



Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

High glucose induces hippocampal neuron impairment through the SKP1/COX7C pathway: A potential mechanism for perimenopausal depression



Ziqi Wang^{a,†}, Zhiyuan Liu^{a,†}, Sijia Feng^a, Xintong Song^a,
Dequan Liu^a, Ning Ma^a, Xinyue Zhang^a, Weiwei Liu^a,
Dan Ohtan Wang^{a,b,*}, Xiaoling Liu^{a,*}, Takashi Ikejima^{a,c,*}

^aWuya College of Innovation, Shenyang Pharmaceutical University, Shenyang 110016, China

^bBiology Program, Science Division, New York University Abu Dhabi, Abu Dhabi 129188, United Arab Emirates

^cKey Laboratory of Computational Chemistry-Based Natural Antitumor Drug Research & Development, Shenyang Pharmaceutical University, Shenyang 110016, China

Received 22 November 2024; received in revised form 19 May 2025; accepted 30 June 2025

KEY WORDS

Perimenopause;
Depression;
Glucose;
Neuron;
COX7C;
SKP1;
Mitochondrion;
Ubiquitination

Abstract Perimenopause raises the risk and incidence of depression, whereas the underlying molecular mechanism remains unclear. Disturbed glucose regulation has been widely documented in depressive disorders, which renders the brain susceptible to various stresses such as estrogen depletion. However, whether and how glucose dysfunction regulates depression-like behaviors and neuronal damage in perimenopausal transition remains unexplored. Here, a prominent depressive phenotype was found in perimenopausal mice induced by the ovarian toxin 4-vinylcyclohexene diepoxide (VCD). The VCD depression susceptible group (VCD^{SS}) and the VCD depression resilient group (VCD^{RES}) were determined using a ROC-based behavioral screening approach. We found that the hippocampus, a crucial region linked to depression, had hyperglycemia and mitochondrial abnormalities. Interestingly, oral administration of the SGLT2 inhibitor empagliflozin (EMPA) and intrahippocampal glucose infusion suggest a close relationship between hyperglycemia in the hippocampus and the susceptibility to depression. We verified that cytochrome *c* oxidase 7c (COX7C) downregulation is a potential cause of the high glucose-induced neuronal injury using proteomic screening and biochemical validations. High glucose

*Corresponding authors.

E-mail addresses: ikejimat@vip.sina.com (Takashi Ikejima), lx191321@126.com (Xiaoling Liu), ohtan.wang@nyu.edu (Dan Ohtan Wang).

[†]These authors made equal contributions to this work.

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

<https://doi.org/10.1016/j.apsb.2025.09.003>

2211-3835 © 2025 The Authors. Published by Elsevier B.V. on behalf of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

causes COX7C to be ubiquitinated in a S-phase kinase associated protein 1 (SKP1)-dependent manner. According to these results, SKP1/COX7C represents a unique therapeutic target and a novel molecular route for treating perimenopausal depression.

© 2025 The Authors. Published by Elsevier B.V. on behalf of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Women during perimenopause, a menopause transition stage (5- to 10-year period preceding the menopause onset and the early post-menopause period), are vulnerable to depression due to the hormonal fluctuation^{1,2}. The incidence of depression peaks in the perimenopause period with highest suicide rates in women among the ages of 45 to 64 years^{3,4}. Although hormone replacement therapy, such as estrogen therapy, is an effective way to treat perimenopausal depression (PMD), there remains considerable reluctance to hormone replacement therapy because of increased risks for breast cancer, heart disease, and stroke^{5,6}. Investigating the pathophysiology of depression during the perimenopausal transition is therefore crucial to enhancing PMD treatment.

Metabolic dysfunction contributes to the severity of psychiatric disorders. Glucose metabolism dominantly functions in maintaining brain cell viability^{7,8}, and metabolic imbalance affects the processes of various neurological diseases, such as depression^{9,10}. Both type 2 diabetes mellitus and depressive disorders exhibit a decrease in cerebral glucose uptake as measured by PET imaging (¹⁸F-fluorodeoxyglucose, ¹⁸F-FDG)¹¹, indicating that impairment of brain glucose metabolism may be an important factor for mental diseases. Downregulation of ATP synthesis, mitochondrial damage, and neuronal dysfunction were found to be caused by disruptions in the brain's glucose metabolism, which included cerebral hyperglycemia, reduced glucose uptake, and changes in the glucose metabolic coupling between neurons and glia¹². Neurons experience spontaneous death in the setting of mitochondrial malfunction due to inadequate ATP generation, which contributes to the pathogenesis of neurodegenerative diseases^{13–16}. Therefore, disturbed glucose metabolism is crucial for the pathogenesis of depression. A series of factors involving electron transport chain obstruction, decreased mitochondrial potential, lower ATP levels, and oxidative damage have all been proven to have negative roles in the development of depression^{17,18}. The systems of cerebral dopamine and serotonin (5-HT), which are essential for mood regulation and the development of depressive symptoms, may also be impacted concurrently by abnormal glucose metabolism¹⁹. What's more, chronically elevated glucose levels damage both mitochondria and mitochondrial DNA, generating toxic products and promoting systemic inflammation, ultimately hastening cell ageing and death²⁰. However, it is yet unclear if and how perimenopausal transition-related changes in brain glucose metabolism could affect depression symptoms.

Brain metabolism dysfunction inherent to the perimenopausal transition has been extensively documented^{21,22}. A 15%–25% reduction in brain metabolic function occurs when estrogen is lost due to ovarian removal or aging, especially for a persistent decline in glucose metabolism^{23,24}. In aged female mice (6–9 months of age, perimenopausal transition phase), hypometabolism of brain glucose was observed, characterized by a decline in glucose

uptake and hexokinase activity, the inactivation of pyruvate dehydrogenase and mitochondrial complex IV activity, which induces impairment of mitochondrial structure and function²⁵. Furthermore, the lactate transport and utilization are also obstructed in parallel to the hypometabolism of glucose and decline in glucose transport in perimenopausal transition²⁵, indicating the transformation of an alternative fuel source for neurons during perimenopausal transition. Hyperglycemia is associated with the hypometabolism of brain glucose in specific areas, including the entorhinal cortex, medial temporal cortex, and hippocampal regions²⁶. The glucose metabolic process appears to be accelerated in ovariectomized mice, as evidenced by the simultaneous occurrence of hypometabolism, mitochondrial malfunction, and oxidative damage^{22,24}. A decrease in cerebral glucose absorption has been seen in the brains of perimenopausal women^{27,28}. They also demonstrated decreased expression of the glucose transporter, such as Glucose transporter (GLUT) 1 and 3, and low glucose uptake, which can be reversed by estrogen supplementation²⁹. Similarly, we previously discovered that peripheral glucose metabolism is impaired in perimenopausal mice, followed by hyperglycemia³⁰. The consequences of estrogen loss during the perimenopause on cerebral glucose levels and depression-like behaviors have received little attention, despite the fact that several brain diseases have been linked to abnormal glucose metabolism as part of their pathogenesis.

In this study, to distinguish the subgroups of depression-prone mice exposed to VCD, we used the ROC algorithm based on depression-like behavior. We revealed that hyperglycemia in the hippocampus determines the susceptibility to depression and neuronal damage. Furthermore, using proteomic sequencing, we identified a possible novel predicted therapeutic target for hyperglycemia-induced brain damage in VCD-induced perimenopausal depression mice: the cytochrome *c* oxidase 7c (COX7C)/S-phase kinase associated protein 1 (SKP1) pathway. Our findings proposed a novel possibility that the perimenopausal period's neuronal mitochondrial damage, which sets off depressive behaviors, is determined by cerebral glucose metabolism. Current research could help explain why mental illnesses coexist with metabolic ailments like depression and diabetes, providing a promising strategy for the development of new drugs to alleviate depressive behaviors in perimenopausal transition.

2. Materials and methods

2.1. Reagents

EMPA was purchased from Aladdin (Shanghai, China), and estradiol (E2) was purchased from Sigma–Aldrich (Taufkirchen, Germany). MG132 and chloroquine (CQ) were obtained from MedChemExpress (Monmouth Junction, NJ, USA), and cycloheximide (CHX), Necrostatin-1 (Nec-1), and Z-VAD were

purchased from ABmole Bioscience (Houston, TX, USA). Anti-COX7C (11411-2-AP), anti-PSD95 (30255-1-AP), anti-SYN (17785-1-AP), anti-BDNF (25699-1-AP), anti-SKP1 (67745-1-Ig), and anti- β -actin (20536-1-AP) antibodies were purchased from Proteintech Bioscience (Chicago, IL, USA). Anti-Flag (2368T), anti-HA (3724T), and anti-Ub (3936T) antibodies were bought from Cell Signaling Technology (Houston, TX, USA).

2.2. Animal models

Five to six-week-old female C57BL/6 mice were acquired from ChangSheng Bioscience (Benxi, China). Mice were maintained in a specific pathogen-free facility at $23 \pm 0.5^\circ\text{C}$, and a 55%–65% humidity, under a 12 h/12 h light/dark cycle. Mice were fed with standard chow and water *ad libitum*. After acclimatization for a week, mice were randomly divided into the SHAM and VCD groups ($n = 30$ per group) by a completely random method. Mice in the VCD group were intraperitoneally injected with 160 mg/kg/day VCD (dissolved in corn oil; YUANYE Bio-Technology, Shanghai, China) continuously for 20 days³¹. The SHAM group received an injection of the same amount of corn oil. The vaginal swab was then used to measure the estrus cycle daily starting on Day 30 following the initial VCD injection. Mice in the VCD group had estrus cycles that were extended to 15 days beginning on Day 45, whereas mice's usual estrus cycles lasted 4–5 days, indicating that VCD mice entered the perimenopausal stage. Animal experiments were approved by the policies of China on the use and care of laboratory animals. In accordance with the standards specified in the Guide for the Care and Use of Laboratory Animals prepared by the Shenyang Pharmaceutical University, every animal received human care (approval No. SCXK 2020–0001, approval date: 28/03/2023).

2.3. Behavioral tests

According to the findings of Wang et al.³² and Yu et al.³³, depression behavioral tests were conducted on Day 35 after intraperitoneal injection of VCD in mice. Forced swim test (FST), tail suspension test (TST), and sucrose preference test (SPT) were used to detect the depressive behaviors of mice in each group starting from Day 45.

In the FST, mice were placed in a transparent cylinder (40 cm height $\times$ 20 cm diameter). Every mouse was made to swim for 6 min at a depth of 18 cm (23 to 25°C) in each water-filled cylinder, and the entire process was captured on camera. The lack of all movements other than those necessary to maintain the mice's heads above the water's surface is known as the immobility period. Using the VisuTrack Animal Behavior Analysis System (Xinlun MDT, Shanghai, China), mice were observed for 6 min, and the length of time they were immobile during the final 4 min was noted.

The TST was performed in a white plastic chamber (55 cm height $\times$ 20 cm width $\times$ 20 cm depth). Each mouse spent 6 min hanging head-down from the tip of its tail with adhesive tape. The end of all trunk and limb motions is known as the immobility period. Similar to FST, TST counts the final 4 min after recording 6 min of remaining time.

Mice were given *ad libitum* access to two bottles containing 1% sucrose solution (w/v) for 24 h in order to perform SPT. After that, a 24-h period was spent administering a bottle of 1%

sucrose solution and another bottle of tap water. To prevent adverse effects, the bottles' positions were replaced halfway through the duration. All mice were fasted and dehydrated for 12 h at the conclusion of the acclimation phase. Following that, SPT was carried out for 24 h in a separate cage where each mouse had unrestricted access to a bottle of tap water and 1% sucrose solution. During the test, the two bottles' positions were alternated to avoid any unintended drinking preferences. Changes in the weight of fluid eaten were used to quantify the intake of sugar and water. The sucrose preference was calculated according to Eq. (1):

$$\text{Sucrose preference (\%)} = \frac{\text{The sucrose consumption (g)}}{\text{The sucrose consumption (g)} + \text{The water consumption (g)}} \times 100 \quad (1)$$

2.4. ^{18}F -FDG PET scanning

After 12 h of fasting, the mice were anesthetized with 1.5% isoflurane vaporized in 70% O_2 delivered through a nose cone. Through the tail vein, the ^{18}F -FDG (37 kBq (1 μCi)/g) supplied by Sun Yat-sen University's First Affiliated Hospital (Guangzhou, China) was administered. Forty minutes after receiving the ^{18}F -FDG injection, the mice were placed in a prone position and examined using a small-animal PET/CT scanner that was equipped with Inveon software (Siemens Medical Solutions, Forchheim, Germany). The static picture acquisition was carried out for 20 min after the scanning conditions (80 kV, 500 μA) were set. After 5 min of low-dose spiral CT detection, 15 min of 3D scanning mode with two-bed positions were spent on Positron Emission Tomography (PET) scanning. The two-dimensional ordered-subsets expectation maximum was used to rebuild all PET images after they had been CT-corrected for emission scatter and attenuation. The ^{18}F -FDG uptake was analyzed by using Inveon research workplace 4.1 software according to Eq. (2):

$$\text{Normalized radioactivity} = \frac{\text{Measured radioactivity in the PET image (kBq/mL)}}{\text{Injected radioactivity (kBq) in units of } [\% \text{ID/mL}]} \quad (2)$$

The Region of Interest (ROI) on the CT images was defined in three dimensions for every small-animal PET scan. The PET scans were co-registered to guarantee anatomic localization consistency. The total brain, prefrontal cortex, parietal cortex, temporal cortex, occipital cortex, hippocampus, amygdala, and hypothalamus were the seven brain areas chosen and projected onto the PET pictures. By repeating the measurements three times, ROIs for each region were displayed along with the average of the three ROIs.

2.5. Biochemical analysis

The contents of glucose and 5-HT (Jianglai Biotech, Shanghai, China), pyruvate, lactate, malondialdehyde (MDA, Jiancheng Biotech, Nanjing, China), ATP level and the activity of mitochondrial complex II and IV (Solarbio, Beijing, China) in the whole brain, hippocampus or mouse hippocampal neuron HT22 cells (iCell Bioscience, Shanghai, China), were determined using the corresponding commercial kits according to the manufacturer's instructions.

2.6. *In vivo* EMPA and E2 treatment

To verify the effect of hippocampal hyperglycemia on perimenopausal depressive behaviors, the VCD mice were orally administered 75 mg/kg EMPA, dissolved in 0.5% CMC-Na for 8 days ($n = 10$) to reduce the cerebral glucose level. Coherently, E2 was administered intraperitoneally at a dose of 0.5 mg/kg every day for four weeks ($n = 10$). After administration, the mice were exposed to a depressive behavior test, and the level of glucose in the hippocampus was examined using a glucose detection kit.

2.7. Stereotaxic surgery for hippocampal cannulation and glucose infusion

Mice were anesthetized with 2% isoflurane in O₂ (4 L/min) delivered through a nose cone and head-fixed in a stereotaxic device (RWD Life Science, Houston, TX, USA). To prevent dehydration, the eyes were coated with erythromycin eye ointment, and a longitudinal cut was made to reveal the skin above the skull after it had been shaved and cleaned with 75% alcohol. After removing the membrane lining the skull, a drop of 30% H₂O₂ was applied, and the skull's surface was roughened using a knife. Four tiny holes were drilled: two for the insertion of anchoring screws and two for the positioning of a stainless-steel cannula directed at the dorsal hippocampus using stereotaxic coordinates (mediolateral, ± 2.4 mm from bregma; dorsoventral, -0.8 mm from the skull surface; and anteroposterior, -2.5 mm from bregma)³⁴. The cannula was secured with dental acrylic cement, and the wound was sutured shut. We placed injectors 1 mm from the cannula tip after giving the mice two weeks to recover. In order to allow the infusion to disseminate, mice were injected with either a glucose solution (2.7 μ g glucose per hippocampal region) or a vehicle (0.9% NaCl) at a rate of 0.1 μ L/min (1 μ L bilateral). We used 0.5 μ L of Evans blue (0.1%) postmortem to validate the proper targeting. Calculated from the average hippocampal glucose concentrations seen in VCD-treated animals, the concentration of infused glucose solution was 2.7 μ g of glucose per hippocampus. There are no changes in cerebral osmotic pressure at this concentration and dosage according to van der Kooij et al.'s discovery¹². Following the glucose infusion, we used FST to detect depressive behavior.

2.8. Injection of adeno-associated virus

Recombinant COX7C adeno-associated viral (AAV) vector (AAV2/9-hSyn-Cox7c) and negative AAV vector (AAV2/9-hSyn-EGFP) were constructed and packed by Shanghai Juding Biotechnology (Shanghai, China) and stored at -80°C . Among the vectors used in this investigation are AAV2/9-hSyn-Cox7c (titre: 3.5×10^{13} vector genomes/mL) and AAV2/9-hSyn-EGFP (titre: 1.6×10^{13} vector genomes/mL). The sequence of vector is as follows (5'-3'): ATGTTGGGCCAGAGTATCCGGAGGTT CACGACCTCCGTGGTCCGTCGCAGCCACTATGAGGAGGGTCCGGGAAGAATTTGCCATTTT CAGTGGAACAA GTGGCGGTTGCTGGCTATGATGACCGTGTACTTTGGATCTGGGTTTGCCGCACCTTTCTTTATAGTAAGACACCAGCTACTTAAAAATAA.

A microinjection syringe, operated by the same microinjector, was used to inject the virus bilaterally into the hippocampal CA2/3 (mediolateral, ± 2.5 mm from bregma; dorsoventral, -2.4 mm from the skull surface; and anteroposterior, -2.18 mm from bregma) at a slow rate of 100 nL/min for 10 min. Ten

minutes after injection, the syringe was gradually withdrawn to allow for diffusion. Mice were subjected to subsequent experiments after at least 3 weeks.

2.9. Quantitative RT-PCR analysis

As previously mentioned, quantitative RT-PCR (qRT-PCR) was carried out⁷. To put it briefly, TRIzol reagent (Thermo Fisher, MA, USA) was utilized to isolate total RNA from hippocampus tissue and cells. The PrimeScript RT-PCR Kit (TaKaRa, Shiga, Japan) was used to reverse-transcribe the RNA using oligo dT-primers. PrimeScript RT Master Mix (TaKaRa, Shiga, Japan) was then used for qPCR on an ABI 7500 Fast (Thermo Fisher, MA, USA). Each sample was examined in triplicate. The $2^{-\Delta\Delta C_t}$ technique was used to measure mRNA levels. β -Actin was utilized as a standardized control to assess the mRNA levels of the genes of interest. The primer sequences are presented in Supporting Information Table S1.

2.10. Histological and immunofluorescence staining

Perfused mice's uteri and brains were removed, and they were subsequently preserved for 24 h in 4% paraformaldehyde. The uterus was initially dehydrated with ethanol and then embedded in paraffin for hematoxylin and eosin (H&E) staining. H&E was used to stain each tissue after it had been divided into 5- μ m-thick slices (Servicebio Technology, Wuhan, China).

Brain portions were dried in 30% sucrose solution, frozen in an OCT freezer at -80°C , and then sectioned using a cryostat into slices that were 15 μ m thick for Nissl staining (CM1950, Leica, Hessian, Germany). Each tissue was stained with Nissl staining solution c.

For immunofluorescence staining, after permeabilization with 0.3% Triton X-100 and 5% BSA in PBS, the slices were blocked in QuickBlock Immunostaining blocking solution (Beyotime, Shanghai, China) before incubation with anti-COX7C antibody (1:200) or anti-SKP1 antibody (1:200) at 4°C overnight. After three washes with PBS, samples were incubated with TRITC-conjugated secondary antibody (1:200, Affinity Bioscience, Jiangsu, China) for 2 h at room temperature. The slices were washed three times with PBS and mounted with DAPI.

A light microscope (Nikon, Tokyo, Japan) was used to capture the H&E and Nissl images, and a confocal microscope (Nikon, Tokyo, Japan) was used to acquire the IHC images. The stained neurons in the Nissl and IHC images were examined using Image J.

2.11. Golgi staining

The Golgi Stain Kit (GP1152, Servicebio Technology) was used to stain the mice's brains. Image J 2.9.0 software was employed to code the images and count the dendritic spines of at least 20 pyramidal neurons in each group in the hippocampus in a double-blind fashion. For each group, Sholl analysis demanded at least six pyramidal neurons in the hippocampus.

2.12. Western blotting

Mouse hippocampus tissues and cells were homogenized in RIPA buffer (Beyotime, Shanghai, China) supplemented with a protease inhibitor complex (Meilunbio, Dalian, China). The protein concentration was evaluated by the BCA Protein Assay Kit (NCM

Biotech, Suzhou, China). All samples were separated using SDS-PAGE gels and then placed onto a Millipore Immobilon-P Membrane (Millipore, Billerica, MA, USA) in accordance with standard procedure. After blocking the membrane for 2 h with 5% skim milk, the primary antibody was incubated for the entire night at 4 °C. The secondary antibody, peroxidase affinity-pure goat anti-rabbit or anti-mouse IgG (Affinity Bioscience, Jiangsu, China), was exposed to ECL (Meilunbio, Dalian, China) after being incubated for 2 h at room temperature. Using Image J software (NIH, New York, NY, USA), target protein band intensities were evaluated and converted to β -actin to provide relative values.

2.13. Proteomic analysis

Protein isolated from hippocampus tissues with SDT lysis buffer (4% SDS, 100 mmol/L DTT, 100 mmol/L Tris-HCl pH 8.0) was denatured by a boiling-ultrasonication-boiling treatment. Centrifuged at $16,000\times g$ for 15 min to remove undissolved cellular debris. The supernatant was collected and measured using a BCA Protein Assay Kit (Bio-Rad, CA, USA). Protein (200 μ g for each sample) digestion was performed with the FASP method. The peptide was desalted with C18 StageTip for further LC-MS analysis.

The Q Exactive Plus mass spectrometer and Easy 1200 nLC (Thermo Fisher, MA, USA) were used to perform LC-MS/MS. Reverse-phase high-performance liquid chromatography (RP-HPLC) separation was performed using the EASY-nLC system (Thermo Fisher, MA, USA) with a self-packed column (75 μ m $\times$ 150 mm; 3 μ m, ReproSil-Pur C18 beads, 120 Å, Dr. Maisch GmbH, Ammerbuch, Germany) and a flow rate of 300 nL/min. The resolution of the full MS scans was 70,000 at m/z 200, while the resolution of the MS/MS scan was 17,500 at m/z 200. For MS and MS/MS, the maximum injection time was set at 50 and 50 ms, respectively. The isolation window was set at 1.6 Th, and the normalized collision energy was 27. The length of dynamic exclusion was 1 min.

Using MaxQuant software version 1.6.0.16, the MS data were examined and compared to the UniProtKB Rattus norvegicus database. Filtered and exported with a false discovery rate of less than 1% at the protein and peptide-spectrum-matched levels, respectively, were the database search results. Using the intensity determination and normalization procedures previously reported^{35–37}, label-free quantification was carried out in MaxQuant. Proteins that had a P -value < 0.05 and a fold change of ≥ 1.5 were categorized as significantly differentially regulated.

Bioinformatics data were analyzed using Perseus software, Microsoft Excel, and R statistical computing software. To annotate the sequences, information was extracted from UniProtKB/Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Ontology (GO). The enriched GO and KEGG pathways were statistically significant ($P < 0.05$). The STRING database and Cytoscape software were also used to develop a protein-protein interaction (PPI) network.

Omics and Text based Target Enrichment and Ranking (OTTER, <http://otter-simm.com/otter.html>) is a novel computational approach for enriching targets from omics data of investigated chemicals. OTTER first performs text mining on the differentially expressed proteins (DEPs) in PubMed abstracts related to keywords. Next, the PPIs between these top-ranked DEPs are considered. Finally, the total score is derived by summing the text and PPI scores, resulting in a final score table for the genes, as previously published³⁸.

2.14. Cell culture

HT22 cells free from mycoplasma contamination were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, CA, USA) containing 10% fetal bovine serum (Vazyme Biotech, Nanjing, China) and 1% penicillin-streptomycin (TBD Science, Tianjin, China) in a cell incubator with 5% CO₂ at 37 °C.

2.15. Cell viability analysis

Cells (8×10^3 cells/well) were cultured in 96-well dishes (Corning, NY, USA) for 24 h before incubation with medium containing 25, 50, 100, 200, and 400 mmol/L glucose (additional supplemented with 0, 25, 75, 175, and 375 mmol/L glucose) for 2, 4, 8, 12, and 24 h. Cells were treated with different doses (7.5–120 μ mol/L) of autophagy inhibitor (CQ), necrosis inhibitor (Nec-1), and apoptosis inhibitors (Z-VAD) to assess the viability of HT22 cells co-cultured with high glucose for 24 h. To estimate the cytotoxicity of glucose and the cause of cell death, cells were exposed to the Cell Counting Kit-8 (Meilunbio) for 1 h at 37 °C in an incubator with 5% CO₂. At a wavelength of 450 nm, the absorbance was calculated.

2.16. Protein stability assay

Cells were treated with 20 μ mol/L MG132 or 20 μ mol/L CQ for 9 h each in order to monitor the stability of COX7C protein degradation by preventing proteasomal or lysosomal degradation. The CHX chase test employed 30 μ mol/L CHX.

2.17. Cell transfection

Following the manufacturer's instructions, cells were cultivated on a 6-well plate (1×10^5 cells/well) for 24 h before being transfected with plasmids to either knockdown SKP1 (siSkp1) or overexpress COX7C (oeCox7c) (Mingsheng Biotech, Shanghai, China) using Lipofectamine 8000 (Beyotime, Shanghai, China). Cells were extracted, and the ensuing tests were carried out 48 h after transfection. Supporting Information Table S2 has a list of the antisense sequence.

2.18. Transmission electron microscopy

Transmission electron microscopy (TEM) was used to examine the mitochondria's integrity and morphological changes in hippocampal tissues and cells. The samples were transferred to Servicebio Biotechnology for additional processing and slicing after being fixed with 2.5% glutaraldehyde that had been pre-cooled. Images were acquired under a transmission electron microscope (HT7700, HITACHI, Japan).

2.19. TUNEL staining

Neuronal cell death in the hippocampal tissues and cells was assessed by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assays using TUNEL Apoptosis Detection Kit (YSFluor 640, Yeasen, Shanghai, China). As directed by the manufacturer, the sections were incubated with TUNEL reagents. Confocal microscopy was used to examine the fluorescence, and Image J software was used to determine the statistics of apoptotic cells.

2.20. Annexin V-FITC/PI staining

Cell apoptosis was tested by flow cytometry using the Annexin V-FITC/PI kit (UElandy, Suzhou, China). According to the requirements of the kit, HT22 cells were resuspended and mixed with 500 μ L of binding buffer. Then, 5 μ L of solution A and 10 μ L of solution B were added and mixed. The reaction was carried out in the dark at room temperature for 15 min, and the samples were analyzed by flow cytometry. FlowJo software was used to analyze streaming data.

2.21. JC-1 staining

The alteration of MMP was assessed using JC-1 staining assays (Beyotime, Shanghai, China) according to the manufacturer's instructions. Hoechst 33342 (Thermo Fisher, MA, USA) staining is performed before staining to determine the position of the cell nucleus.

2.22. Mito-tracker deep red staining

Cells grown on 24-well chamber slides were stained with 1 mol/L MitoTracker Deep Red (Thermo Fisher, MA, USA) for 30 min. After 15 min of 4% paraformaldehyde fixation, cells were permeabilized for 5 min using 0.2% Triton X-100. Cells were blocked with QuickBlock Immunostaining blocking solution for 30 min, and incubated with anti-COX7C antibody (1:200) or anti-SKP1 antibody (1:200) at 4 °C overnight. Secondary antibodies labeled with FITC or TRITC were then added to the cells for 2 h before being mounted with DAPI and examined using a confocal microscope.

2.23. Immunoprecipitation (IP) and ubiquitination assays

Cells were transfected with the indicated plasmids for 24 h and were lysed with lysis buffer for WB and immunoprecipitation (APExBIO, Houston, TX, USA) at 4 °C for 30 min. The samples underwent a 10-min centrifugation at 12,000 $\times$ g, an overnight incubation with the primary antibody at 4 °C, and a 3-h incubation with 40 μ L protein A/G agarose at 4 °C. The buffer was then used to wash the beads three times. The immunocomplexes underwent Western blotting analysis after being eluted in SDS loading buffer.

2.24. Statistical analysis

All data were analyzed using GraphPad software (Version 6.01, La Jolla, CA). Student's *t*-test with Welch's correction and one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's correction were used to compare group results statistically. The data displayed in the charts were mean $\pm$ standard error of the mean (SEM), and *P* < 0.05 was deemed significant.

3. Results

3.1. A subpopulation of mice treated with VCD is vulnerable to depression accompanying with high-glucose levels in the hippocampus

In order to investigate the relationship between cerebral glucose metabolism and depressive behaviors, we established a perimenopausal mouse model by using an ovarian toxic VCD as

previously reported³⁹. Mice were intraperitoneally injected with VCD for continuous 20 days and then tested with vaginal smear examination at the indicated time point (Supporting Information Fig. S1A). Mice injected with VCD had prolonged estrous cycles from Day 45 to Day 60, as determined by vaginal smear tests (Fig. S1B). The uterine wall thickness decreased in VCD-treated mice, according to the consistent results of uterine H&E staining (Fig. S1C). These data demonstrate the successful establishment of the perimenopausal model by VCD injection. Furthermore, we observed that VCD injection had no discernible damage to other primary organs, including heart, liver, spleen, lung, and kidney (Fig. S1D)⁴⁰.

Subsequently, a series of behavioral tests, including FST, TST, and SPT, were conducted to evaluate the depression-like behaviors in VCD-induced perimenopausal mice. Compared to SHAM mice, VCD-induced perimenopausal mice showed a longer period of immobility in the FST and TST and a reduced preference for sucrose in the SPT (Fig. 1A–C and Fig. S1E). Significant relationships were found between immobility time in FST and TST and sucrose preference (Fig. S1F), indicating good test coherence for assessing depression-like behavior in VCD mice. Nonetheless, there were significant individual differences in the depressive behaviors of VCD mice. In order to define cutoff criteria based on the three behavioral tests, we used the receiver operating characteristic (ROC) algorithm to identify a subset of VCD mice that are more susceptible to VCD-induced depression (Fig. 1D)^{41,42}. Mice were assigned a depression score (D-score: 0–3) based on how many positive criteria they met (Fig. S1G and S1H). The majority of SHAM mice had low D-scores (0 and 1), whereas the VCD animals had higher D-scores (2 and 3) (Fig. 1E). The VCD mice were specifically divided into two groups: the VCD^{RES} (D-score = 1 and 2) and the VCD^{SS} (D-score = 3). The VCD^{SS} group was assigned to about 36.7% of the VCD mice (D-score = 3). There is no depression-like phenotype in the SHAM mice (about 56.7%; D-score = 0) (Fig. 1F). This approach was used in later screenings for depression-like behavior in VCD mice. At the same time, the measurement of 5-HT levels in the hippocampal region also showed a significant rise in the VCD^{SS}, suggesting a possible rise in depressive symptoms (Fig. S1I).

The direct effect of VCD on depression was ruled out, and the particular effect of VCD-induced follicular failure in female perimenopausal depressive disorders was confirmed, as male mice injected with VCD did not exhibit any discernible alterations in depression-like behaviors (Fig. S1J). The association between perimenopausal status and sadness was also confirmed when we observed depression-like behaviors in the bilateral ovariectomy (OVX)-induced perimenopausal mice (Fig. S1K).

We used ¹⁸F-FDG PET scanning in several brain areas linked to emotion regulation, such as the cortex, hippocampus, amygdala, and hypothalamus in the VCD^{SS} and VCD^{RES} mice, to assess the uptake of glucose in the brains of perimenopausal depression-like mice. Our results showed that the VCD^{SS} and VCD^{RES} mice had significantly lower glucose absorption in a number of different brain areas (Fig. 1G and H). Notably, the hippocampal glucose level rose in both OVX-induced perimenopausal mice (Fig. S1L) and VCD^{SS} mice (Fig. 1I). Furthermore, the immobility duration in FST was directly correlated with the hippocampal glucose level (Fig. 1J), highlighting the potential involvement of hippocampal hyperglycemia in perimenopausal depression.

To identify the potential mechanisms contributing to the elevated glucose content observed in the hippocampus, we

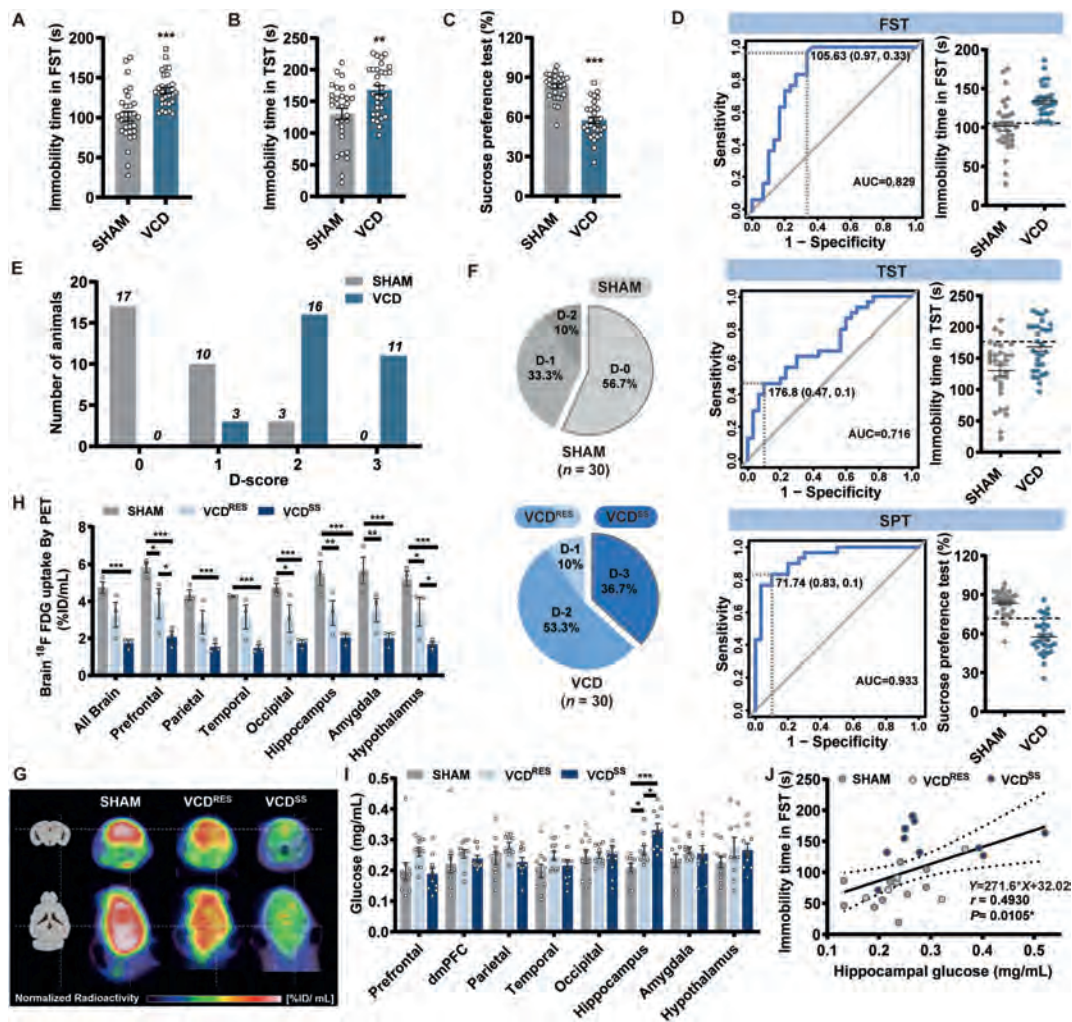


Figure 1 Intraperitoneal injection of VCD develops depression-like behavior and increases glucose level in the hippocampus. (A, B) The immobility time in FST and TST in mice after VCD treatment ($n = 30$). (C) The percentage of sucrose preference in SPT ($n = 30$). (D) ROC algorithm to establish cutoff criteria based on FST, TST, and SPT. Dash line represents cutoff value based on the Youden index. AUC, area under curve ($n = 30$). (E) Number of animals distributed among different D-score ($n = 30$). (F) Distribution of groups according to the depression score (D-1, D-score = 1; D-2, D score = 2; D-3, D score = 3). VCD mice that met all three positive criteria (D-3) were classified as the VCD^{SS} phenotype. VCD mice with D-1 and D-2 were classified as the VCD^{RES} phenotype. The SHAM group included only D-0 mice. (G, H) ¹⁸F-FDG uptake in the whole brain was examined by PET scans obtained by overlay from the individual PET signals. The results were quantified for the whole brain and other brain regions ($n = 3$ per group). (I) Glucose level in different brain regions in three groups of mice ($n = 10$). (J) The relationship between hippocampal glucose levels and immobility time in the FST ($n = 3$). Significance was determined by Student's *t* test in A, B and C; one-way ANOVA analysis with Tukey's *post hoc* test in H and I; or Pearson's correlation coefficient in J. Regression lines are shown with the 95% confidence interval (dotted lines) in I. Data are expressed as mean $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

conducted an assessment of glucose metabolism. VCD^{SS} mice showed increased anaerobic glycolysis and gluconeogenesis along with lower pyruvate and higher L-lactate levels (Fig. S1M–S1O). However, the glucose transporters were impaired in VCD^{SS} mice (Fig. S1O). We supposed that the enhancement of anaerobic glycolysis induced a higher level of lactate as the main source for gluconeogenesis, which may lead to slower consumption but increased production of glucose, thereby triggering cerebral hyperglycemia. But there were no such changes in the VCD^{RES} mice. Altogether, these data highlight the importance of the hippocampus and its glucose metabolic dynamics as the primary focus of the research efforts, aiming at elucidating the mechanisms underlying perimenopausal depression.

3.2. Perimenopausal depression-like mice show mitochondrial damage and apoptosis in hippocampal neurons

In order to validate the neuronal synaptic alterations in VCD^{SS} mice, we employed Golgi staining in conjunction with Sholl analysis, providing a method for examining the morphology and quantifying the number of dendritic spines within neurons⁴³. The results demonstrated a significant decrease in the density of dendritic spines and the number of branches, suggesting impaired synaptic structure within hippocampal neurons of VCD^{SS} mice (Fig. 2A and B, Supporting Information Fig. S2A and S2B). Western blotting analysis revealed a statistically significant reduction in the protein levels of synapse-associated proteins

(postsynaptic density 95 (PDS95) and synaptophysin (SYN)) and brain-derived neurotrophic factor (BDNF) in the VCD^{SS} group (Fig. S2C and S2D). Furthermore, given the crucial function of glucose metabolism in mitochondrial function, we assessed mitochondrial morphology, MDA levels and ATP content in the hippocampus. Mitochondrial ultrastructural anomalies in hippocampal neurons were examined. Hippocampal neurons in VCD^{SS} animals showed significant morphological alterations, including vacuolization and swelling of the mitochondria as well as reductions in mitochondrial density, volume, and length (Fig. 2C and D). In line with this, VCD^{SS} mice had clear mitochondrial dysfunction and oxidative damage, as seen by decreased ATP synthesis and increased MDA levels (Fig. 2E and F). These observed outcomes indicate the presence of mitochondrial damage in hippocampal neurons of depression-like mice. Then we employed TUNEL staining to confirm the apoptosis of hippocampal neurons. Our analysis revealed a significant increase in apoptosis of hippocampal neurons in VCD^{SS} mice with pronounced TUNEL-positive cells observed specifically in the CA2/3

region (Fig. 2G and H). All of these findings confirmed that perimenopausal depression-like mice exhibit apoptosis and mitochondrial damage to their hippocampal neurons.

3.3. Dynamic regulation of cerebral glucose level impacts neuronal damage and depression-like phenotype in perimenopausal mice

Inspired by our finding that hippocampal hyperglycemia is closely correlated to depression-like behaviors in perimenopausal mice, we further explored whether pharmacological intervention on cerebral glucose levels influences neuronal damage in the hippocampus and depression-like behaviors in perimenopausal mice. SGLT2 inhibitor EMPA, which inhibits absorption of brain glucose and alleviates hyperglycemia-induced cognitive impairments¹², had been used to decrease glucose levels in VCD^{SS} mice, and E2 balanced hormone fluctuations during perimenopause to treat depressive behavior (Supporting Information Fig. S3A). Hippocampal glucose levels significantly decreased after EMPA

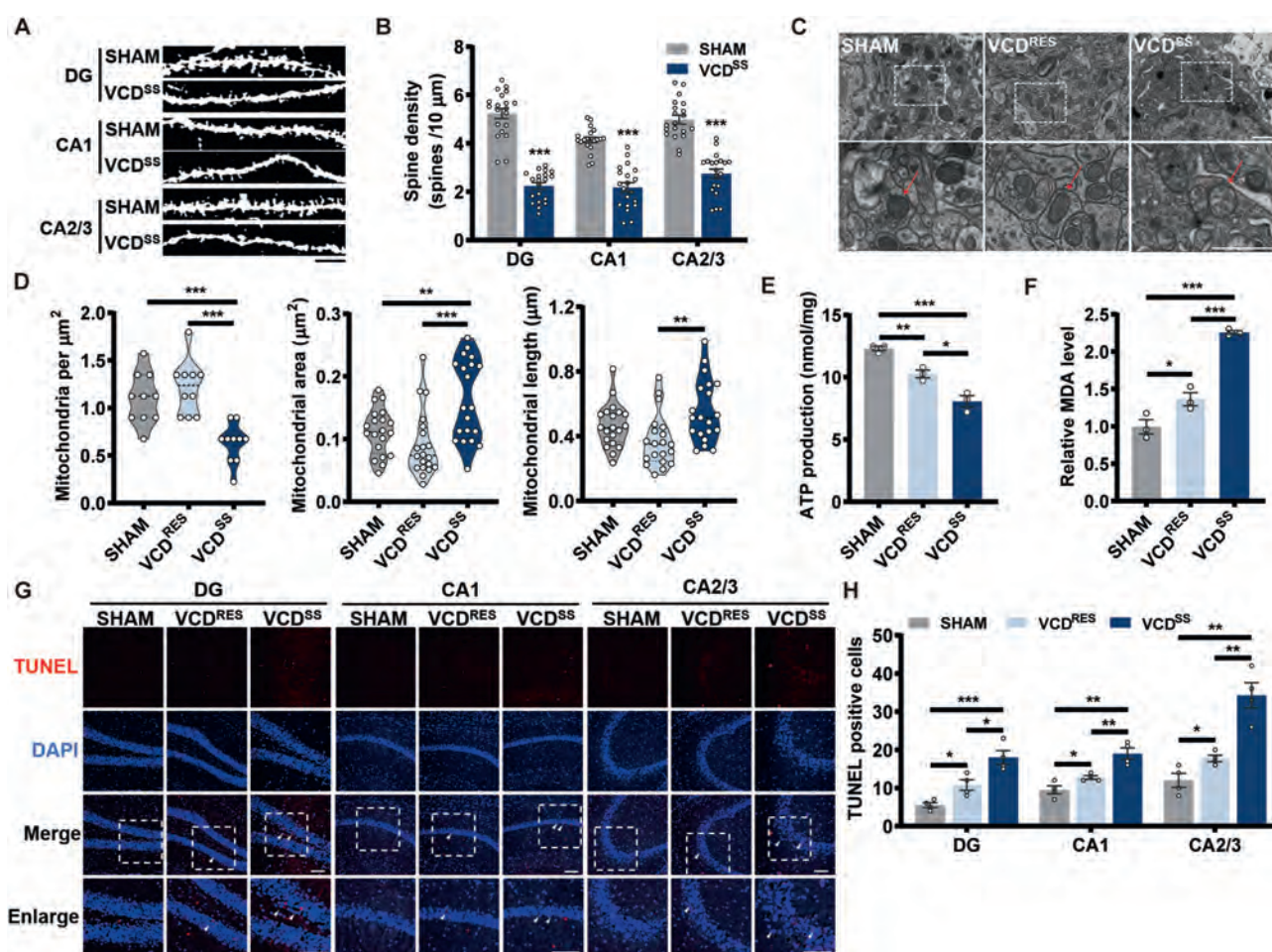


Figure 2 Mitochondrial damage and neuronal apoptosis in the hippocampus of VCD^{SS} mice. (A, B) Representative images and densities of basilar dendritic spines from hippocampal pyramidal neurons (DG, CA1, and CA2/3) in SHAM and VCD^{SS} group ($n = 20$). Scale bars: far below, 5 μm. (C) Transmission electron microscope (TEM) analysis of the mitochondrial morphology in the hippocampus of all groups. Red arrow indicates the mitochondrion. Scale bars: far below, 1 μm. (D) Quantitative measures of the density, size and length of mitochondria ($n = 10$ or 20). (E) The production of ATP in three groups of mice ($n = 3$). (F) Relative MDA level in the hippocampus in three groups of mice ($n = 3$). (G, H) TUNEL staining for evaluating neuronal apoptosis in the hippocampal DG, CA1 and CA2/3 regions ($n = 4$). Scale bars: far below, 100 μm. Significance was determined by Student's t test in B and one-way ANOVA analysis with Tukey's *post hoc* test in D, E, F and H. Data are expressed as mean $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

therapy (Fig. S3B), and behavioral tests and 5-HT content in VCD^{SS} mice showed a reduction in the depression-like phenotype (Fig. 3A–C and Fig. S3C). Additionally, our research showed that EMPA-treated VCD^{SS} mice had much higher dendritic spine density and more branches of hippocampus neurons, suggesting that lowering glucose levels reduces synaptic abnormalities in

hippocampus neurons (Fig. 3D and E, Fig. S3D–S3F). Moreover, in VCD^{SS} animals, EMPA therapy reduced neuronal damage by significantly increasing ATP and Nissl body content, decreasing MDA levels, and apoptosis hippocampal neurons (Fig. 3F–I, Fig. S3G–S3J). Similar outcomes were also shown with E2 therapy, which can lower blood glucose levels while repairing

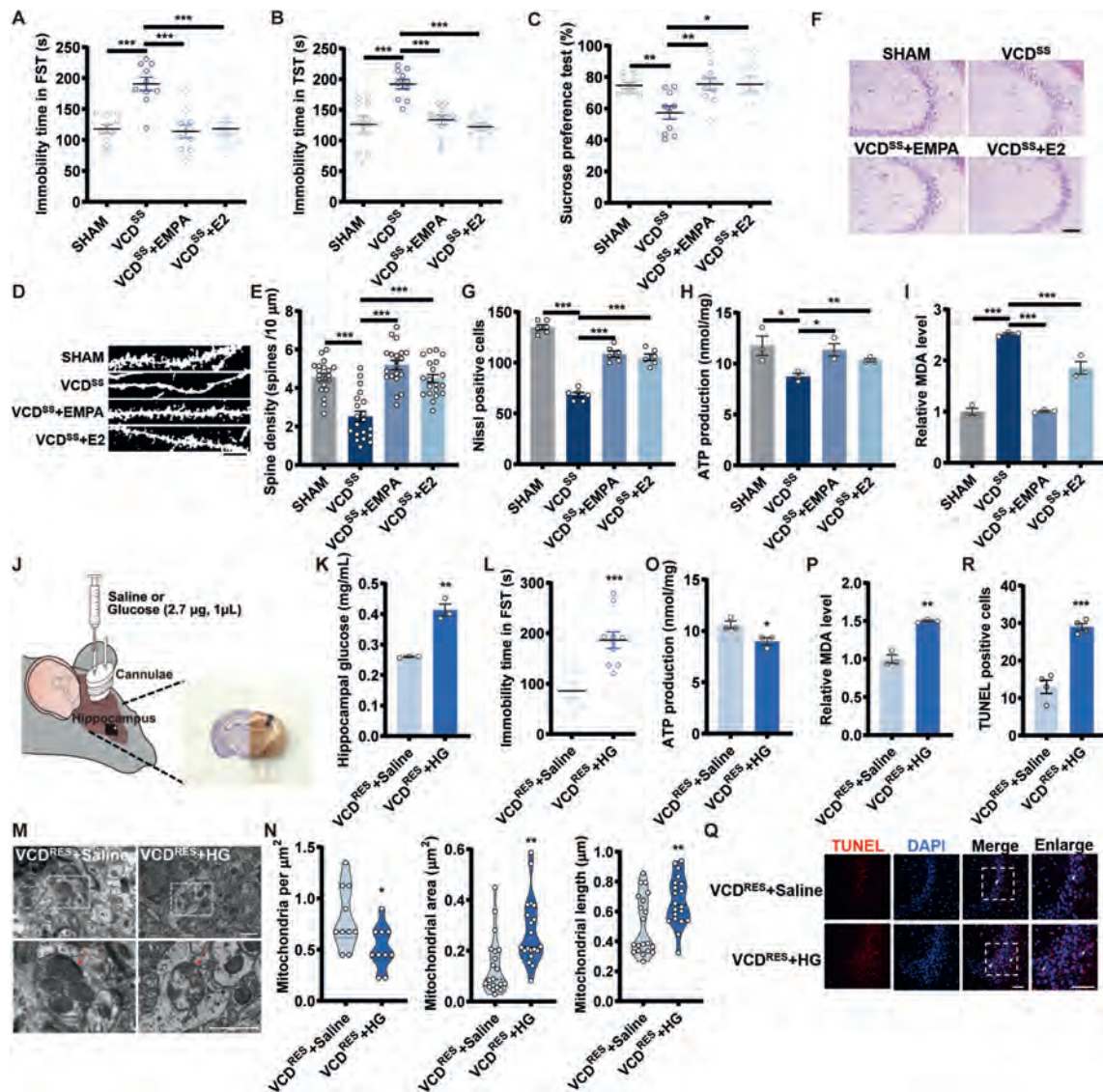


Figure 3 Interfering with glucose levels in the hippocampus affects depressive behaviors and neuronal damage in VCD mice. (A, B) The immobility time in FST and TST in the SHAM and VCD mice treated with EMPA or E2 ($n = 10$). (C) The percentage of sucrose preference in SPT ($n = 10$). (D, E) Representative images and densities of basal dendritic spines from CA2/3 hippocampal pyramidal neurons in the SHAM and VCD mice treated with EMPA or E2 ($n = 20$). Scale bars: far below, 5 μm . (F, G) Nissl staining image and quantification of Nissl bodies in the hippocampal CA2/3 subregion ($n = 6$). Scale bars: far below, 100 μm . (H) The production of ATP ($n = 3$). (I) Relative MDA level in the hippocampus ($n = 3$). (J) Verification of the hippocampal infusion of glucose or saline (right). A representative image of Evans blue staining in the injection site (blue, left). The corresponding Nissl-stained histological plate. Image courtesy of 2001 ACADEMIC PRESS. The Mouse Brain in Stereotaxic Coordinates. (K) The hippocampus glucose levels in the VCD^{RES} mice exposed to glucose or saline intrahippocampal infusion ($n = 3$). (L) The immobility time in FST in the VCD^{RES} mice exposed to glucose or saline intrahippocampal infusion ($n = 10$). (M) TEM analysis of the mitochondrial morphology in the hippocampal CA2/3 region. Red arrow indicates the mitochondria. Scale bars: far below, 1 μm . (N) Quantitative measures of the density, volume and length of mitochondrion ($n = 10$ or 20). (O) The production of ATP in the hippocampus ($n = 3$). (P) Relative MDA level in the hippocampus ($n = 3$). (Q, R) TUNEL staining for evaluating neuronal apoptosis in the hippocampal CA2/3 region ($n = 4$). Scale bars: far below, 100 μm ; Significance was determined by Student's t test in K, L, N, O, P and R; one-way ANOVA analysis with Tukey's *post hoc* test in A, B, C, E, G, H and I. Data are expressed as mean $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

brain damage. These findings suggest that lowering hippocampal glucose levels in rats that resemble perimenopausal depression may have a neuroprotective impact.

On the other hand, by administering intrahippocampal glucose infusion to the CA2/3 region of the hippocampus in VCD^{RES} mice, we were able to further investigate whether hippocampal hyperglycemia was directly related to the susceptibility of depression during the perimenopausal stage (Fig. 3J). Evans blue and glucose measurement kits were used to successfully validate the experimental schedule depicted in Supporting Information Fig. S4A with respect to infusion sites and modeling (Fig. 3J and K). As expected, compared to saline infusion, intra-hippocampal glucose infusion exacerbated the depression-like behaviors in VCD^{RES} animals, as evidenced by a longer immobility duration in FST and a decreased level of 5-HT in the hippocampus (Fig. 3L and Fig. S4B). Additionally, VCD^{RES} mice subjected to glucose infusion showed substantial mitochondrial damage and malfunction, as indicated by an increase in mitochondrial swelling and a decrease in mitochondrial density, volume, and length (Fig. 3M and N). The hippocampus of VCD^{RES} mice given glucose infusion showed a decrease in ATP content and an increase in MDA levels (Fig. 3O and P). Furthermore, as shown by a marked reduction in Nissl bodies and an increase in apoptosis of hippocampal neurons, glucose infusion worsened neuronal damage in the hippocampus (Fig. 3Q and R, Fig. S4C and S4D). Following glucose infusion, VCD^{RES} mice also showed a significant drop in the expression of BDNF and synapse-associated proteins in their hippocampal neurons (Fig. S4E and S4F). Collectively, these findings underscored the critical role of hippocampal glucose in modulating depression-like behaviors and neuronal damage in perimenopausal depression-like mice. We speculated that hyperglycemia in the hippocampus might be detrimental to neuronal synaptic structure and function in perimenopausal transition.

3.4. COX7C is reduced in the hippocampus of perimenopausal depression-like mice

To identify the principal targets associated with depression in perimenopausal mice, an unbiased quantitative proteomic screening was carried out in the hippocampus of SHAM and VCD^{SS} mice (Fig. 4A and B). As compared to SHAM mice, the results indicated that 46 and 28 DEPs out of 6053 total proteins were up- and down-regulated in VCD^{SS} mice, respectively. These DEPs' GO research revealed a number of metabolic functions, such as oxidative phosphorylation, glucose metabolism, and ATP synthesis (Fig. 4C). The DEPs were subjected to computational analysis using OTTER website calculation based on metabolism³⁸ and PPI (Fig. 4D and E). The analysis results demonstrated that COX7C, a subunit of electron transport chain (ETC) complex IV, was located in the heart of the interaction network and had a high OTTER analysis score (Fig. 4D and E). As a key factor in stability of the ETC and ATP production⁴⁴, COX7C is speculated as a potential target of interest in hyperglycemia-induced neuronal damage. SKP1 is significantly upregulated in depression-like mice and has a close interaction with COX7C in the PPI analysis with contrary protein level. As an important linker protein within the E3 ubiquitin ligase machinery, SKP1 plays a crucial role in modulating protein degradation⁴⁵. Consequently, there is a compelling reason for undertaking further investigative efforts focused on elucidating the mechanisms underlying COX7C's

interaction with SKP1 and the subsequent impact on protein degradation.

3.5. Hippocampal hyperglycemia decreases the expression of COX7C in perimenopausal depression-like mice

To substantiate the preceding speculation, we employed Western Blotting analysis and confocal microscopy to observe the level of COX7C in hippocampus of VCD mice. Of note, VCD^{SS} mice showed reduced hippocampal COX7C expression, whereas VCD^{RES} mice did not (Fig. 5A–D). Intriguingly, VCD^{SS} mice's hippocampal levels of *Cox7c* mRNA remained unchanged (Fig. 5E). Considering that COX7C is a fundamental enzyme for ETC complexes IV, we further evaluated the activity of ETC complex IV in the hippocampus of VCD^{SS} mice. In line with COX7C expression, ETC complex IV activity was markedly downregulated in VCD^{SS} mice but showed no abnormalities in VCD^{RES} animals (Fig. 5F). Subsequently, we further investigated whether the level of glucose could regulate the expression of COX7C in the hippocampus of VCD mice. The results showed that the reduced expression of COX7C in VCD^{SS} mice was dramatically restored by EMPA and E2 therapy. Moreover, intrahippocampal glucose infusion led to a visible reduction in COX7C level in VCD^{RES} mice (Fig. 5G and H). According to these findings, COX7C may be able to identify the harmful contribution of hyperglycemia to perimenopausal depression-like mice's hippocampus neuronal damage.

3.6. Overexpression of COX7C attenuates neuronal apoptosis induced by high glucose

To verify our speculation, we examined the impact of COX7C on high glucose (HG)-induced neuronal injury in mouse hippocampus neuron HT22 cells *in vitro*. Untreated cells are designated as the control (CON) group. To create HT22 cell damage models, we subjected HT22 cells to varying HG (25, 75, and 175 mmol/L) doses for 24 h. We found a significant decrease in cell viability with 175 mmol/L glucose intervention for 24 h, whereas treatment with an osmolarity control (175 mmol/L mannitol) for 24 h did not significantly influence cell viability (Supporting Information Fig. S5A). Consequently, 175 mmol/L glucose treatment for 24 h was chosen for subsequent experiment. Three programmed cell death inhibitors, including Z-VAD (apoptosis inhibitor), Nec-1 (necrosis inhibitor), and CQ (autophagy inhibitor), were applied to ascertain the mode of death of HT22 treated with HG (Fig. S5B). The results showed that Z-VAD (15 μ mol/L) was able to decrease cell death induced by HG treatment (Fig. S5C). This result indicated that HG treatment might induce apoptosis of HT22 cells. The expression of COX7C was significantly reduced in the HG group in a time-dependent manner, while the mRNA level of *Cox7c* remained unchanged (Fig. 6A, Fig. S5D and S5E). To further examine the involvement of COX7C in regulating neuronal damage, we infected HT22 cells (CON and HG group) with an over-expressed plasmid encoding *Cox7c* (Fig. S5F and S5G). The overexpression of COX7C did not influence viability of cells with normal glucose but significantly improved the viability in HG-treated cells (Fig. 6B). Mitochondrial ultrastructural analysis using TEM revealed that overexpression of COX7C increased the density, volume, and length of mitochondria in HG-treated cells while decreasing vacuolization and swelling in cells with normal glucose levels (Fig. 6C and D). COX7C overexpression in HG-treated cells consistently resulted in a considerable increase in the

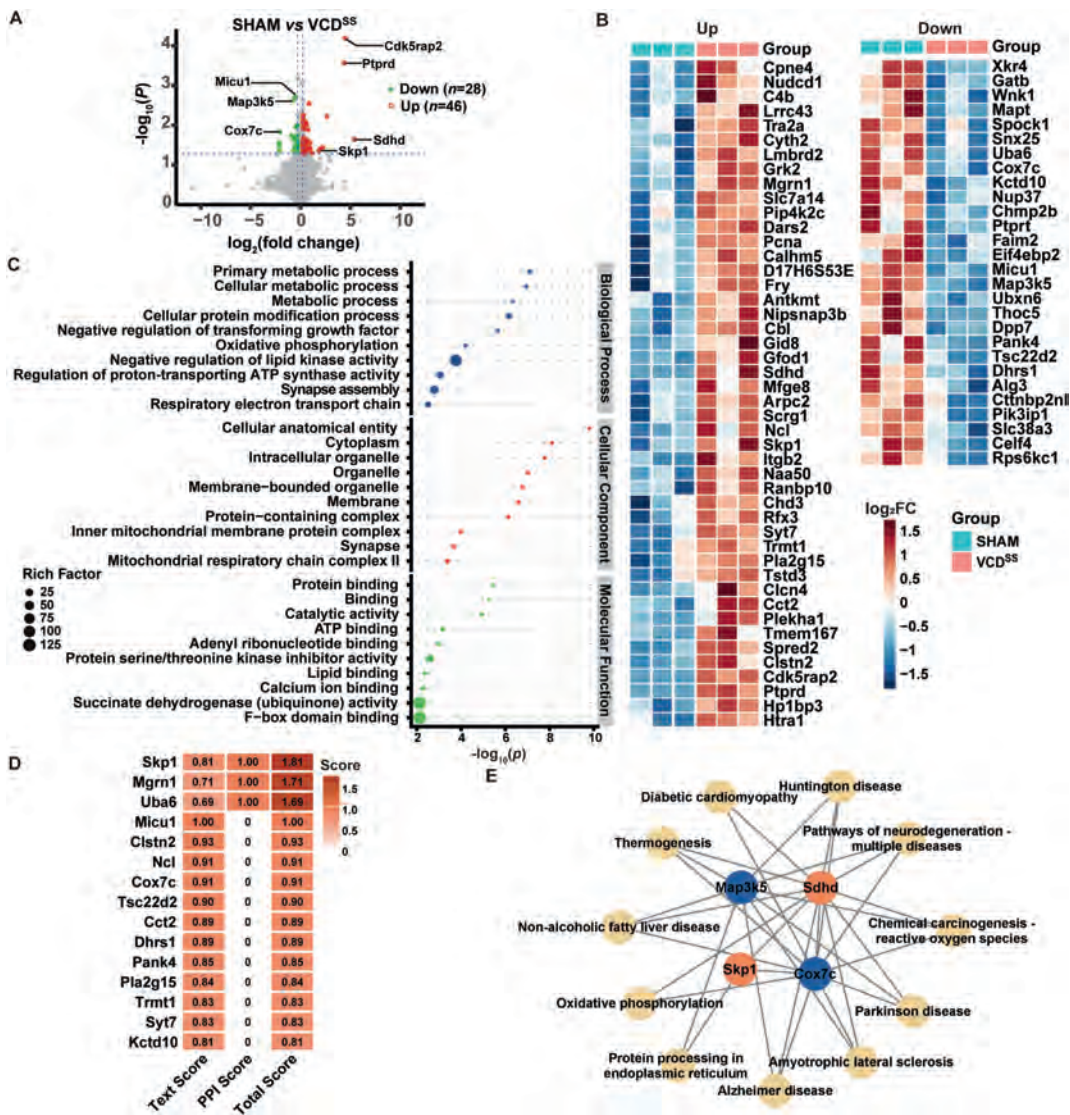


Figure 4 Proteomic alteration and identified COX7C in the hippocampus. (A) A volcano plot of DEPs. Green and red dots indicate significantly down- and up-regulated proteins, respectively ($n = 3$). (B) Heatmap of DEPs showing expression trends. The significance of enrichment is scaled by color intensity. (C) The result of GO enrichment analysis of DEPs identified in SHAM and VCD^{SS} mice. (D) The heatmap of OTTER analysis of DEPs. (E) Links between common PPI network targets.

ratio of red/green fluorescence, as demonstrated by JC-1 staining, suggesting that COX7C repaired the mitochondrial membrane potential compromised by HG (Fig. 6E and F). Furthermore, COX7C overexpression increased ATP production and ETC complex IV activity in HG-treated cells, suggesting that the reduced level of COX7C participated in high glucose-induced mitochondrial dysfunction (Fig. 6G and H). COX7C overexpression also significantly reduced MDA level in HG-treated cells (Fig. 6I). Additionally, TUNEL-positive cells and apoptosis rate in the HG-treated group decreased with the overexpression of COX7C (Fig. 6J–M). These results imply that COX7C plays a crucial role in the neuronal harm brought on by HG.

3.7. High glucose induces SKP1-dependent ubiquitination and degradation of COX7C

Given that the mRNA level of *Cox7c* remained unaltered despite the considerable reduction in COX7C protein following HG

therapy, it was assumed that the decreased expression of COX7C might be connected to protein degradation. The degradation of the COX7C protein was monitored in HG-treated cells using CHX, an inhibitor of protein synthesis, at intervals of 0, 3, 6, and 9 h. Our results revealed an accelerated degradation rate of COX7C protein in HG-treated cells (Fig. 7A and B). In parallel, we employed the proteasome inhibitor MG132 and the lysosome inhibitor CQ to clarify the fundamental mechanism of COX7C protein degradation. Applying MG132 decreased the reduction in COX7C brought on by HG treatment, suggesting that the ubiquitin–proteasome pathway regulated the increased COX7C protein degradation (Fig. 7C and D). We postulated that SKP1 would be a possible target in the degradation of COX7C based on proteomic screening study. We first looked at SKP1 expression in VCD mice and HG-treated HT22 cells to confirm this theory. According to our findings, the hippocampus of VCD^{SS} mice and cells exposed to HG had significantly higher levels of SKP1 protein and mRNA expression (Fig. 7E and F, Supporting

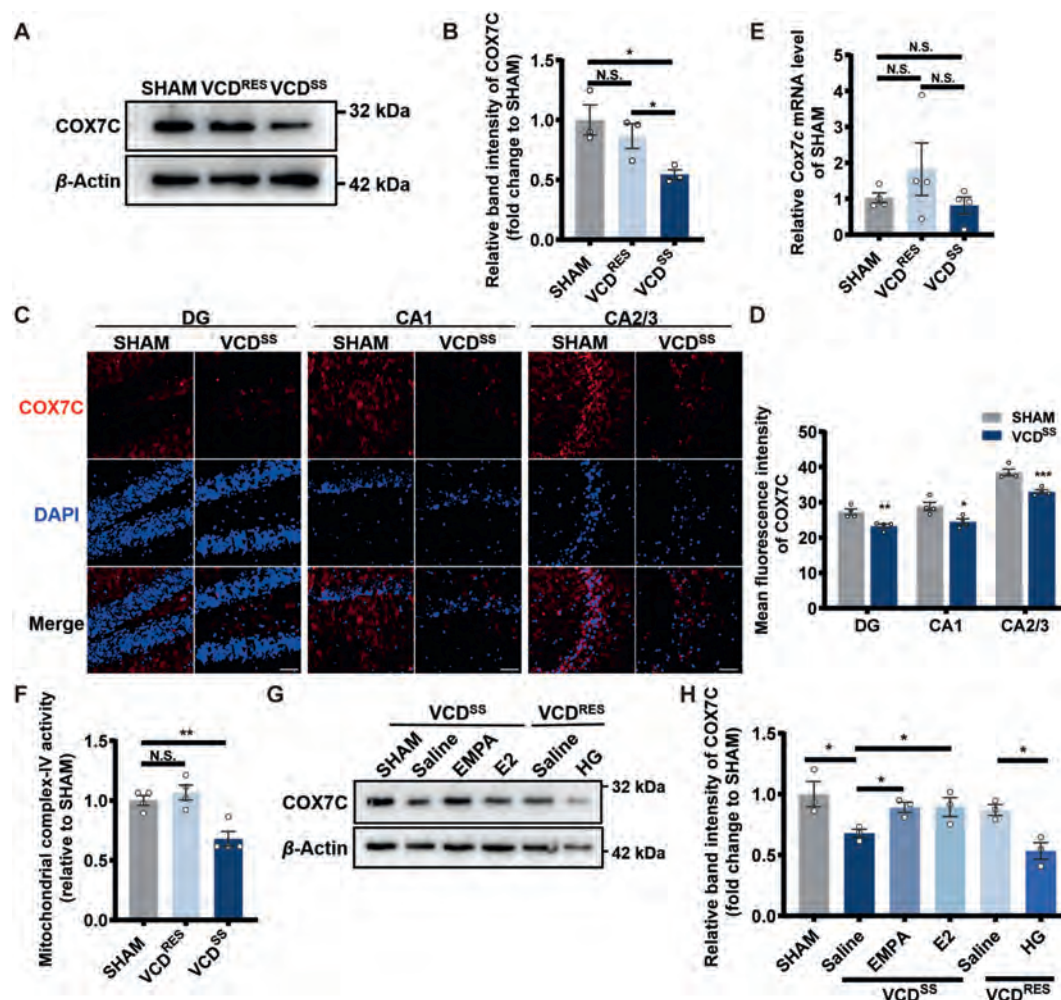


Figure 5 COX7C is reduced in the hippocampus of perimenopausal depression mice. (A, B) Protein level of COX7C in the hippocampus in SHAM, VCD^{SS} and VCD^{RES} mice ($n = 3$). β -Actin is used as a loading control. (C, D) COX7C (Red) staining and mean fluorescence intensities of COX7C in hippocampus ($n = 4$). Scale bars: far below, 50 μ m. (E) Relative mRNA level of *Cox7c* in the hippocampus in VCD-treated mice. β -Actin is used as a loading control ($n = 4$). (F) Relative activity of mitochondrial complex IV in the hippocampus in SHAM and VCD-treated mice ($n = 4$). (G, H) Protein level of COX7C in the hippocampus in VCD^{SS} mice treated with EMPA or E2 and VCD^{RES} exposed to glucose or saline infusion ($n = 3$). β -Actin is used as a loading control. Significance was determined by Student's t test in D, and one-way ANOVA analysis with Tukey's *post hoc* test in B, E, F and H. Data are expressed as mean $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$; N.S., not significant.

Information Fig. S6A–S6C). Consistently, a decrease in COX7C expression and an increase in SKP1 expression were found in the hippocampus of OVX^{SS} mice (Fig. S6D and S6E). Subsequently, we investigated the correlation between COX7C and SKP1. The co-staining of mitochondria with SKP1 and COX7C revealed an increased expression of SKP1 and decreased expression of COX7C. The co-localization analysis showed that HG treatment promoted the co-localization pattern of COX7C and SKP1 on mitochondria (Fig. 7G and H). These results suggested that following HG stimulation in HT22 cells, the binding interface between COX7C and SKP1 is mostly found in the mitochondria. Therefore, we carried out co-immunoprecipitation (Co-IP) assays to further support the connection between COX7C and SKP1. The Co-IP results showed that the anti-COX7C antibody was able to immunoprecipitate SKP1 and the anti-SKP1 antibody could also immunoprecipitate COX7C (Fig. 7I). To clarify

whether SKP1 mediated COX7C ubiquitination degradation after HG treatment, SKP1 expression was knocked down using siRNA in HG-treated HT22 cells after CHX intervention for indicated time. SKP1 knockdown dramatically reduced the increased degradation rate of COX7C brought on by HG treatment (Fig. 7J and K, Fig. S6F and S6G). Additionally, using IP analysis, we looked into the function of SKP1 and the precise mechanism behind COX7C degradation by ubiquitination in HG-treated cells. Notably, we found that knockdown of SKP1 reversed the HG-induced increase in COX7C ubiquitination level, supporting the regulatory role of SKP1 in this process (Fig. 7L). To further determine the functional domain of SKP1 in regulating COX7C ubiquitination and degradation, we prognosticated the ubiquitination sites (<http://gpsubser.biocuckoo.cn/index.php>) within COX7C selected option “Cullin RING”, specifically identifying lysine residues K63 with a higher predictive score (Fig. S6H). To

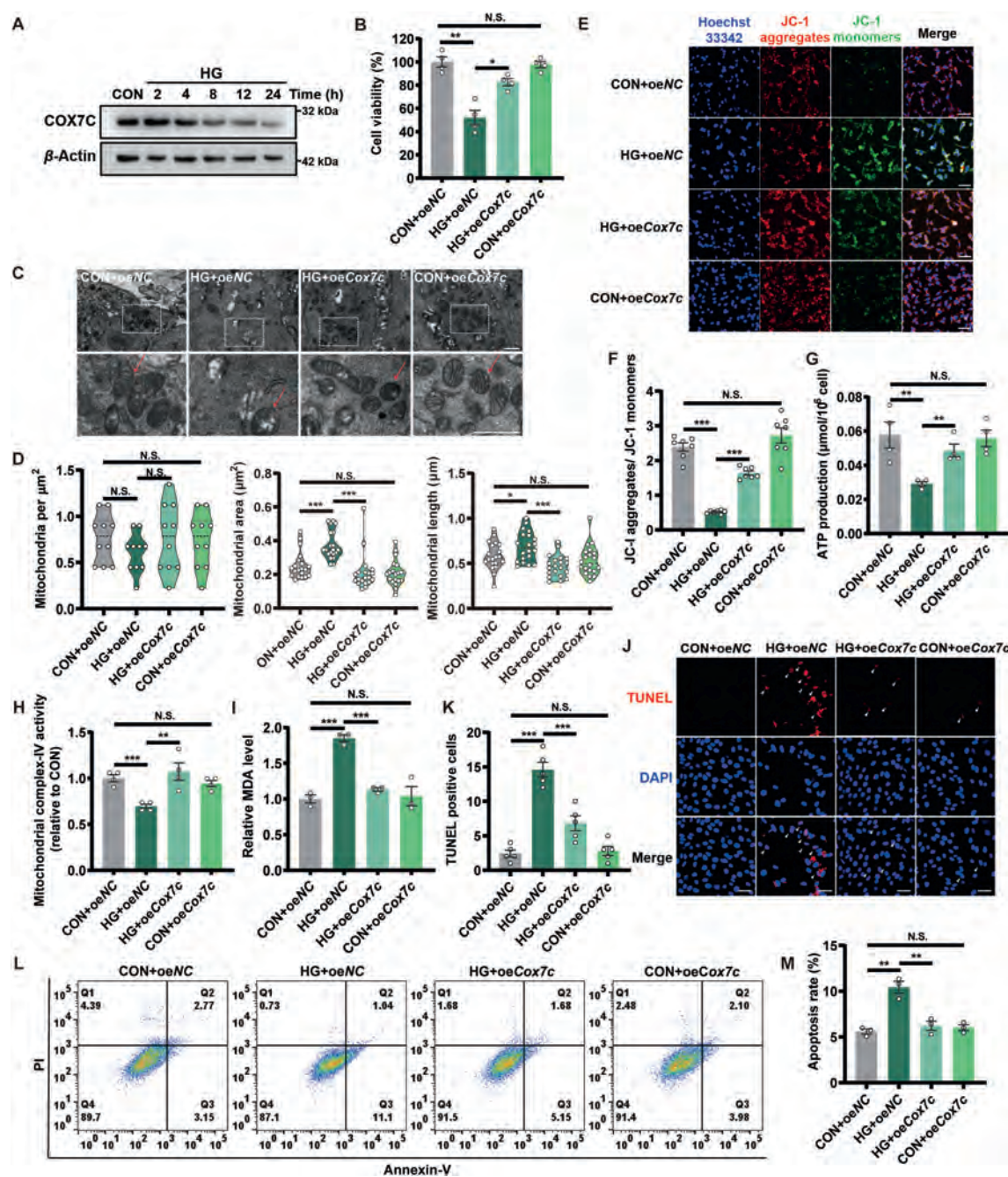


Figure 6 Overexpression of COX7C improves neuronal apoptosis in HG-damaged HT22 cells. (A) Western-blotting analysis of COX7C expression in HG treatment for different h. (HG, high glucose). β -Actin is used as a loading control. (B) Cell viability in HG-treated cells with COX7C overexpression (oeCox7c) examined by CCK-8 assay ($n = 4$). (C) TEM analysis of the mitochondrial morphology in HG-treated cells after overexpression of COX7C. Red arrow indicates the mitochondria. Scale bars: far below, 1 μ m. (D) Quantitative measures of the density, volume and length of mitochondrion in C ($n = 10$ or 20). (E, F) Representative images and statistics of HT-22 cells loaded with the mitochondrial membrane potential indicator JC-1 and Hoechst 33342 ($n = 7$). Scale bars: far below, 50 μ m. (G) The production of ATP in HG-treated cells with COX7C overexpression ($n = 3$). (H) Relative mitochondrial complex IV activity level in HG-treated cells with COX7C overexpression ($n = 4$). (I) Relative MDA level in HG-treated cells with COX7C overexpression ($n = 3$). (J, K) TUNEL staining of HG-treated cells with COX7C overexpression and quantification of TUNEL positive cells ($n = 5$). Scale bars: far below, 50 μ m. (L, M) Cell apoptosis was evaluated by flow cytometry after staining with Annexin V and PI dyes ($n = 3$). Significance was determined by one-way ANOVA analysis with Tukey's *post hoc* test. Data are expressed as mean $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; N.S., not significant.

confirm the degree of COX7C ubiquitination, a pcDNA vector containing the Flag-tagged *Cox7c* mutant (K63R) was created and introduced into HG-treated cells. K63 was the putative

ubiquitination site related to SKP1 inside COX7C, according to the IP analysis, which revealed that the COX7C mutant reduced COX7C ubiquitination (Fig. 7M). These results indicated that

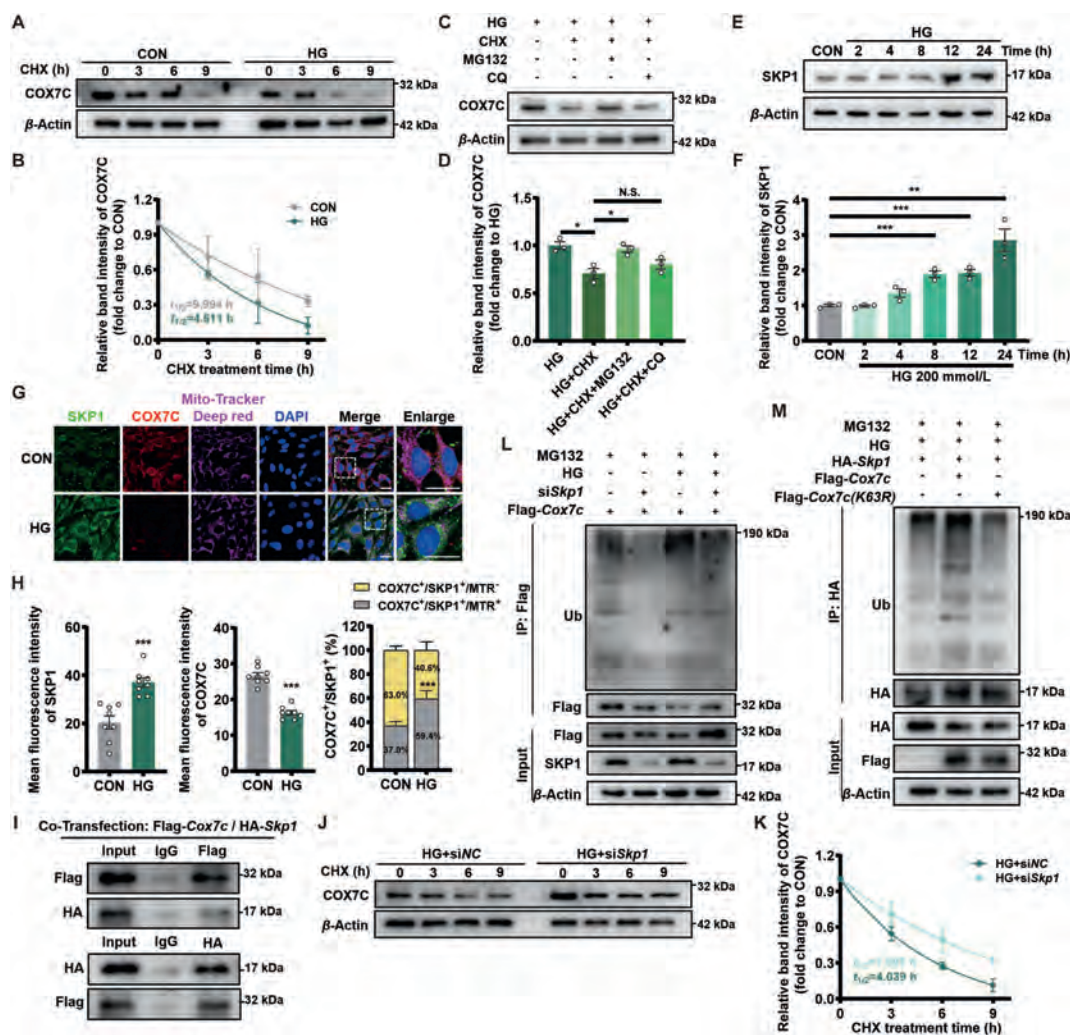


Figure 7 Higher doses of glucose induce COX7C ubiquitination degradation in a SKP1-dependent manner. (A, B) COX7C protein expression in HG-treated HT22 cells with CHX 30 μ mol/L for indicated durations ($n = 3$). β -Actin is used as a loading control. (C, D) The expression and quantification of COX7C protein in HG-exposed cells pretreated with MG132 (20 μ mol/L) or CQ (20 μ mol/L) after CHX (30 μ mol/L) treatment for 9 h ($n = 3$). β -Actin is used as a loading control. (E, F) Western-blotting analysis of SKP1 in HG-treated cells for different durations ($n = 3$). β -Actin is used as a loading control. (G) Co-staining of COX7C (red), SKP1 (green), mitochondria (Mito-tracker deep red, magenta) and nucleus (DAPI, blue) in HG-treated cells. Scale bar: 25 μ m. (H) Mean fluorescence intensities of SKP1 (left), COX7C (middle) and co-localization of SKP1 and COX7C on mitochondria (right) ($n = 8$). (I) Co-IP of COX7C with SKP1 in HG-treated cells cotransfected with plasmids encoding Flag-Cox7c and HA-Skp1. The lysates were immunoprecipitated with anti-Flag or anti-HA antibody, followed by Western blotting. (J, K) COX7C protein expression in HG-treated cells transfected with siSkp1 or siNC after CHX treatment for indicated durations ($n = 3$). β -Actin is used as a loading control. (L) Ubiquitination of exogenous COX7C in HG-treated HT22 cells co-transfected with plasmid encoding Flag-tagged Cox7c and siSkp1 with MG132 treatment. The lysates were immunoprecipitated with anti-HA antibody, followed by Western blotting with anti-ubiquitin antibody. (Ub, ubiquitin). (M) Ubiquitination of COX7C in HG-treated HT22 cells cotransfected with plasmids encoding HA-tagged Skp1 with Flag-tagged Cox7c or Flag-tagged Cox7c mutant (K63R) with MG132 treatment. The lysates were immunoprecipitated with anti-HA antibody, followed by Western blotting with anti-ubiquitin antibody; Significance was determined by Student's t test in H, and one-way ANOVA analysis with Tukey's *post hoc* test in D and F. Data are expressed as mean $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; N.S., not significant.

SKP1 mediated COX7C degradation by ubiquitination in HG-induced neuronal damage.

3.8. Overexpression of COX7C in hippocampal neurons improves depression-like behaviors and neuronal damage in perimenopausal mice

To elucidate whether replenishing COX7C is required for alleviating depression-like behaviors in perimenopausal mice, we

injected AAV2/9-hSyn-Cox7c and AAV2/9-hSyn-EGFP virus into the hippocampal CA2/3 region of VCD^{SS} and HG-treated VCD^{RES} mice to specifically increase COX7C expression in hippocampal neurons (Fig. 8A and Supporting Information Fig. S7A). In Fig. S7A and a surgical operation was carried out. After 28 days of virus injection, *in vivo* imaging techniques were used to validate the accuracy of the injection location and the transfection efficacy (Fig. 8A). Following AAV-Cox7c injection, there was a substantial upregulation of COX7C expression in the

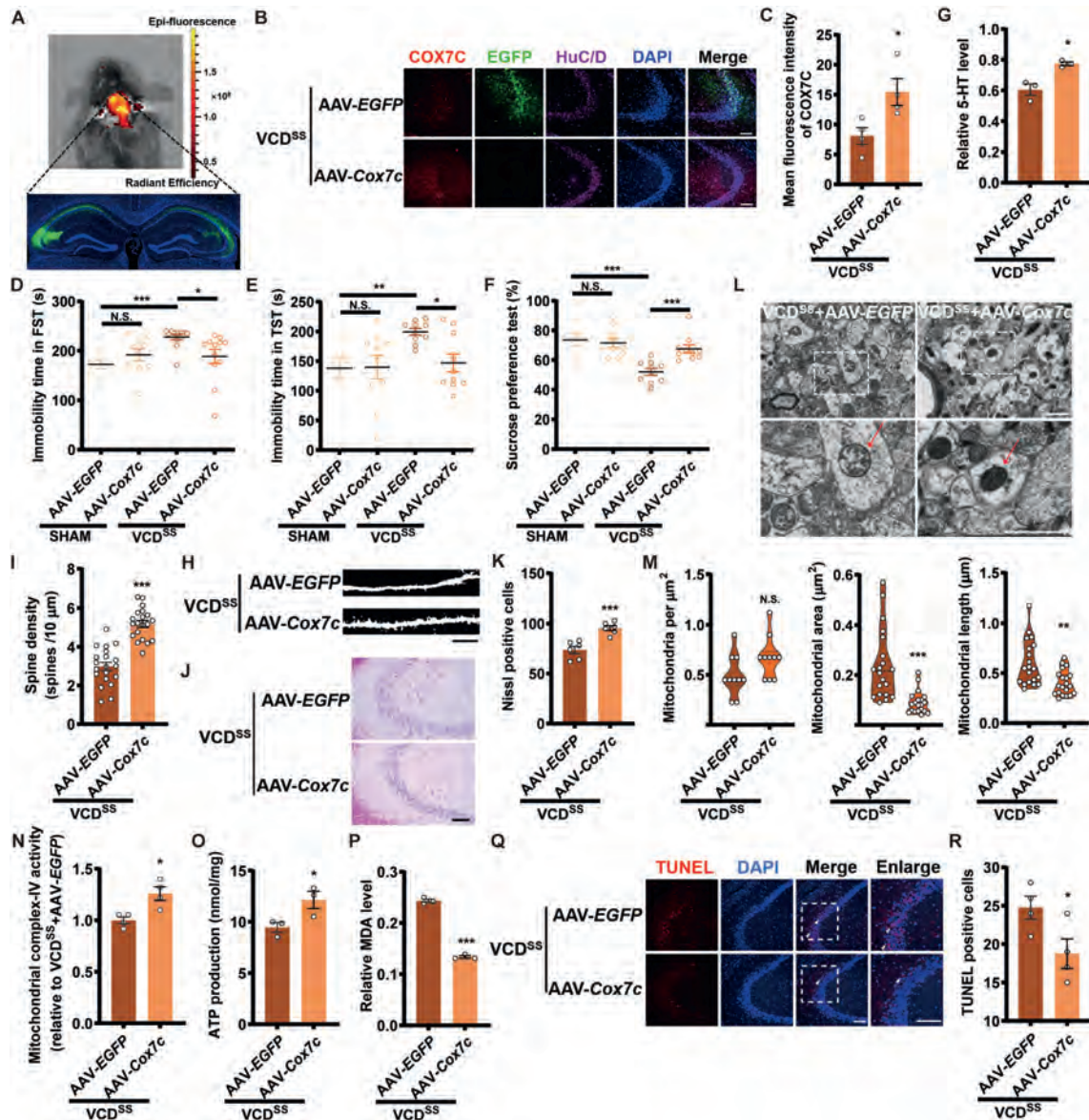


Figure 8 Overexpression of COX7C in hippocampal neurons alleviates the neuronal damage and depression-like behaviors in perimenopausal mice. (A) The evidence of successful adenovirus infusion using *in vivo* imaging techniques (right). The corresponding EGFP histological plate in the hippocampal CA2/3 region (left). (B, C) COX7C (red) immunofluorescence staining in hippocampal CA2/3 region co-stained with neuron marker HuC/D (magenta), AAV-EGFP (green), and DAPI (blue) in the VCD^{SS} mice injected AAV2/9-hSyn-EGFP or AAV2/9-hSyn-Cox7c ($n = 4$). Scale bars: far below, 100 μm . The mean fluorescence intensity of COX7C in the CA2/3 region was quantified. (D, E) The immobility time in FST and TST in the VCD^{SS} mice transfected with AAV-Cox7c or AAV-EGFP ($n = 10$). (F) The percentage of sucrose preference in SPT ($n = 10$). (G) Relative 5-HT level in the hippocampus ($n = 3$). (H, I) Representative images and quantification of basilar dendritic spines from CA2/3 hippocampal pyramidal neurons in the VCD^{SS} mice transfected with AAV-Cox7c or AAV-EGFP ($n = 20$). Scale bars: far below, 5 μm . (J, K) Nissl staining and quantification of Nissl bodies in the hippocampal CA2/3 subregion ($n = 6$). Scale bars: far below, 100 μm . (L) TEM analysis of the mitochondrial morphology in the hippocampal CA2/3 region. Red arrow indicates the mitochondria. Scale bars: far below, 1 μm . (M) Quantitative measures of the density, volume and length of mitochondrion in C ($n = 10$ or 20). (N) Relative mitochondrial complex IV activity in the hippocampus ($n = 4$). (O) The production of ATP in the hippocampus ($n = 3$). (P) Relative MDA level ($n = 3$) in the hippocampus. (Q, R) TUNEL staining and quantification of TUNEL positive cells in the hippocampal CA2/3 region ($n = 4$). Scale bars: far below, 100 μm ; Significance was determined by Student's *t* test in C, G, I, K, M, N, O, P and R; one-way ANOVA analysis with Tukey's *post hoc* test in D, E and F. Data are expressed as mean $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; N.S., not significant.

neurons of the hippocampus CA2/3 area (Fig. 8B and C). AAV-Cox7c injection had no obvious influence on depression-like behaviors in SHAM mice. However, AAV-Cox7c-injected VCD^{SS} mice displayed a reduced immobility time in FST and TST and

higher preference for sucrose in the SPT, indicating the alleviation of depression-like behaviors in VCD^{SS} mice (Fig. 8D–F). Similar results were observed in HG-treated VCD^{RES} mice with decreased duration of immobility in FST after AAV-Cox7c injection

(Fig. S7B). In VCD^{SS} and HG-treated VCD^{RES} mice, AAV-*Cox7c* injection also increased the amount of 5-HT in the hippocampal region (Fig. 8G and Fig. S7C). Moreover, overexpression of COX7C in hippocampal CA2/3 neurons had been demonstrated to alleviate impaired synaptic structure with increased the density of dendritic spines (Fig. 8H and I). The number of Nissl bodies was also enhanced by AAV-*Cox7c* injection in VCD^{SS} and HG-treated VCD^{RES} mice with increased expression of synapse-associated proteins and BDNF (Fig. 8J and K, Fig. S7D–S7I). In addition, COX7C upregulation restored the mitochondrial morphology and function, indicated by the decrease in vacuolization and swelling, as well as the increase in the density, volume and length of mitochondria (Fig. 8L and M). In VCD^{SS} mice, COX7C overexpression also increased the ATP level and ETC complex IV activity (Fig. 8N and O). Correspondingly, the oxidative damage and neuronal apoptosis were also downregulated by AAV-*Cox7c* injection in VCD^{SS} mice with decreased level of MDA and TUNEL-positive cell number (Fig. 8P–R). Taken together, these evidences demonstrate that the supplementation of COX7C expression in hippocampal CA2/3 neurons reduces neuronal apoptosis and alleviates depression-like phenotype in perimenopausal mice.

4. Discussion

Vulnerability to depression is increased during perimenopausal transition, while its pathogenesis is still elusive. Disturbed glucose metabolism that affects depression occurrence is widely documented, offering a series of potential therapeutic alternatives. However, it remains to be clarified whether glucose dysfunction is associated with neuronal damage and depression-like behaviors and how hyperglycemia contributes to individual susceptibility to the aversive consequences of perimenopausal depression. This study showed that hippocampal glucose levels were elevated in mice with perimenopausal depression, and that reducing cerebral glucose levels could alleviate the severity of depressive symptoms and neuronal injury in VCD^{SS} mice. Furthermore, the study revealed that through SKP1-mediated degradation of COX7C, elevated hippocampal glucose levels increased vulnerability to depression-like behaviors and neuronal injury. In particular, increasing COX7C expression in hippocampal neurons emerged as an effective therapeutic strategy to combat depressive behavior and neuronal damage in perimenopausal transition. Taken together, we discovered that hyperglycemia in the hippocampus induced mitochondrial dysfunction and oxidative damage to neurons, which contributed to depression-like behaviors in VCD-induced perimenopausal transition mice *via* SKP1-dependent COX7C degradation by ubiquitination.

Perimenopause is a crucial period in the lives of women in the age group of 45–64. As documented in previous studies, perimenopausal women are more prone to depression and irritation because of hormonal oscillations and external sociocultural elements that disrupt their emotional well-being⁴⁶. Therefore, the objective of this study was to pinpoint the precise mechanism behind perimenopausal depression and create viable strategies to alleviate its symptoms. To emulate the perimenopausal state, the ovarian toxicant VCD was administered to mice by intraperitoneal injection. By reducing the immature ovarian follicle reserve and maintaining the structural integrity of the follicle-depleted ovarian tissue, a VCD model replicated the perimenopausal transition in mice in comparison to OVX operation, producing a profile that

was comparable to that of most perimenopausal humans³⁹. Here, VCD (160 mg/kg/day, for 20 days) was applied to construct perimenopausal mouse model, which has been proven no obvious toxicity in other major organs^{47–49}. In addition, it was shown that VCD injection has minimal toxicity to non-ovarian tissues according to organ coefficients quantification⁴⁰. VCD injection (160 mg/kg/day) has no obvious direct effects on the normal functions of liver, kidney and brain^{47,49,50}. Furthermore, it was shown that after 24 h of application of VCD (250 mg/kg), most of it was excreted through urine, the remaining was mainly distributed in fat and ovary^{51–53}. Therefore, we speculated that the function of VCD injection on neuronal damage in the hippocampus in female mice was largely related to the impairment of estrogen-mediated effects.

Mounting evidences have revealed cerebral dysfunction in VCD-induced perimenopausal mice. Specifically, VCD administration decreased hippocampal 5-HT levels and slightly increased corticosterone levels in response to acute stress⁵⁴. Furthermore, VCD-induced follicular depletion increased hippocampal A β levels and exacerbated cognitive impairment associated with aging and menopause in rats^{55,56}. Therefore, here we applied VCD-induced perimenopausal mouse model to elucidate the mechanisms underlying depression-like behaviors and neuronal damage. Critically, we identified a subpopulation of mice (VCD^{SS} group, 36.7%) that were more susceptible to VCD-induced depression-like behaviors and neuronal damage using a behavior screening method based on the ROC algorithm, which facilitated more precise identification of relevant targets contributing to perimenopausal depression.

In this study, we revealed that the glucose uptake was decreased in numbers of regions of brain, including hippocampus, hypothalamus and cortex. However, there was no significant change in glucose content in other regions, except for hippocampus showed the hyperglycemia. Therefore, we focus on investigating the role of hyperglycemia in hippocampus in neuronal damage during PMD pathological process. Here, we speculated that the high level of glucose in hippocampus might be attributed to the increasing gluconeogenesis in hippocampus. It is shown that hippocampus may require more glucose to maintain normal neuronal activity when ATP supply is insufficient^{57,58}. However, the glycogen content in the brain is relatively low, mainly stored in the form of lactate in astrocytes, which is consumed within a few minutes without exogenous glucose^{59,60}. This proved that the brain's ability to provide glucose through glycogen breakdown is poor^{59,60}. Therefore, we believe that gluconeogenesis is the main pathway for glucose production when glucose uptake decreases in hippocampus⁶¹. Meanwhile, hippocampus showed more obvious neuronal damage than other regions in OVX-induced perimenopausal mice^{62–64}. Our study also pointed out a significant neuronal damage and mitochondrial dysfunction in the hippocampus of VCD mice. As a result, we hypothesized that the hippocampus of VCD mice experienced mitochondrial damage and decreased ATP synthesis, which set off a vicious cycle in which neurons need more glucose for anaerobic glycolysis to produce ATP, which in turn causes increased glucose intake. Therefore, the gluconeogenesis pathway in the hippocampus of VCD^{SS} mice might be abnormally activated. Moreover, our previous study showed that compared to other cerebral regions, hippocampus exhibited a significant damage to blood–brain barrier (BBB) in OVX-induced perimenopausal depression mice. We supposed that the BBB impairment might be related to the hyperglycemia in hippocampus⁶⁵. It was shown that

BBB damage induced the dysfunction of glucose transporters, which impaired the glucose metabolism in the hippocampus of aged mice^{66–68}. However, the specific mechanism underlying hyperglycemia in the hippocampus needs to be further investigated.

In the brain, glucose is primarily transported from the peripheral circulation to astrocytes that convert glucose to lactate by anaerobic metabolism, serving as an energy source for the neighboring neurons^{9,69}. Neurons primarily utilize pyruvate converted to lactate for ATP production *via* oxidative phosphorylation⁷⁰. A number of glucose dysfunctions, such as aberrant glucose and glucose intermediates like lactate and pyruvate, the inhibition of oxidative phosphorylation, and decreased ATP production, are brought on by disruptions in glucose uptake, conversion, and utilization. This study revealed an unexpected finding in the hippocampus of VCD^{SS} mice, where glucose uptake decreased but glucose levels increased. More investigation revealed a generalized downregulation of glucose transporter mRNA levels in the hippocampus, accompanied by an upregulation of mRNA expression for key enzymes involved in anaerobic glycolysis and gluconeogenesis. We supposed that this paradoxical increase in hippocampal glucose levels in VCD^{SS} mice might result from aberrant activation of anaerobic glycolysis and gluconeogenesis pathways, leading to the accumulation of tissue glucose. The abnormal activation of gluconeogenesis pathway may be related to estrogen receptors (ERs) fluctuations. It is shown that ERs, including ER α , ER β and GPER, are mainly distributed in the brain regions related to learning and memory, especially in hippocampus and hypothalamus, which contributes to the bioenergetics system, including glucose transport, glucose metabolism, mitochondrial respiration and ATP production^{71–75}. OVX or VCD induced downregulation of ER expression and function in the hippocampus during the perimenopausal transition period^{62,76}. The changes of ERs seem to be an important factor in glucose metabolism, especially for inhibiting gluconeogenesis, during perimenopausal neuropathological transition^{77,78}. Rhonda et al.⁷⁹ reported that ER β conditional knockout increased the gluconeogenesis in the hippocampus *via* enhancing enolase 1 gene expression (a key rate limiting enzyme of gluconeogenesis), which modulates hippocampal neuropathology in mid-age female mice with cognitive impairment.

On the other hand, we found that although anaerobic glycolysis was promoted with higher lactate and lower pyruvate levels in the hippocampus, the oxidative phosphorylation was impaired with lower ATP production. We supposed that the deficiency of energy supply might enhance glycogenolysis of astrocytes, a vital source of glucose for the brain under stress⁸⁰. The enhanced glycogenolysis contributes to elevating lactate and glucose level for ATP production in hippocampus where glycolysis is the primary mechanism with high ATP/ADP ratios⁸¹. This paradoxical phenomenon has also been widely documented in various neurologic diseases. For example, elevated glucose concentrations coupled with reduced glucose uptake in the hippocampus were observed in the spatially and cognitively impaired mice exposed to chronic stress⁹, which might be due to increased cerebral glucose demand and possible neuronal dysfunction. Xu et al.⁸² suggested that similar phenomena in Alzheimer's disease (AD) patients might be explained primarily by the likelihood that elevated brain glucose levels precede the downregulation of GLUT expression, together with impairments in glycolysis and the tricarboxylic acid cycle in the brain, which in turn promote enhanced glycogenolysis. These findings indirectly support our hypothesis.

The hyperglycemia in the hippocampus has been implicated in regulating depression and cognitive dysfunction. However, the specific mechanism underlying the impaired effect of high glucose on neuronal damage is elusive. Here, we revealed that the hyperglycemia in the hippocampus worsened depression-like behaviors and neuronal mitochondrial dysfunction in VCD-induced perimenopausal female mice. We supposed that the level of glucose in the hippocampus might determine the neuronally damaged susceptibility to estrogen deficiency during perimenopausal transition. Additionally, male rodent models also showed the same tendency. It was shown that elevated glucose levels in the hippocampus induced spatial and cognitive impairment in chronic stress-induced male mice⁹. Furthermore, higher levels of glucose in the hippocampus promoted tau hyperphosphorylation in neurons, which contributed to diabetic cognitive dysfunction and induced depression-like behaviors in male diabetic rats^{83,84}. By improving glucose metabolism in the brain, hypoglycemic medications like metformin have been demonstrated to reduce depressive-like behaviors in elderly rats^{85,86}. The current study pointed out a decrease in COX7C expression in hippocampal neurons exposed to high glucose stress, and COX7C overexpression conferred protective effects against neuronal damage *in vitro* and *in vivo*. Based on these findings, we speculated that hyperglycemia in the hippocampus might be correlated with COX7C expression in neurons, which provides a therapeutic avenue to alleviate depressive behaviors or cognitive dysfunction in high glucose-stressed rodents.

Mitochondrial dynamics (fusion/fission) is important for ATP production through regulating the balance between oxidative phosphorylation (OXPHOS) and reductive synthesis in response to nutrient availability and bioenergetics demand^{87,88}. It was shown that when nutrient availability exceeding the requirement for sustaining ATP, the biosynthesis of macromolecular precursors will proceed to support the physiological function⁸⁹. Disruption of mitochondrial dynamics impairs the capacity for ATP generation by OXPHOS or reductive synthesis for cell proliferation^{87,90,91}. In this study, we found that high glucose impaired the function of OXPHOS by causing COX7C degradation, resulting in the decline in ATP production. But we did not evaluate whether high glucose regulates mitochondrial dynamics to change ATP production and the biosynthesis of precursor substances in neurons. Many studies have found that high glucose treatment leads to excessive mitochondrial division, resulting in rupture and swelling of the mitochondrial inner membrane, and a decrease in ATP production^{92–94}. This is consistent with our findings that swelling and decreased density of mitochondria were found in the hippocampal neurons and high glucose-treated HT22 cells. Therefore, we speculate that excessive mitochondrial fission may also occur in the hippocampal neurons of VCD^{SS} mice and has a vital role in ATP production and reproductive synthesis, but the specific phenomenon still needs further testing.

COX7C is a key component of cytochrome *c* oxidase (Cox), which is located at the terminal end of the mitochondrial respiratory chain. It facilitates complex IV construction and control and is essential for electron transport in the mitochondrial respiratory chain⁹⁵. Current research efforts were focused on elucidating its role in diabetes⁹⁶ and AD⁹⁷. In addition, regulating the lifespan of the COX7C protein was critical for maintaining the integrity of the mitochondrial proteome and ensuring the stability of mitochondrial function⁹⁵. Jia et al.⁴⁴, found that COX7C exerted a critical role in protecting mitochondrial function during cerebral ischemia/reperfusion injury. In order to treat mood problems

brought on by social isolation, resveratrol has also been demonstrated to increase the expression of *Cox7c* mRNA, which modulates spinal plasticity and mitochondrial function⁹⁸. Given its critical regulatory function in mitochondrial homeostasis, COX7C was hypothesized to be a promising target for protection against perimenopausal depression in this study. Specifically, in the VCD^{SS} mice, targeted overexpression of COX7C in the hippocampal neurons *via* AAV injection was proven to protect neurons from oxidative damage. However, more research is needed to determine the mechanism by which elevated glucose controls the expression of COX7C.

Here, we revealed that SKP1-dependent ubiquitin–proteasomal pathways may be responsible for the COX7C protein's destruction brought on by high glucose. We verified the direct interaction between SKP1 and COX7C through Co-IP experiments, and we discovered that SKP1 downregulation successfully reduced the ubiquitination degradation of COX7C following high glucose stress. This helped to clarify a crucial pathway linked to the neuronal damage seen in VCD-induced perimenopausal mice. SKP1, a key scaffold protein within the SKP1–CUL1–F-box protein ubiquitin ligase complex, plays a fundamental role in regulating protein ubiquitination processes essential for various cellular functions such as cell cycle progression, signal transduction, and transcriptional regulation^{99,100}. Consequently, we supposed that SKP1-mediated degradation of COX7C induced by high glucose stress might trigger alterations in mitochondrial morphology, membrane integrity and function, ultimately contributing to abnormal glucose metabolism in the brain and subsequent neuronal damage.

In this study, we revealed that high glucose induced mitochondrial dysfunction and neuronal damage *via* activating SKP1-dependent COX7C degradation. The results showed that high glucose-induced COX7C decrease aggravated the impairment of oxidative phosphorylation function and the reduction of ATP production relying on aerobic glycolysis. The phenomenon of glucose hypometabolism induced by high glucose seems to be similar with low glucose on ATP production. It is widely acknowledged that low glucose supply induced by glucose deprivation causes cellular homeostasis disturbance and eventual neuronal death^{101–103}. Moreover, low glucose has a deleterious influence on astrocytes, characterized by inducing astrocyte overactivation and mitochondrial dysfunction, which contributes to neuronal injury and astrocyte senescence in AD and diabetic disease^{104–106}. Multiple researches confirm that low glucose regulates mitochondrial function and ATP production^{107–109}. Although it is largely unclear whether low glucose regulates SKP1–COX7C pathway in neuronal damage, there are some researches indicate that low glucose may influence the level of COX7C and SKP1 function. It was shown that the expression of COX7C in vascular endothelial cells decreased after glucose deprivation⁴⁴. The expression of the substrate recognition subunit FBOX3 connected to SKP1 was increased in HT22 cells in low glucose condition¹¹⁰, which may cause changes in downstream protein ubiquitination levels. But whether low glucose influences the level of SKP1 is elusive. Taken together, we suppose that low glucose may trigger mitochondrial dysfunction and neuronal damage similar to high glucose, but whether low glucose influences SKP1/COX7C pathway is largely unknown and needs to be further investigated.

In addition, we did not further investigate the specific mechanism of SKP1 increase in this study, but other data from our synchronous study suggested that high glucose might increase

SKP1 transcription level mainly in an aryl hydrocarbon receptor (AhR)-dependent manner. As an estrogen deficiency-sensitive protein, AhR has always been reported to increase and activate in estrogen deficiency models. We found that specific knockout of AhR in hippocampal neurons reduced the expression of SKP1 in the hippocampus of VCD^{SS} mice, and AhR played a role in SKP1 transcription. These results will be presented in subsequent research.

Studies had shown that proteins located in the inner mitochondrial membrane could be recognized by ubiquitin ligases and subsequently translocated to the outer membrane. Once at the outer membrane, these proteins underwent ubiquitin ligase binding and subsequent degradation¹¹¹. Based on this premise, our study suggested that under HG stress, COX7C, localized on the inner mitochondrial membrane, underwent translocation to the outer membrane where it subsequently interacted with SKP1, thereby initiating its degradation process. Notably, the present study did not address the specific mechanisms underlying SKP1-mediated degradation of COX7C. In order to obtain a more thorough understanding of the network of proteins involved in the pathophysiology of perimenopausal depression, future research should focus on clarifying the binding mode between SKP1 and COX7C as well as identifying the F-box protein or proteins that are in charge of COX7C recognition.

5. Conclusions

Our findings revealed a close relationship between hippocampal glucose levels with depression-like behaviors in VCD-induced perimenopausal mice. The hyperglycemia in the hippocampus aggravated vulnerability to VCD stress-induced neuronal damage and depression-like behaviors. Mechanistically, our study identified COX7C as a key target affecting mitochondrial integrity and function of hippocampal neurons in perimenopausal depression mice. We observed that elevated glucose levels in the hippocampal neurons exacerbated COX7C ubiquitination degradation *via* SKP1-mediated pathways, ultimately contributing to neuronal damage. In addition, this study offers fresh perspectives that could guide tactics to safeguard women's mental health.

Acknowledgments

We thank all members of Ikejima and Liu lab for useful discussions. We thank Prof. Jinkai Wang for assistance of PET examination. This project is supported by a grant from National Nature Science Foundation of China (82104260 to Xiaoling Liu), Xingliao Talents Program (XLYC1802007, China) and Department of education of Liaoning Province (1911520092, China) to Dan Ohtan Wang.

Author contributions

Takashi Ikejima, Xiaoling Liu and Dan Ohtan Wang made substantial contribution to the conceptualization, supervision, and interpretation of the data. Ziqi Wang and Zhiyuan Liu led the design and application of the experiments, the data analysis, and drafted the manuscript of this report. Sijia Feng, Xingtong Song, Dequan Liu, Ning Ma, and Xinyue Zhang performed the experiments and data collection. Weiwei Liu revised the manuscript. All authors contributed to the discussion and manuscript review.

Availability of data and materials

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Data are located in controlled access data storage at Shenyang Pharmaceutical University. Proteomic sequencing data were deposited to the ProteomeXchange Consortium (<https://proteomecentral.proteomexchange.org>) via the iProX partner repository with the dataset identifier PXD057078.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supporting information

Supporting information to this article can be found online at <https://doi.org/10.1016/j.apsb.2025.09.003>.

References

- Soares CN. Can depression be a menopause-associated risk?. *BMC Med* 2010;**8**:79.
- Bromberger JT, Schott L, Kravitz HM, Joffe H. Risk factors for major depression during midlife among a community sample of women with and without prior major depression: are they the same or different?. *Psychol Med* 2015;**45**:1653–64.
- Curtin SC, Warner M, Hedegaard H. *Increase in suicide in the United States, 1999–2014*, vol. 241. NCHS Data Brief; 2016. p. 1–8.
- Schmidt PJ, Ben Dor R, Martinez PE, Guerrieri GM, Harsh VL, Thompson K, et al. Effects of estradiol withdrawal on mood in women with past perimenopausal depression: a randomized clinical trial. *JAMA Psychiatry* 2015;**72**:714–26.
- Lobo RA. Hormone-replacement therapy: current thinking. *Nat Rev Endocrinol* 2017;**13**:220–31.
- Boardman HM, Hartley L, Eisinga A, Main C, Roque i Figuls M, Bonfill Cosp X, et al. Hormone therapy for preventing cardiovascular disease in post-menopausal women. *Cochrane Database Syst Rev* 2015;**2015**:CD002229.
- Morella IM, Brambilla R, More L. Emerging roles of brain metabolism in cognitive impairment and neuropsychiatric disorders. *Neurosci Biobehav Rev* 2022;**142**:104892.
- Saito T, Tamura M, Chiba Y, Katsuse O, Suda A, Kamada A, et al. Regional cerebral glucose metabolism in systemic lupus erythematosus patients with major depressive disorder. *J Neurol Sci* 2017;**379**:127–30.
- Zhang S, Lachance BB, Mattson MP, Jia X. Glucose metabolic crosstalk and regulation in brain function and diseases. *Prog Neurobiol* 2021;**204**:102089.
- Cunnane SC, Trushina E, Morland C, Prigione A, Casadesus G, Andrews ZB, et al. Brain energy rescue: an emerging therapeutic concept for neurodegenerative disorders of ageing. *Nat Rev Drug Discov* 2020;**19**:609–33.
- Roberts RO, Knopman DS, Cha RH, Mielke MM, Pankratz VS, Boeve BF, et al. Diabetes and elevated hemoglobin A1c levels are associated with brain hypometabolism but not amyloid accumulation. *J Nucl Med* 2014;**55**:759–64.
- van der Kooy MA, Jene T, Treccani G, Miederer I, Hasch A, Voelxen N, et al. Chronic social stress-induced hyperglycemia in mice couples individual stress susceptibility to impaired spatial memory. *Proc Natl Acad Sci U S A* 2018;**115**:E10187–96.
- Halim ND, McFate T, Mohyeldin A, Okagaki P, Korotchkina LG, Patel MS, et al. Phosphorylation status of pyruvate dehydrogenase distinguishes metabolic phenotypes of cultured rat brain astrocytes and neurons. *Glia* 2010;**58**:1168–76.
- Lopez-Fabuel I, Le Douce J, Logan A, James AM, Bonvento G, Murphy MP, et al. Complex I assembly into supercomplexes determines differential mitochondrial ROS production in neurons and astrocytes. *Proc Natl Acad Sci U S A* 2016;**113**:13063–8.
- Austad SN, Ballinger S, Buford TW, Carter CS, Smith Jr DL, Darley-Usmar V, et al. Targeting whole body metabolism and mitochondrial bioenergetics in the drug development for Alzheimer's disease. *Acta Pharm Sin B* 2022;**12**:511–31.
- Dash UC, Bhol NK, Swain SK, Samal RR, Nayak PK, Raina V, et al. Oxidative stress and inflammation in the pathogenesis of neurological disorders: mechanisms and implications. *Acta Pharm Sin B* 2025;**15**:15–34.
- Kolar D, Kleteckova L, Brozka H, Vales K. Mini-review: brain energy metabolism and its role in animal models of depression, bipolar disorder, schizophrenia and autism. *Neurosci Lett* 2021;**760**:136003.
- Cao X, Li LP, Wang Q, Wu Q, Hu HH, Zhang M, et al. Astrocyte-derived ATP modulates depressive-like behaviors. *Nat Med* 2013;**19**:773–7.
- Cataldo Bascunan LR, Lyons C, Bennet H, Artner I, Fex M. Serotonergic regulation of insulin secretion. *Acta Physiol* 2019;**225**:e13101.
- Picard M, Juster RP, McEwen BS. Mitochondrial allostatic load puts the 'gluc' back in glucocorticoids. *Nat Rev Endocrinol* 2014;**10**:303–10.
- Brinton RD, Yao J, Yin F, Mack WJ, Cadenas E. Perimenopause as a neurological transition state. *Nat Rev Endocrinol* 2015;**11**:393–405.
- Yin F, Yao J, Sancheti H, Feng T, Melcangi RC, Morgan TE, et al. The perimenopausal aging transition in the female rat brain: decline in bioenergetic systems and synaptic plasticity. *Neurobiol Aging* 2015;**36**:2282–95.
- Yao J, Hamilton RT, Cadenas E, Brinton RD. Decline in mitochondrial bioenergetics and shift to ketogenic profile in brain during reproductive senescence. *Biochim Biophys Acta* 2010;**1800**:1121–6.
- Yao J, Irwin R, Chen S, Hamilton R, Cadenas E, Brinton RD. Ovarian hormone loss induces bioenergetic deficits and mitochondrial beta-amyloid. *Neurobiol Aging* 2012;**33**:1507–21.
- Ding F, Yao J, Rettberg JR, Chen S, Brinton RD. Early decline in glucose transport and metabolism precedes shift to ketogenic system in female aging and Alzheimer's mouse brain: implication for bioenergetic intervention. *PLoS One* 2013;**8**:e79977.
- Rasgon NL, Silverman D, Siddarth P, Miller K, Ercoli LM, Elman S, et al. Estrogen use and brain metabolic change in postmenopausal women. *Neurobiol Aging* 2005;**26**:229–35.
- Mosconi L, Berti V, Quinn C, McHugh P, Petrongolo G, Osorio RS, et al. Perimenopause and emergence of an Alzheimer's bioenergetic phenotype in brain and periphery. *PLoS One* 2017;**12**:e0185926.
- Rasgon NL, Geist CL, Kenna HA, Wroolie TE, Williams KE, Silverman DH. Prospective randomized trial to assess effects of continuing hormone therapy on cerebral function in postmenopausal women at risk for dementia. *PLoS One* 2014;**9**:e89095.
- Khayum MA, Moraga-Amaro R, Buwalda B, Koole M, den Boer JA, Dierckx R, et al. Ovariectomy-induced depressive-like behavior and brain glucose metabolism changes in female rats are not affected by chronic mild stress. *Psychoneuroendocrinology* 2020;**115**:104610.
- Liu XL, Wang ZQ, Song XT, Chang XY, Zu E, Ma XW, et al. Crocetin alleviates ovariectomy-induced metabolic dysfunction through regulating estrogen receptor beta. *J Agric Food Chem* 2021;**69**:14824–39.
- Lohff JC, Christian PJ, Marion SL, Hoyer PB. Effect of duration of dosing on onset of ovarian failure in a chemical-induced mouse model of perimenopause. *Menopause* 2006;**13**:482–8.
- Wang Y, Liu Y, Xiong JW, Di TT, Yuan ZH, Wu J, et al. Reduced serotonin impairs long-term depression in basolateral amygdala complex and causes anxiety-like behaviors in a mouse model of perimenopause. *Exp Neurol* 2019;**321**:113030.

33. Yu S, Zhang LX, Wang YY, Yan JM, Wang Q, Bian HS, et al. Mood, hormone levels, metabolic and sleep across the menopausal transition in VCD-induced ICR mice. *Physiol Behav* 2023;**265**:114178.
34. Lesnikov VA, Tsvetkova IP. Stereotaxic coordinates of the mouse hypothalamus. *Fiziol Zh SSSR Im I M Sechenova* 1985;**71**:798–804.
35. Schwanhaussier B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, et al. Global quantification of mammalian gene expression control. *Nature* 2011;**473**:337–42.
36. Luber CA, Cox J, Lauterbach H, Fancke B, Selbach M, Tschoep J, et al. Quantitative proteomics reveals subset-specific viral recognition in dendritic cells. *Immunity* 2010;**32**:279–89.
37. Cox J, Hein MY, Luber CA, Paron I, Nagaraj N, Mann M. Accurate proteome-wide label-free quantification by delayed normalization and maximal peptide ratio extraction, termed MaxLFQ. *Mol Cell Proteomics* 2014;**13**:2513–26.
38. Xu H, Zhao HF, Ding CY, Jiang DF, Zhao ZJ, Li Y, et al. Celastrol suppresses colorectal cancer via covalent targeting peroxiredoxin 1. *Signal Transduct Target Ther* 2023;**8**:51.
39. Brooks HL, Pollow DP, Hoyer PB. The VCD mouse model of menopause and perimenopause for the study of sex differences in cardiovascular disease and the metabolic syndrome. *Physiology (Bethesda)* 2016;**31**:250–7.
40. Cao LB, Leung CK, Law PW, Lv Y, Ng CH, Liu HB, et al. Systemic changes in a mouse model of VCD-induced premature ovarian failure. *Life Sci* 2020;**262**:118543.
41. Zhao Y, Chen YL, Guo CH, Li PP, Cheng Z, Zheng L, et al. Chronic stress dysregulates the Hippo/YAP/14-3-3eta pathway and induces mitochondrial damage in basolateral amygdala in a mouse model of depression. *Theranostics* 2024;**14**:3653–73.
42. Zou KH, O'Malley AJ, Mauri L. Receiver-operating characteristic analysis for evaluating diagnostic tests and predictive models. *Circulation* 2007;**115**:654–7.
43. Fang WT, Zhang J, Hong LY, Huang WB, Dai XM, Ye QY, et al. Metformin ameliorates stress-induced depression-like behaviors via enhancing the expression of BDNF by activating AMPK/CREB-mediated histone acetylation. *J Affect Disord* 2020;**260**:302–13.
44. Jia JJ, Deng JW, Jin HQ, Yang J, Nan D, Yu ZM, et al. Effect of D,L-3-n-butylphthalide on mitochondrial Cox7c in models of cerebral ischemia/reperfusion injury. *Front Pharmacol* 2023;**14**:1084564.
45. Thompson LL, Rutherford KA, Lepage CC, McManus KJ. The SCF complex is essential to maintain genome and chromosome stability. *Int J Mol Sci* 2021;**22**:8544.
46. Lee DY, Andreescu C, Aizenstein H, Karim H, Mizuno A, Kolobaric A, et al. Impact of symptomatic menopausal transition on the occurrence of depression, anxiety, and sleep disorders: a real-world multi-site study. *Eur Psychiatry* 2023;**66**:e80.
47. Avila BM, Zanini BM, Luduvico KP, Oliveira TL, Hense JD, Garcia DN, et al. Effect of senolytic drugs in young female mice chemically induced to estropause. *Life Sci* 2024;**357**:123073.
48. Flaws JA, Salyers KL, Sipes IG, Hoyer PB. Reduced ability of rat preantral ovarian follicles to metabolize 4-vinyl-1-cyclohexene diepoxide *in vitro*. *Toxicol Appl Pharmacol* 1994;**126**:286–94.
49. Wright LE, Christian PJ, Rivera Z, Van Alstine WG, Funk JL, Bouxsein ML, et al. Comparison of skeletal effects of ovariectomy versus chemically induced ovarian failure in mice. *J Bone Miner Res* 2008;**23**:1296–303.
50. Muhammad FS, Goode AK, Kock ND, Arifin EA, Cline JM, Adams MR, et al. Effects of 4-vinylcyclohexene diepoxide on periparturient and adult Sprague-Dawley rats: ovarian, clinical, and pathologic outcomes. *Comp Med* 2009;**59**:46–59.
51. Chhabra RS, Elwell MR, Peters A. Toxicity of 4-vinyl-1-cyclohexene diepoxide after 13 weeks of dermal or oral exposure in rats and mice. *Fundam Appl Toxicol* 1990;**14**:745–51.
52. National Toxicology P. Toxicology and carcinogenesis studies of 4-vinyl-1-cyclohexene diepoxide (CAS No. 106-87-6) in F344/N Rats and B6C3F1 mice (dermal studies). *Natl Toxicol Program Tech Rep Ser* 1989;**362**:1–249.
53. Smith BJ, Carter DE, Sipes IG. Comparison of the disposition and *in vitro* metabolism of 4-vinylcyclohexene in the female mouse and rat. *Toxicol Appl Pharmacol* 1990;**105**:364–71.
54. Rodrigues-Santos I, Kalil-Cutti B, Anselmo-Franci JA. Low corticosterone response to stress in a perimenopausal rat model is associated with the hypoactivation of PaMP region of the paraventricular nucleus and can be corrected by exogenous progesterone supplementation. *Neuroendocrinology* 2022;**112**:467–80.
55. Platholi J, Marongiu R, Park L, Yu FM, Sommer G, Weinberger R, et al. Hippocampal glial inflammatory markers are differentially altered in a novel mouse model of perimenopausal cerebral amyloid angiopathy. *Front Aging Neurosci* 2023;**15**:1280218.
56. Koebele SV, Mennenga SE, Hiroi R, Quihuis AM, Hewitt LT, Poisson ML, et al. Cognitive changes across the menopause transition: a longitudinal evaluation of the impact of age and ovarian status on spatial memory. *Horm Behav* 2017;**87**:96–114.
57. Butterfield DA, Halliwell B. Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. *Nat Rev Neurosci* 2019;**20**:148–60.
58. Carr Jr ME, Powers PL. Effect of glycosaminoglycans on thrombin- and atroxin-induced fibrin assembly and structure. *Thromb Haemostasis* 1989;**62**:1057–61.
59. Evans RD, Brown AM, Ransom BR. Glycogen function in adult central and peripheral nerves. *J Neurosci Res* 2013;**91**:1044–9.
60. Falkowska A, Gutowska I, Goschorska M, Nowacki P, Chlubek D, Baranowska-Bosiacka I. Energy metabolism of the brain, including the cooperation between astrocytes and neurons, especially in the context of glycogen metabolism. *Int J Mol Sci* 2015;**16**:25959–81.
61. Yip J, Geng XK, Shen JM, Ding YC. Cerebral gluconeogenesis and diseases. *Front Pharmacol* 2016;**7**:521.
62. Fang YY, Zeng P, Qu N, Ning LN, Chu J, Zhang T, et al. Evidence of altered depression and dementia-related proteins in the brains of young rats after ovariectomy. *J Neurochem* 2018;**146**:703–21.
63. Ko S, Jang WS, Jeong JH, Ahn JW, Kim YH, Kim S, et al. (–)-Gallocatechin gallate from green tea rescues cognitive impairment through restoring hippocampal silent synapses in postmenopausal depression. *Sci Rep* 2021;**11**:910.
64. Ye Z, Cudmore RH, Linden DJ. Estrogen-dependent functional spine dynamics in neocortical pyramidal neurons of the mouse. *J Neurosci* 2019;**39**:4874–88.
65. Bogush M, Heldt NA, Persidsky Y. Blood brain barrier injury in diabetes: unrecognized effects on brain and cognition. *J Neuro-immune Pharmacol* 2017;**12**:593–601.
66. Chen Y, Joo J, Chu JM, Chang RC, Wong GT. Downregulation of the glucose transporter GLUT 1 in the cerebral microvasculature contributes to postoperative neurocognitive disorders in aged mice. *J Neuroinflamm* 2023;**20**:237.
67. Ishida H, Takemori K, Dote K, Ito H. Expression of glucose transporter-1 and aquaporin-4 in the cerebral cortex of stroke-prone spontaneously hypertensive rats in relation to the blood–brain barrier function. *Am J Hypertens* 2006;**19**:33–9.
68. Wang Q, Huang XM, Su YX, Yin GW, Wang SY, Yu B, et al. Activation of Wnt/beta-catenin pathway mitigates blood–brain barrier dysfunction in Alzheimer's disease. *Brain* 2022;**145**:4474–88.
69. Harris JJ, Jolivet R, Attwell D. Synaptic energy use and supply. *Neuron* 2012;**75**:762–77.
70. Ivanov AI, Malkov AE, Waseem T, Mukhtarov M, Buldakova S, Gubkina O, et al. Glycolysis and oxidative phosphorylation in neurons and astrocytes during network activity in hippocampal slices. *J Cereb Blood Flow Metab* 2014;**34**:397–407.
71. Brinton RD. Estrogen-induced plasticity from cells to circuits: predictions for cognitive function. *Trends Pharmacol Sci* 2009;**30**:212–22.
72. Liao TL, Tzeng CR, Yu CL, Wang YP, Kao SH. Estrogen receptor-beta in mitochondria: implications for mitochondrial bioenergetics and tumorigenesis. *Ann N Y Acad Sci* 2015;**1350**:52–60.

73. Simpkins JW, Yi KD, Yang SH, Dykens JA. Mitochondrial mechanisms of estrogen neuroprotection. *Biochim Biophys Acta* 2010;**1800**: 1113–20.
74. Uddin MS, Rahman MM, Jakaria M, Rahman MS, Hossain MS, Islam A, et al. Estrogen signaling in Alzheimer's disease: molecular insights and therapeutic targets for Alzheimer's dementia. *Mol Neurobiol* 2020;**57**:2654–70.
75. Yao J, Brinton RD. Estrogen regulation of mitochondrial bioenergetics: implications for prevention of Alzheimer's disease. *Adv Pharmacol* 2012;**64**:327–71.
76. Pestana-Oliveira N, Kalil B, Leite CM, Carolino ROG, Debarba LK, Elias LLK, et al. Effects of estrogen therapy on the serotonergic system in an animal model of perimenopause induced by 4-vinylcyclohexen diepoxide (VCD). *eNeuro* 2018;**5**:ENEURO.0247-17.2017.
77. Brinton RD. The healthy cell bias of estrogen action: mitochondrial bioenergetics and neurological implications. *Trends Neurosci* 2008;**31**:529–37.
78. Yan H, Yang WB, Zhou FH, Li XP, Pan Q, Shen Z, et al. Estrogen improves insulin sensitivity and suppresses gluconeogenesis via the transcription factor foxo1. *Diabetes* 2019;**68**:291–304.
79. Itoh N, Itoh Y, Meyer CE, Suen TT, Cortez-Delgado D, Rivera Lomeli M, et al. Estrogen receptor beta in astrocytes modulates cognitive function in mid-age female mice. *Nat Commun* 2023;**14**:6044.
80. Dienel GA. Brain glucose metabolism: integration of energetics with function. *Physiol Rev* 2019;**99**:949–1045.
81. Kleinridders A, Ferris HA, Reyzer ML, Rath M, Soto M, Manier ML, et al. Regional differences in brain glucose metabolism determined by imaging mass spectrometry. *Mol Metab* 2018;**12**:113–21.
82. Xu J, Begley P, Church SJ, Patassini S, McHarg S, Kureishy N, et al. Elevation of brain glucose and polyol-pathway intermediates with accompanying brain-copper deficiency in patients with Alzheimer's disease: metabolic basis for dementia. *Sci Rep* 2016;**6**:27524.
83. Qu ML, Zuo LH, Zhang MR, Cheng P, Guo ZJ, Yang JY, et al. High glucose induces tau hyperphosphorylation in hippocampal neurons via inhibition of ALKBH5-mediated Dgkh m⁶A demethylation: a potential mechanism for diabetic cognitive dysfunction. *Cell Death Dis* 2023;**14**:385.
84. Su WJ, Li JM, Zhang T, Cao ZY, Hu T, Zhong SY, et al. Microglial NLRP3 inflammasome activation mediates diabetes-induced depression-like behavior via triggering neuroinflammation. *Prog Neuropsychopharmacol Biol Psychiatry* 2023;**126**:110796.
85. Lin YB, Dai XM, Zhang J, Chen XC. Metformin alleviates the depression-like behaviors of elderly apoE4 mice via improving glucose metabolism and mitochondrial biogenesis. *Behav Brain Res* 2022;**423**:113772.
86. Lam YY, Tsai SF, Chen PC, Kuo YM, Chen YW. Pioglitazone rescues high-fat diet-induced depression-like phenotypes and hippocampal astrocytic deficits in mice. *Biomed Pharmacother* 2021;**140**: 111734.
87. Ryu KW, Fung TS, Baker DC, Saoi M, Park J, Febres-Aldana CA, et al. Cellular ATP demand creates metabolically distinct subpopulations of mitochondria. *Nature* 2024;**635**:746–54.
88. Chan DC. Mitochondrial dynamics and its involvement in disease. *Annu Rev Pathol* 2020;**15**:235–59.
89. Schworer S, Berisa M, Violante S, Qin WG, Zhu JJ, Hendrickson RC, et al. Proline biosynthesis is a vent for TGFβ-induced mitochondrial redox stress. *EMBO J* 2020;**39**:e103334.
90. Zhong ZW, Wang K, Zhong T, Wang JW. Mitochondrial fission regulates midgut muscle assembly and tick feeding capacity. *Cell Rep* 2025;**44**:115505.
91. Zhou XG, Wang J, Yu L, Qiao G, Qin DL, Yuen-Kwan Law B, et al. Mitophagy and cGAS–STING crosstalk in neuroinflammation. *Acta Pharm Sin B* 2024;**14**:3327–61.
92. Gonzalez-Blanco C, Lockwood AC, Jimenez B, Iglesias-Fortes S, Marques P, Garcia G, et al. Resveratrol protects pancreatic beta cell and hippocampal cells from the aggregate-prone capacity of hIAPP. *Sci Rep* 2024;**14**:27523.
93. Hu Y, Zhou YL, Yang YJ, Tang HH, Si Y, Chen ZY, et al. Metformin protects against diabetes-induced cognitive dysfunction by inhibiting mitochondrial fission protein DRP1. *Front Pharmacol* 2022;**13**: 832707.
94. Tang WX, Yan CY, He SX, Du MY, Cheng B, Deng B, et al. Neuron-targeted overexpression of caveolin-1 alleviates diabetes-associated cognitive dysfunction via regulating mitochondrial fission–mitophagy axis. *Cell Commun Signal* 2023;**21**:357.
95. Krishna S, Arrojo EDR, Capitanio JS, Ramachandra R, Ellisman M, Hetzer MW. Identification of long-lived proteins in the mitochondria reveals increased stability of the electron transport chain. *Dev Cell* 2021;**56**:2952–65.e9.
96. Melero R, Quiroz-Rodriguez ME, Lara-Hernandez F, Redon J, Saez G, Briongos-Figuero LS, et al. Genetic interaction in the association between oxidative stress and diabetes in the Spanish population. *Free Radic Biol Med* 2023;**205**:62–8.
97. Xicota L, Lagarde J, Eysert F, Grenier-Boley B, Rivals I, Botte A, et al. Modifications of the endosomal compartment in fibroblasts from sporadic Alzheimer's disease patients are associated with cognitive impairment. *Transl Psychiatry* 2023;**13**:54.
98. Zhao JL, Ye LH, Liu ZY, Cui YF, Deng D, Bai SS, et al. Protective effects of resveratrol on adolescent social isolation-induced anxiety-like behaviors via modulating nucleus accumbens spine plasticity and mitochondrial function in female rats. *Nutrients* 2022;**14**:4542.
99. Thompson LL, Rutherford KA, Lepage CC, McManus KJ. Aberrant SKP1 expression: diverse mechanisms impacting genome and chromosome stability. *Front Cell Dev Biol* 2022;**10**:859582.
100. Baek K, Scott DC, Henneberg LT, King MT, Mann M, Schulman BA. Systemwide disassembly and assembly of SCF ubiquitin ligase complexes. *Cell* 2023;**186**:1895–911.e21.
101. Bai X, Tan TY, Li YX, Li Y, Chen YF, Ma R, et al. The protective effect of cordyceps sinensis extract on cerebral ischemic injury via modulating the mitochondrial respiratory chain and inhibiting the mitochondrial apoptotic pathway. *Biomed Pharmacother* 2020;**124**: 109834.
102. Su J, Liu J, Yan XY, Zhang Y, Zhang JJ, Zhang LC, et al. Cytoprotective effect of the UCP2–SIRT3 signaling pathway by decreasing mitochondrial oxidative stress on cerebral ischemia–reperfusion injury. *Int J Mol Sci* 2017;**18**:1599.
103. Xie J, Kittur FS, Li PA, Hung CY. Rethinking the necessity of low glucose intervention for cerebral ischemia/reperfusion injury. *Neural Regen Res* 2022;**17**:1397–403.
104. Gao L, Yang WY, Qi H, Sun CJ, Qin XM, Du GH. Unveiling the anti-senescence effects and senescence-associated secretory phenotype (SASP) inhibitory mechanisms of *Scutellaria baicalensis* Georgi in low glucose-induced astrocytes based on boolean network. *Phyto-medicine* 2022;**99**:153990.
105. Gao RN, Chen Z, Wu YB, Chen RY, Zheng WR, Qi LQ, et al. SIRT3 alleviates mitochondrial dysfunction induced by recurrent low glucose and improves the supportive function of astrocytes to neurons. *Free Radic Biol Med* 2022;**193**:405–20.
106. Gao RN, Ren LJ, Zhou Y, Wang LJ, Xie YZ, Zhang MJ, et al. Recurrent non-severe hypoglycemia aggravates cognitive decline in diabetes and induces mitochondrial dysfunction in cultured astrocytes. *Mol Cell Endocrinol* 2021;**526**:111192.
107. Circu ML, Maloney RE, Aw TY. Low glucose stress decreases cellular NADH and mitochondrial ATP in colonic epithelial cancer cells: influence of mitochondrial substrates. *Chem Biol Interact* 2017;**264**:16–24.

108. Ortiz-Rodriguez JM, Martin-Cano FE, Gaitskell-Phillips GL, Silva A, Ortega-Ferrusola C, Gil MC, et al. Low glucose and high pyruvate reduce the production of 2-oxoaldehydes, improving mitochondrial efficiency, redox regulation, and stallion sperm function. *Biol Reprod* 2021;**105**:519–32.
109. Zarpellon PS, Murat C, Leao RM. Reduction in mitochondrial ATP synthesis mimics the effect of low glucose in depolarizing neurons from the subpostremal nucleus of the solitary tract of rats. *J Bioenerg Biomembr* 2024;**56**:483–93.
110. Gao Y, Xiao XY, Luo J, Wang JW, Peng QL, Zhao J, et al. E3 ubiquitin ligase FBXO3 drives neuroinflammation to aggravate cerebral ischemia/reperfusion injury. *Int J Mol Sci* 2022;**23**:13648.
111. Azzu V, Brand MD. Degradation of an intramitochondrial protein by the cytosolic proteasome. *J Cell Sci* 2010;**123**:578–85.