Long-term exercise training down-regulates m^6^A RNA demethylase FTO expression in the hippocampus and hypothalamus: an effective intervention for epigenetic modification

Shu-Jing Liu^1†, Tong-Hui Cai^2†, Chun-Lu Fang^1†, Shao-Zhang Lin^2, Wen-Qi Yang^1, Yuan Wei^1, Fu Zhou^1, Ling Liu^1, Yuan Luo^1, Zi-Yi Guo^1, Ge Zhao^1, Ya-Ping Li^1 and Liang-Ming Li^1*

Abstract

Background: Exercise boosts the health of some brain parts, such as the hippocampus and hypothalamus. Several studies show that long-term exercise improves spatial learning and memory, enhances hypothalamic leptin sensitivity, and regulates energy balance. However, the effect of exercise on the hippocampus and hypothalamus is not fully understood. The study aimed to find epigenetic modifications or changes in gene expression of the hippocampus and hypothalamus due to exercise.

Methods: Male C57BL/6 mice were randomly divided into sedentary and exercise groups. All mice in the exercise group were subjected to treadmill exercise 5 days per week for 1 h each day. After the 12-week exercise intervention, the hippocampus and hypothalamus tissue were used for RNA-sequencing or molecular biology experiments.

Results: In both groups, numerous differentially expressed genes of the hippocampus (up-regulated: 53, down-regulated: 49) and hypothalamus (up-regulated: 24, down-regulated: 40) were observed. In the exercise group, increased level of N6-methyladenosine (m^6^A) was observed in the hippocampus and hypothalamus (p < 0.05). Furthermore, the fat mass and obesity-associated gene (FTO) of the hippocampus and hypothalamus were down-regulated in the exercise group (p < 0.001). In addition, the Fto co-expression genes of the mouse brain were studied and analyzed using database to determine the potential roles of exercise-downregulated FTO in the brain.

Conclusion: The findings demonstrate that long-term exercise might elevates the levels of m^6^A-tagged transcripts in the hippocampus and hypothalamus via down-regulation of FTO. Hence, exercise might be an effective intervention for epigenetic modification.

Keywords: Exercise, Hippocampus, Hypothalamus, RNA-sequencing, FTO

Introduction

The brain is the master organ of the central nervous system that modulates body organ functioning. The hippocampus and hypothalamus are parts of the brain crucial for the body’s physiological functions. The hippocampus is a highly plastic region associated with...
stress response, learning, and memory [1, 2], and the hypothalamus is a critical central regulatory center for blood sugar, energy balance, and water balance [3]. Dysfunction of these parts can lead to adverse effects.

Exercise targets various aspects of brain function and broadly influences brain health. Studies on humans and animals suggested that physical exercise improves spatial learning and memory [4, 5]. Some studies reported that exercise controls obesity by enhancing hypothalamic leptin sensitivity [6, 7]. However, the cellular and molecular effects of exercise on the hippocampus and hypothalamus remain unknown. Therefore, it is important to study the effect of exercise on gene expression in the brain, and find a non-drug method to maintain brain health.

Recently, epigenetic regulation in various biological functions and pathogenesis of diseases has gained attention. m⁶A is one of the most common post-transcriptional RNA modifications in mRNA, represents another novel epigenetic marker, play critical roles in the regulation of gene expression. Through mutual interplay with methyltransferases, demethylases and m⁶A binding proteins to balance the m⁶A level, and to insure the mRNA transcripts can be properly spliced, transported, transcripts, and degraded [8]. Exercise as a lifestyle intervention can fine-tune gene expressions and biological processes via epigenetic modifications [9]. In the present study, a transcriptome profiling technology RNA-sequencing was used to identify differentially expressed genes of the hippocampus and hypothalamus in exercise training models. In the exercise group, an increased level of m⁶A was observed in the hippocampus and hypothalamus. Furthermore, the m⁶A RNA methylation regulator expression was assessed. Bioinformatic analysis showed that the Fto gene was down-regulated in the hippocampus and hypothalamus, which responds to exercise. Based on previous bioinformatics analyses, we further confirm Fto expression and other m⁶A RNA methylation regulators in the hippocampus and hypothalamus of exercise mice using qPCR and western blot analysis.

* FTO is associated with an increased risk of diabetes and obesity [10]. Recently, the *FTO* gene and its expression product have attracted widespread interest due to its identification as an m⁶A RNA demethylase [11–13]. FTO is highly expressed in the brain and likely involved in many nuclear RNA processing events, such as mRNA translation, splicing, and metabolism [11, 13]. Previous study showed that highly intensive exercise decreases the skeletal muscle FTO mRNA [14]. However, the evidence on the effects of long-term exercise on *FTO* expression is scarce. Hence, evidence on the molecular biological mechanisms of exercise-induced changes of FTO-m⁶A expression on brain function and the biological process has been provides in this study.

**Experimental procedure**

**Animals and diet administration**

The C57BL/6 mice were provided by the Experimental Animal Center of Sun Yat-sen University (Guangzhou, China). Eight-week-old male C57BL/6 J mice were randomly divided into sedentary (n=24) and exercise (n=24) groups. The mice in the exercise group were trained on a treadmill 5 days per week for 12 weeks (Fig. 1). The schedule was as follows: 5 min of warm-up at 0–12 m/min, 50 min of the main exercise at 12 m/min (moderate-intensity exercise with 75% maximum oxygen consumption), and 5 min of cool down at 12–0 m/min [15]. The mice in the sedentary group were controls. And the control mice were exposed to treadmill noise and vibration without running. The mice were fed a standard diet and water ad libitum in a 12 h-light/12 h-dark cycle at the Guangzhou Sport University. Five days after the final exercise training, mice were assessed for body composition and metabolic status. The mice were euthanized under anesthesia (sodium pentobarbital 50 μg/g) for collection of the hippocampus and hypothalamus tissues.

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![Fig. 1 Construction of 12-week exercise training model in exercise mice and exercise protocol](image-url)
This study was approved by the Institutional Animal Care and Use Committee of Guangzhou Sport University (2021DWLL-05).

Metabolic assessments
Mice were placed in an Oxymax Comprehensive Lab Animal Monitoring System (Columbus Instruments, USA) to detect the oxygen consumption (VO2), the carbon dioxide production (VCO2), respiratory exchange ratio (RER), energy intake, and energy expenditure. The body composition was assessed using the EchoMRI quantitative magnetic resonance (QMR) method (EchoMRI-500H, USA).

Library construction for RNA-sequencing
The quantity and purity of the total RNA were analyzed using the Bioanalyzer 2100 and RNA 1000 Nano LabChip Kit (Agilent, USA) with RIN number > 7.0. Poly(A) RNA was purified from the total RNA (5 μg) using poly-T oligo-attached magnetic beads with two rounds of purification. Subsequently, the mRNA was fragmented into small pieces using divergent cations under elevated temperatures. The cleaved RNA fragments were then reverse transcribed using the mRNA Seq sample preparation kit (Illumina, USA) to create the final cDNA library [the average insert size for the libraries was 300 bp (±50 bp)]. In addition, the paired-end sequencing was performed on an Illumina Novaseq™ 6000 (LC-Bio Technology CO., Ltd., Hangzhou, China) following the vendor's recommended protocol.

RNA extraction and quantitative real-time PCR (qPCR)
Total RNA from the mouse hippocampus and hypothalamus tissues were extracted using HiPure Universal RNA Kit (Magen, China). The cDNA was synthesized from 1 μg of total RNA using PrimeScript™ RT Reagent Kit with gDNA Eraser (TaKaRa, Japan). qPCR analysis was conducted using the Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, USA). The relative cycle threshold (CT) values were normalized using β-actin for the housekeeping as an internal control.

Protein extraction and western blot
The mouse hippocampus and hypothalamus tissues were lysed on ice using RIPA lysis buffer (100 mm NaCl, 20 mm Tris, pH8.0, 1 mm EDTA, pH8.0, 0.5% Triton X-100, and 0.5% Nonidet P-40) containing a protease and phosphatase inhibitor cocktail (Beyotime Biotechnology, China). These tissues were quantified using the BCA Protein Assay Kit (Pierce, Germany). The same amount of protein (15 μg) was resolved on a 12% SDS-PAGE under a denaturing condition, transferred onto a PVDF membrane, and blocked in 5% non-fat milk. The blots were cut prior to hybridisation with antibodies. After the tissues were incubated with FTO antibody (Cat#: 98768, Santa Cruz Biotech, USA) or β-actin antibody (Cat#: 60008-1-Ig, ProteinTech Group, USA) overnight at 4 °C and secondary antibody (Peroxidase-conjugated Affinipure Goat Anti Mouse/Rabbit IgG, ProteinTech Group, USA) for 2 h at room temperature, the bands were exposed using enhanced chemiluminescence (Pierce, USA) and X-ray film. Quantitative data were obtained using ImageJ software.

m6A level
The m6A RNA methylation status of the mouse hippocampus and hypothalamus were detected using enzyme-linked immunoassay (ELISA) with an EpiQuik™ m6A RNA Methylation Quantification Kit (Epigebtek, China) following the manufacturer’s protocol. The detected signal was quantified by reading the absorbance in a microplate spectrophotometer. The amount of m6A is proportional to the OD intensity measured.

Bioinformatic analysis of Fto gene co-expression network
The transcriptome and expression profiles of Fto and other m6A RNA methylation regulators were analyzed using the R package “Llimma” (R software version R3.6.3). The Fto gene co-expression network in the mouse brain were analyzed using the coexpedia database. To explore the functional annotation and pathway enrichment of the co-expression network in the brain, the Gene Ontology

| Table 1 | Mouse specific primer sequences used for qPCR (β-actin for the housekeeping as an internal control) |
|---------|-----------------------------------------------------------------------------------|
| Gene    | Forward sequence | Reverse sequence |
| Fto     | GCCAGTGACGCTCTCAACAGG               | CCAACATGCGAAAGATTCAGGA               |
| Alkbh5  | GGGGTGTCGAAAACCTGTGC               | GCCATGTGAGCTGCTCA               |
| Mettl3  | GAGCTAGGTGATGGGCAGACGG              | GCCAGGAGCAATCTCAGGG               |
| Mettl4  | GGGGAAGCCCTGACCTTGGG               | ACCCACTTTTCGCAAGCATA               |
| Wtap    | TCTTGTGATCATGCGGTAGCA               | GCGTAAACCTCCAGGGCATC               |
| Rbm15   | GAAGAGCCAGAGCGCAACG               | GAGGTCACCTCTGCAAGCAGAT               |
| Zc3h13  | GGAACTCAAGAAGACGCT               | CGAGATTCCTGAGCGGTAC               |
| Ythdf1  | ACAGTTACCCCTCGATGAGT               | GGTAGTGAAGATAACCGGATGGGA               |
| Ythdf3  | TGGACACAAACCGGTTACCA               | TGTTTTCTTTTCTCCTCCCTACGC               |
| Ythdf2  | GGTCGGAATCATATACCTG               | GAAGTAACGAAAGATGGCATG               |
| Hnrnpc  | GGCAGACAGCTTACACAAACAA             | CTGTTGGGCTCAAGTAAGGTTTC               |
| Ythdc1  | CCAAAAGCAAGGGTGTAT               | TCATTCCAGGGATTTGAGAT               |
| β-actin | TGTTGTCGTGACCAAGGCGT               | CCATGTCGTCAGGTTGGAAC               |
Statistical analyses
All experimental data were analyzed using the SPSS 20.0 software, and the results were expressed as mean ± SEM. Kolmogorov–Smirnov and Shapiro–Wilk normality tests were performed and homogeneity of variance was tested with the Levene. Statistical significance of differences between the two groups were calculated using Student’s t-test. When variables did not fulfill assumptions of normality, the Kruskal–Wallis test was applied. p < 0.05 were considered significant and marked with an asterisk (*).

Results
Characterization of mice with 12-week exercise training
A long-term aerobic exercise training mouse model with moderate-intensity was used to analysis the effect of exercise on weight, body compose, and metabolic parameters. Compared with the mice in the sedentary group, those in the exercise group showed lower body weight (p < 0.05) and fat percentages (p < 0.05) after 12-week exercise training (Fig. 2a). In addition, the VCO₂ (p < 0.01) and RER (p < 0.001) decreased in the exercise group compared to that in the sedentary group (Fig. 2e and f). But there is no statistical difference in energy intake, energy expenditure, and VO₂ between the exercise group and the sedentary group (Fig. 2b–d).

Alteration of hippocampal and hypothalamic RNA-sequencing in mice with 12-week exercise training
RNA-sequencing determined the transcriptome and expression profiles in the hippocampus and hypothalamus of 5 mice with 12-week exercise training (exercise group) and 5 control mice (sedentary group). Heatmaps and volcano maps showed significant differentially expressed genes in the hippocampus and hypothalamus between the two groups (Fig. 3). As shown in Fig. 3c, 102 differentially expressed genes were observed in the hippocampus: 53 and 49 genes were up-regulated and down-regulated, respectively, in the exercise group compared to that in the sedentary group. However, 64 differentially expressed genes were observed in the hypothalamus: 24 and 40 genes were up-regulated and down-regulated, respectively, in the exercise group compared to that in the sedentary group (Fig. 3d).

Genes with significant changes (|log₂-fold-change| > 1 and normalized p < 0.05) were identified in the exercise and sedentary group, and their functions were annotated using GO and KEGG pathway analyses [16]. The results
reported that differentially expressed genes in the hippocampus majorly enriched in biological process (BP), including “cell adhesion,” “biological process” and “ventricular cardiac muscle tissue morphogenesis”; cellular component (CC), including “membrane,” “integral component of membrane” and “cytoplasm”; and molecular function (MF), including “protein binding,” “metal ion binding,” and “calcium ion binding” (Fig. 4a). In addition, differentially expressed genes in the hypothalamus majorly enriched in BP, including “biological process,” “regulation of transcription, DNA-templated,” and “positive regulation of transcription by RNA polymerase II”; CC, including “nucleus,” “cytoplasm,” and “membrane”; and MF, including “protein binding,” “metal ion binding,”
Fig. 4 Visualization analysis of GO and KEGG of the exercise group compared to the sedentary group in hippocampus and hypothalamus.  

**a, b** GO analysis classified regulators into BP, CC, and MF groups. **c, d** KEGG pathway enrichment. n = 5 per group, p < 0.05
and “molecular function” (Fig. 4b). Furthermore, genes involved in the KEGG pathways of the hippocampus enriched “hypertrophic cardiomyopathy signaling pathway,” “dilated cardiomyopathy signaling pathway,” “cardiac muscle contraction signaling pathway,” “adrenergic signaling in cardiomyocytes signaling pathway,” “viral myocarditis signaling pathway,” and “cell adhesion molecules signaling pathway” (Fig. 4c). Similarly, genes involved in the KEGG pathway of the hypothalamus enriched “phosphoenolpyruvate carboxykinase binding” (Fig. 6b). Furthermore, Fto/co-expression genes involved in the KEGG pathway of the hypothalamus enriched “vasopressin regulated water reabsorption signaling pathway,” “synaptic vesicle cycle signaling pathway,” “protein processing in endoplasmic reticulum signaling pathway,” and “CAMP signaling pathway” (Fig. 6c). Therefore, FTO plays diverse physiological and pathological functions in the brain tissues. Further, exercise may play a role in brain functions and biological processes by regulating the FTO expression.

**Discussion**

Physical exercise has substantial beneficial effects not only on physical health but also on brain function. Most studies suggested the importance of exercise on the brain, particularly the hippocampus and hypothalamus. For example, long-term exercise can prevent cognitive dysfunction induced by obesity [17] or aging [18] and improve spatial learning and memory ability. Endurance exercise can alter the gene expression status of the hippocampus, thereby affecting human cognitive function [19]. In addition, exercise ameliorates the hypothalamic leptin resistance [20] and insulin resistance [21] to affect the energy balance. However, the molecular mechanisms through which exercise affects brain function are unclear. The development of high-throughput sequencing provides a beneficial tool to study the role of exercise in regulating the biological processes in the brain by altering the gene expressions.

The molecular mechanisms of exercise that regulates brain function were investigated. We used an exercise mouse model to observe the effects of exercise on gene expression in the hippocampus and hypothalamus. Using high-throughput sequencing technology, differential genes were found to be involved in many important cellular functions and signaling pathways. For example, some enriched functions of the differentially expressed genes in the hippocampus were associated with the synaptic transmission process (GO: 0099025, GO: 0099029, GO: 0099576, GO: 0060080, GO: 0099151, and GO: 0051932), indicating that exercise may regulate synaptic activity. In addition, some enriched functions of the differentially expressed genes in the hypothalamus were associated with the neural function (GO: 0032809 and GO: 0043005), neurogenesis (GO: 0021626 and GO: 0014037), and glucagon secretion regulation (GO: 0070029), suggesting that exercise promotes hypothalamic health and its function. Regarding the KEGG pathway, the “cell adhesion molecules signaling pathway” plays a crucial role in the hippocampal neuronal survival, differentiation, axonal growth, and synaptic development [22, 23].

Recently, the significance of epigenetic regulation in various biological functions and disease pathogenesis...
has increased. As an epigenetic marker, the reversible m6A is the most prevalent post-transcriptional regulation of mammalian gene expression. m6A is abundant in the nervous system, and the cellular dynamics of m6A are associated with neural function, neurogenesis, and neuronal survival [24–26]. The dysregulation of m6A is related to many biological processes, including neurodevelopment and neurodegenerative diseases. Reportedly, the upregulation of m6A occurs with brain maturation [27], behavioral experience [28], and memory formation [29]. In this study, a high level of m6A was observed in the hippocampus and hypothalamus of mice in the exercise group (Fig. 5a). Since the dynamic equilibrium of m6A is governed by m6A-related components, such as methyltransferases, demethylases, and reading proteins, the expression of 13 m6A RNA methylation regulator genes, including METTL3, METTL14, WTAP, RBM15, ZC3H13, FTO, ALKBH5, YTHDF1, YTHDF3, YTHDC2, YTHDF2, YTHDC1, and HNRNPC were analyzed in the hippocampus and hypothalamus of mice in the exercise group. The result showed that only Fto was down-regulated in the hippocampus and hypothalamus of mice in the exercise group. The result showed that only Fto was down-regulated in the hippocampus and hypothalamus of mice in the exercise group (Fig. 5a). Since the dynamic equilibrium of m6A is governed by m6A-related components, such as methyltransferases, demethylases, and reading proteins, the expression of 13 m6A RNA methylation regulator genes, including METTL3, METTL14, WTAP, RBM15, ZC3H13, FTO, ALKBH5, YTHDF1, YTHDF3, YTHDC2, YTHDF2, YTHDC1, and HNRNPC were analyzed in the hippocampus and hypothalamus of mice in the exercise group. The result showed that only Fto was down-regulated in the hippocampus and hypothalamus of mice in the exercise group. The result showed that only Fto was down-regulated in the hippocampus and hypothalamus of mice in the exercise group. The result showed that only Fto was down-regulated in the hippocampus and hypothalamus of mice in the exercise group. The result showed that only Fto was down-regulated in the hippocampus and hypothalamus of mice in the exercise group. The result showed that only Fto was down-regulated in the hippocampus and hypothalamus of mice in the exercise group.
in exercise group (Fig. 5b and c). In addition, western blot experiment was performed, confirming the finding (Fig. 5d).

FTO as an m6A demethylase is a crucial component of m6A modification [30, 31]. Several studies suggested that FTO knockdown with siRNA increased the amount of m(6)A in mRNA, and FTO overexpression decreased the amount of m(6)A in human cells [12]. The above evidence proves that FTO expression may contribute to m6A levels. Hence, presumably, elevated levels of m6A in the hippocampus and hypothalamus after exercise are due to the downregulation of FTO. Although polymorphisms within the intron 1 of the FTO gene were first reported to be associated with obesity [10, 32, 33], the physiological role of the FTO gene remains unclear. FTO is widely found in central and peripheral tissues of mammals [34]. In peripheral tissues, FTO is related to energy metabolism [35, 36] and cancer progression [37–39]. In central tissues, FTO is highly expressed in the brain and essential for development of the central nervous system in humans [40, 41]. Numerous preclinical evidence reported that altered FTO expression is partially responsible for energy balance, epilepsy, neurodevelopment, and neurodegenerative diseases. In animal studies, FTO can activate the phosphorylation of Tau, which is one of the markers of Alzheimer’s disease (AD) [42]. In human studies, the genetic variation in the introns of the FTO gene possibly contributes to the risk of AD [43, 44]. However, specific mechanism of the FTO gene variants that contribute to the risks of AD is still unclear and requires further research. Moreover, the FTO inhibitor can regulate the neuronal excitability with anticonvulsant activity [45], and is responsible for glioblastoma progression [46]. Axonal FTO is reportedly involved in neuronal development by regulating the m6A modification of axonal mRNA [47]. Decreasing FTO in the dorsal hippocampus aids in memory formation [29]. However, the loss of FTO leads to impairment of neuronal differentiation and a processing defect of brain-derived neurotrophic factor (BDNF) within the hippocampus, which increasing anxiety and impairing the working memory [48]. In addition, the complete or neural-specific Fto gene deletion results in
### Table 2  Fto co-expression genes in the mouse brain

| Gene symbol | Description | Entrez ID |
|-------------|-------------|-----------|
| Mark4       | MAP/microtubule affinity-regulating kinase 4 | 232944 |
| H2-D1       | Histocompatibility 2, D region locus 1 | 14964 |
| Zfp385a     | Zinc finger protein 385A | 29813 |
| Pex19       | Peroxisomal biogenesis factor 19 | 19298 |
| Arhgdia     | Rho GDP dissociation inhibitor (GDI) alpha | 192662 |
| Vamp2       | Vesicle-associated membrane protein 2 | 22318 |
| Ncor2       | Nuclear receptor co-repressor 2 | 20602 |
| Rab11b      | RAB11B, member RAS oncogene family | 19326 |
| Rad23b      | RAD23 homolog B, nucleotide excision repair protein | 19359 |
| Ubqln4      | Ubiquitin 4 | 94232 |
| Stxbp1      | Syntaxin binding protein 1 | 20910 |
| Cux1        | Cut-like homeobox 1 | 13047 |
| Tyro3       | TYRO3 protein tyrosine kinase 3 | 22174 |
| Hdgf        | Heparin binding growth factor | 15191 |
| Rab2          | Ras homolog family member T2 | 214952 |
| Atp6v0a1    | ATPase, H+ transporting, lysosomal V0 subunit A1 | 11075 |
| Gabbr1      | Gamma-aminobutyric acid (GABA) B receptor, 1 | 54393 |
| Grina       | Glutamate receptor, ionotropic, N-methyl D-aspartate-associated protein 1 | 66168 |
| Elmo2       | Engulfment and cell motility 2 | 140579 |
| Tpcc1       | Two pore channel 1 | 252972 |
| Syn1        | Synapsin 1 | 20964 |
| Mlf2        | Myeloid leukemia factor 2 | 30853 |
| Coro2b      | Coronin, actin binding protein, 2B | 235431 |
| Pacs2       | Phosphofurin acidic cluster sorting protein 2 | 217893 |
| Tubb4a      | Tubulin, beta 4A class IVA | 22153 |
| Pde4a       | Phosphodiesterase 4A, cAMP specific | 18577 |
| Acin1       | Apoptotic chromatin condensation inducer 1 | 56215 |
| Ttc7b       | Tetracontapeptide repeat domain 7B | 104718 |
| Sdc3        | Syndecan 3 | 20970 |
| Synv1       | Synovial apoptosis inhibitor 1, synoviolin | 74126 |
| Klc2        | Kinesin light chain 2 | 16594 |
| Ap2a1       | Adaptor-related protein complex 2, alpha 1 subunit | 11771 |
| Pigs        | Phosphatidylinositol glycan anchor biosynthesis, class S | 276846 |
| Cnot3       | CCR4-NOT transcription complex; subunit 3 | 232791 |
| Tex261      | Testis expressed gene 261 | 21766 |
| Tspan7      | Tetraspanin 7 | 21912 |
| Hk1         | Hexokinase 1 | 15275 |
| Kitc2       | Kinesin family member C2 | 16581 |
| Zfp362      | Zinc finger protein 362 | 230761 |
| Ppm1g       | Protein phosphatase 1G (formerly 2C), magnesium-dependent, gamma isof orm | 14208 |
| Sfas2       | Splicing factor 3a, subunit 2 | 20222 |
| Sec24c      | Sec24 related gene family, member C (S. cerevisiae) | 218811 |
| Cabp1       | Calcium binding protein 1 | 29867 |
| Ulk1        | Unc-51 like kinase 1 | 22241 |
| Spryd3      | SPRY domain containing 3 | 223918 |
| Prkaca      | Protein kinase, cAMP dependent, catalytic, alpha | 18747 |
| Tgoln1      | Trans-Golgi network protein | 22134 |
| Tpcc1       | Two pore channel 1 | 252972 |
| Add1        | Adducin 1 (alpha) | 11518 |
postnatal growth retardation of mice [34]. The m^6^A RNA demethylase FTO alleviates the deficits in dopaminergic neurotransmission in response to arsenite exposure [49]. Further research found that mice with low expression of FTO remain sensitive to the anorexigenic effects of leptin [51]. All these studies strongly suggest that FTO plays vital roles in the physiological and pathological functions of the brain.

Although most studies have focused on the impact of FTO overexpression or knockdown in the brain, the genes that related to FTO are still important as they perform many subsequent molecular functions and biological processes. It has been reported that FTO as a transcriptional coactivator promotes gene transcription, ultimately affecting adipose tissue development [36]. However, the mechanism of FTO interaction with downstream genes to further regulate nerve function remain largely unknown.

FTO could be regulated not only by nutrition but also by exercise. Previous studies found that physical activity might weaken the effect of the FTO variant on BMI [52–56]. In addition, gender also influences the FTO genotype on exercise for weight loss. It is observed that males carrying the FTO risk allele lose more weight after a 12-week regular exercise [57]. An acute decreased skeletal muscle FTO mRNA expression was observed after high-intensity exercise by Danaher et al. [14]. Most researchers focus on the reducing obesity risk caused by FTO gene polymorphisms under exercise, while there are few on its function. In the present study, we indicated that exercise attenuates FTO expression. FTO, a demethylase, plays an important role in energy metabolism. The abnormal FTO expression modifies the level of m^6^A of target genes and is involved in many physiological and pathological processes. Overall, the study reported for the first time that long-term exercise can down-regulated the FTO expression in the hippocampus and hypothalamus, indicating that FTO may be a promising key player between exercise and the brain.

However, it is unclear whether exercise-induced FTO downregulation can regulate downstream target genes and the biological processes. Hence, the Fto/co-expression genes were downloaded from the database for GO enrichment and KEGG signal pathway analyses. Based on the results of bioinformation analyses, the significant enrichment pathway primarily correlated with vasopressin-regulated signaling pathway, water reabsorption signaling pathway, synaptic vesicle cycle signaling pathway, endocrine signaling pathway, calcium reabsorption signaling pathway, protein processing in endoplasmic reticulum signaling pathway, salivary secretion signaling pathway, cAMP signaling, insulin secretion signaling pathway, and morphine addiction signaling pathway (Fig. 6c). The result suggests that FTO and its co-expression genes are involved in many important biological processes in the brain. In addition, the known and unknown proteins co-expressed with FTO may be regulated by FTO-m^6^A to alter their expression and function. Thus, exercise may regulate the expression and function of the related genes via FTO-dependent demethylation of mRNA m^6^A.

We found that the Vamp2 gene expression in mouse brain is involved in two KEGG signaling pathways, including insulin secretion and synaptic vesicle cycle. Vesicle-associated membrane protein 2 (VAMP2) has been implicated in the insulin-regulated trafficking of GLUT4 in insulin-sensitive cells. VAMP2 inhibited insulin-stimulated GLUT4 translocation and decreased insulin sensitivity [58, 59]. Insulin-sensitive tissue or cells include liver, skeletal muscle, adipocytes, and hypothalamus. In addition, VAMP2 may have important roles in synaptic trafficking in the hippocampus [60]. The epileptogenesis is dramatically attenuated in hippocampus of Vamp^+/− mice [61]. Hence, presumably, long-term exercise may regulate the expression and function of VAMP2 in hypothalamus and hippocampus via FTO-dependent demethylation of mRNA m^6^A, but further research is needed to confirm that VAMP2 is a target of FTO. Hence, FTO could be a valuable therapeutic target for brain diseases in the future.

**Conclusion**

The gene changes after exercise training were confirmed using RNA-sequencing analysis. Long-term exercise training showed increased level of m^6^A and

| Gene symbol | Description | Entrez ID |
|-------------|-------------|-----------|
| Atg13       | Autophagy related 13 | 51897     |
| Atp1b2      | ATPase, Na+/K+ transporting, beta 2 polypeptide | 11932     |
| Mapk8ip3    | Mitogen-activated protein kinase B interacting protein 3 | 30957     |
| Wbp2        | WW domain binding protein 2 | 22378     |
| Rnf208      | Ring finger protein 208 | 68846     |
down-regulated FTO expressions in the hippocampus and hypothalamus. Lifestyle intervention such as exercise might be an effective intervention for epigenetic modification. In addition, reviewed of studies on the role and co-expression genes of Fto in mice brain revealed that the relationship between FTO and downstream genes is not completely reported, requiring additional research to elucidate their roles in the brain in response to exercise. Nevertheless, further research is warranted to understanding the signaling pathways of FTO involved in and their impacts on brain health.

Abbreviations
m6A: N6-methyladenosine; FTO: Fat mass and obesity-associated gene; VO2: Oxygen consumption; VCO2: Carbon dioxide production; RER: Respiratory exchange ratio; GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; mRNA: Messenger RNA; METTL3: Methyltransferase-like 3; METTL14: Methyltransferase-like 14; Wtap: Wilms’ tumor 1 associated protein; RBM15: RNA binding motif protein 15; ZC3H13: Zinc finger CCCH domain-containing protein 13; ALKBH5: Alkylation repair homolog protein 5; YTHDF1: YTH domain-containing family protein 1; YTHDF3: YTH domain-containing family protein 3; YTHDC2: YTH domain-containing protein 1; YTHDF2: YTH domain-containing family protein 2; YTHDC1: YTH domain-containing protein 1; HNRPWC: Heterogeneous nuclear ribonucleoprotein C; AD: Alzheimer’s disease; BDNF: Brain-derived neurotrophic factor.

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Author contributions
LML designed the research; SJL, CLF, LL, YL, ZYG, GZ, YPL, and SZL performed the research; THC, CLF, WQY, YY, and FZ analyzed data; LML, SJL, and THC wrote the main manuscript text and prepared Figs. 1, 2, 3, 4, 5, 6. All authors read and approved the final manuscript.

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Availability of data and materials
The RNA-sequencing datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The data used to bioinformatic analysis of Fto gene co-expression network can be accessed at coexpedia database: https://www.coexpedia.org/mm_single.php?gene=FTO.

Declarations
Ethics approval and consent to participate
This study is reported in accordance with the ARRIVE guidelines (https://arriveguidelines.org). All animal experiments in this study were in strict accordance with relevant guidelines and regulations in the Guide for the Care and Use of Laboratory Animals and approval by the Ethics Committee of Guangzhou Sport University, and the permit number of ethics approval was 2021IDWLL-05.

Consent for publication
Not applicable.

Competing interests
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Author details
1 Center for Scientific Research and Institute of Exercise and Health, Guangzhou Sport University, Guangzhou, China. 2 The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou 510260, China.

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