day 21 and day 14 and did not change significantly in the other time points compared to chow diet in cerebral cortex. Thus, we reasoned that increased astrocytes and microglia may contribute to IL-1β production [43]. HFD induces neuroinflammation in the cerebral cortex through microglia and astrocytes activation. However, unchanged of GFAP, vimentin and Iba-1 level were observed in cerebellum at day 7 after HFD. This phenomenon in our study is consistent with IL-1β results in cerebellum that did not show significant difference between HFD and chow diet. Finally, our results clearly demonstrate region-dependent and time-dependent changes in specific brain regions in response to the HFD.

Currently, many reports have indicated that MAPK signaling are involved in response to metabolic stresses changes of extracellular or intracellular signals, such as glucose, circulating free fatty acids (FFAs) and intake HFD, which affect cell and organ metabolism [17, 44]. In agreement with previous research reported that an increase in the activity of ERK1/2 after long-term HFD (20weeks) in hippocampus [19], our studies also revealed that 21 days HFD feeding increased the level of phospho-ERK1/2 significantly in cerebral cortex. However, other evidence have shown that after 16weeks of HFD feeding decrease ERK activity in the entire brain [20]. Recently, research indicates that stimulation of ERK1/2 can cause neural cell death [22, 45, 46]. Thus, our results suggest that upregulation of ERK1/2 activity in short-term HFD may contribute to apoptosis happening, gliosis and increased APP level. In addition, many new findings in mice and humans suggest that JNK activation might induce insulin resistance and lead to obesity [47, 48]. Meanwhile, NF-κB inflammatory regulation signaling pathway is up-regulated in the brain tissue of 14weeks or 16weeks HFD-feeding mice [20, 49] as well NF-κB inflammatory signaling is unchanged in subcutaneous white adipose tissue after short-term high-fat overfeeding [16]. On the other hand, our results showed that after 7 days or 21 days consumption of HFD led to a significant decrease in phospho-NF-κB p65 and phospho-JNK simultaneously. Thus, we reasoned that the differences of results are mainly due to the experimental duration and tissue location.

Interestingly, we also observed the increased activities of MEKK1/ERK1/2 in cerebellum. In consistent with Liu et al. have reported phospho-p38MAPK increase after extending HFD feeding [20, 49], our results demonstrated that p38MAPK activity was enhanced significantly after 7 days HFD. Some findings presented in this Review show that activation of p38MAPK lead to insulin resistance, obesity and metabolic diseases [48]. Therefore, activation of MEKK1/ERK1/2 and p38MAPK aggravated the vulnerability of cerebellum to 7 days HFD feeding.

However, the limitations of current study should be stated. First, because similar results have been described in some literature [12, 13, 19], we did not explore the changes of metabolic parameters, such as glucose, cholesterol, insulin and triglyceride levels in blood, which may reflect changes of metabolic variables in short-term HFD-fed mice. Nevertheless, more recent studies suggest that cholesterol and triglyceride can induce neuroinflammation [40, 50]. Therefore, it is worth investigating the associations between metabolism and apoptosis in brain over time following short-term HFD. Second, gender and biological sex affect the pathogenesis of metabolic disorders, such as diabetes, obesity, insulin resistance and hyperglycemia [51, 52]. We used male mice only for the experiment. Therefore, whether there is an association between the effects of short-term HFD on brain and mice gender should be evaluated. Third, blood–brain barrier (BBB) remains intact after one-week or 12 month exposure to high-fat diet [53, 54]. Although we observed the up-regulation of
ERK1/2 protein in brain of HFD mice, ERK1/2 inhibitors cannot be used to prove whether decreased ERK1/2 will reverse apoptosis in brain after short-term HFD. Because no sufficient evidence shows that ERK1/2 inhibitors will cross the BBB. We believe that nanoparticle technology may contribute to facilitating inhibitors delivery to transverse the BBB in the future.

In conclusion, we discovered that short periods (7 days, 14 days and 21 days) consumption of HFD caused some alterations of APP protein, apoptosis, glial cell, IL-1β and MAPK signaling in cerebral cortex and cerebellum of young mice. Interestingly, apoptotic molecules changes have started from the first week. In addition, it is necessary to identify the exact roles of ERK1/2 and APP up-regulation in different regions of brain after short-term HFD.

Abbreviations


AUTHOR CONTRIBUTIONS

Jun-Ling Wang: Formal analysis, writing original draft. Wei-Guang Chen: Formal analysis. Chao-Jin Xu: Formal analysis, Writing - original draft. Mei-Qi Li, and Li-Zhao performed partial WB.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest related to this study.

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REFERENCES


Supplementary Figure 1. Quantitation optical density of GFAP⁺ astrocytes (A) and Iba1⁺ microglia (B) staining of mice fed chow diet and high fat diet in different time points. The images (2–3 sections per animal) were captured and quantified using the NIH Image J software package. *p < 0.01 versus the chow diet fed control group.