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Article

Resveratrol supplementation attenuates cognitive and molecular alterations under maternal high-fat diet intake: multigenerational epigenetic inheritance.

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Abstract: Environmental factors as maternal high-fat diet (HFD) intake can increase the risk of age-related cognitive decline in adult offspring. The epigenetic mechanisms are a possible link between diet effect and neurodegeneration across generations. Here, we found a significant decrease in triglyceride levels in a high-fat diet with resveratrol HFD+RV group and the offspring. Firstly, we obtained better cognitive performance in HFD+RV groups and their offspring. Molecularly, a significant increase in 5-mC levels, as well as increased gene expression of *Dnmt1* and *Dnmt3a* in HFD+RV F1 group, were found. Furthermore, a significantly increased of m^{6}A levels in HFD+RV F1 were found, and there were changes in gene expression of its enzymes (*Mettl3* and *Fto*). Moreover, we found a decrease in gene expression levels of pro-inflammatory markers such as *Il1-β, Il-6, Tnf-α, Cxcl-10, Mcp-1* and *Tgf-β*1 in HFD+RV and HFD+RV F1 groups. Moreover, there was increased gene expression of neurotrophins such as *Ngf* and *Nt3* and its receptors *TrkA* and *TrkB*. Likewise, an increase in protein levels of BDNF and p-Akt in HFD+RV F1 was found. These results suggest that maternal RV supplementation under HFD intake prevents cognitive decline in SAMP8 adult offspring, promoting a reduction in triglycerides and leptin plasma levels, changes in the pro-inflammatory profile, restoring the epigenetic landscape as well as synaptic plasticity.

Keywords: cognitive decline**;** epigenetics; HFD; aging; SAMP8; m^{6}A, multigenerational inheritance.

1. Introduction

Due to the advanced age in modern societies, research in aging-hallmarks that lead to neurodegeneration and Alzheimer's disease (AD), it is an important scientific field [1]. Aging has been linked to progressive brain deterioration, leading to impaired cognitive function and increasing vulnerability to death[2]. This cognitive impairment is commonly accompanied by a different molecular alteration like inflammation, synaptic dysfunction, and epigenetic modification, among others [3]. Therefore, pleiotropic compounds to rescue them is a very important field of research [4].

In this regard, nutrition and supplementation are important components of healthy brain aging, particularly in those with dementia [5]. It is well-established that the consumption of a high-fat diet (HFD) increases the risk of several chronic diseases, including age-related cognitive decline [6]. Accordingly, studies have evidenced the link between HFD and neurodegeneration through several altered pathways. Among them, we found the inflammation and synaptic plasticity [7]. In fact, inflammation and synaptic dysfunction are two of the most important events in neurodegeneration

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and AD; further inflammation can alter synaptic plasticity engendering cognitive impairment [8]9]. Microglia activation is considering the main hallmark of neuroinflammation that releases pro-inflammatory cytokines such as Interleukin 1 beta (IL1-ß), Interleukin 6 (IL-6), or Tumor necrosis factor-α (TNF-α) [10]. Likewise, the synaptic plasticity is primary mediated by neurotrophins, among them, brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT3), allowing the permissive conditions under which plasticity can appear [11,12].

On the other hand, evidence suggests that epigenetic mechanisms are an important contributor to the pathogenesis of age-related neurodegenerative diseases [13,14]. Likewise, there is a relationship between the effect of neuroinflammation, synaptic dysfunction and the epigenetic landscape [15]. Recently, it has been described that nutrients can affect epigenetic marks such as DNA methylation (5-mC), hydroxymethylation (5-hmC) as well as RNA N^6-methyladenosine methylation (m^6A), thereby promoting changes in gene expression of critical genes associated with the pathophysiology of several age-related diseases, including AD [16,17]. Thus, epigenetics has emerged as a tool for understanding a broad range of human diseases, such as type 2 diabetes mellitus, obesity, inflammation, and neurodegenerative disorders [14,18,19]. In utero, a HFD supplementation in rodents causes a metabolic syndrome-like (MeS) that can be transmitted across generations [20,21]. Moreover, several studies have demonstrated that HFD exposure promoted epigenetic modifications of leptin, persisting for multiple generations [22,23,24]. Overall, those key events demonstrate the active involvement in the maintenance of neuronal integrity, synapses and cognitive function for the individual and his offspring [25,26].

Resveratrol (RV) (3,5,4'-trihydroxy-trans-stilbene) is a natural phytoalexin produced in grapes, peanuts, and its derivatives, with a plethora of beneficial effects as an anti-inflammatory and synaptic plasticity inductor, including epigenetic modifications [27,28]. RV also exerts neuroprotection in several neuropathological conditions [29,30,31]. Previous results from our group have demonstrated that RV modulates key pathway for the correct neuronal function, improving cognitive decline in the senescence-accelerated mouse prone 8 (SAMP8) and its offspring [32]. Indeed, the pleiotropic mechanism of action of RV is not completely described, although increasing evidence in animal models bring to light the beneficial effects of polyphenols such a RV across generations [33,34].

The SAMP8 mouse is a well-established aging animal model, generated by phenotypic selection from the genetic pool of AKR/J mice [35]. The SAMP8 strain manifests behavioral alterations, age-related cognitive decline, as well as neuropathological AD hallmarks [36]. Jointly with AD hallmarks, several key events associated with neurodegeneration are presented in SAMP8 mice such as neuroinflammation [35], and synaptic dysfunction [13,37]. Of note, those changes are accompanied by epigenetic alterations [17,38]. Furthermore, several studies demonstrated that non-pharmacological interventions such as environmental enrichment (EE), diet supplementation can induce changes in the epigenome, reducing cognitive decline and modifying key neurodegenerative pathways altered in SAMP8 [39,40].

Concretely, the present work aimed to prove the neuroprotective effects of maternal RV supplementation under HFD intervention in SAMP8 offspring through neuroinflammation, synaptic dysfunction as well as delving deep into the epigenetic patterns modulated by a RV supplementation. Therefore, we demonstrated that SAMP8 under HFD enriched with RV showed a preserved cognitive decline across generations, being an important insight for RV use In public health Interventions favoring healthy aging.

2. Results

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2.1. Body weight progression in SAMP8 HFD+RV and its offspring.

Body weight evolution was measured starting from 1 month of age, and then we measured for 14 weeks. All mice groups increased body weight in time. Furthermore, no differences between HFD and HFD+RV groups were found (Figure 1B). Next, we measured the triglycerides (TG) and leptin plasma levels of SAMP8 mice to determine the induction of the MeS after HFD. We found a significant decreased TG levels in SAMP8 HFD+RV directly supplemented, and its offspring, in comparison with HFD mice (Figure 1C). Remarkably, we found a significant decreased of leptin plasma levels in HFD+RV groups in comparison with the HFD group, confirming the effectiveness of RV as a preventive for some of signs characteristics of MeS across generations (Figure 1D).

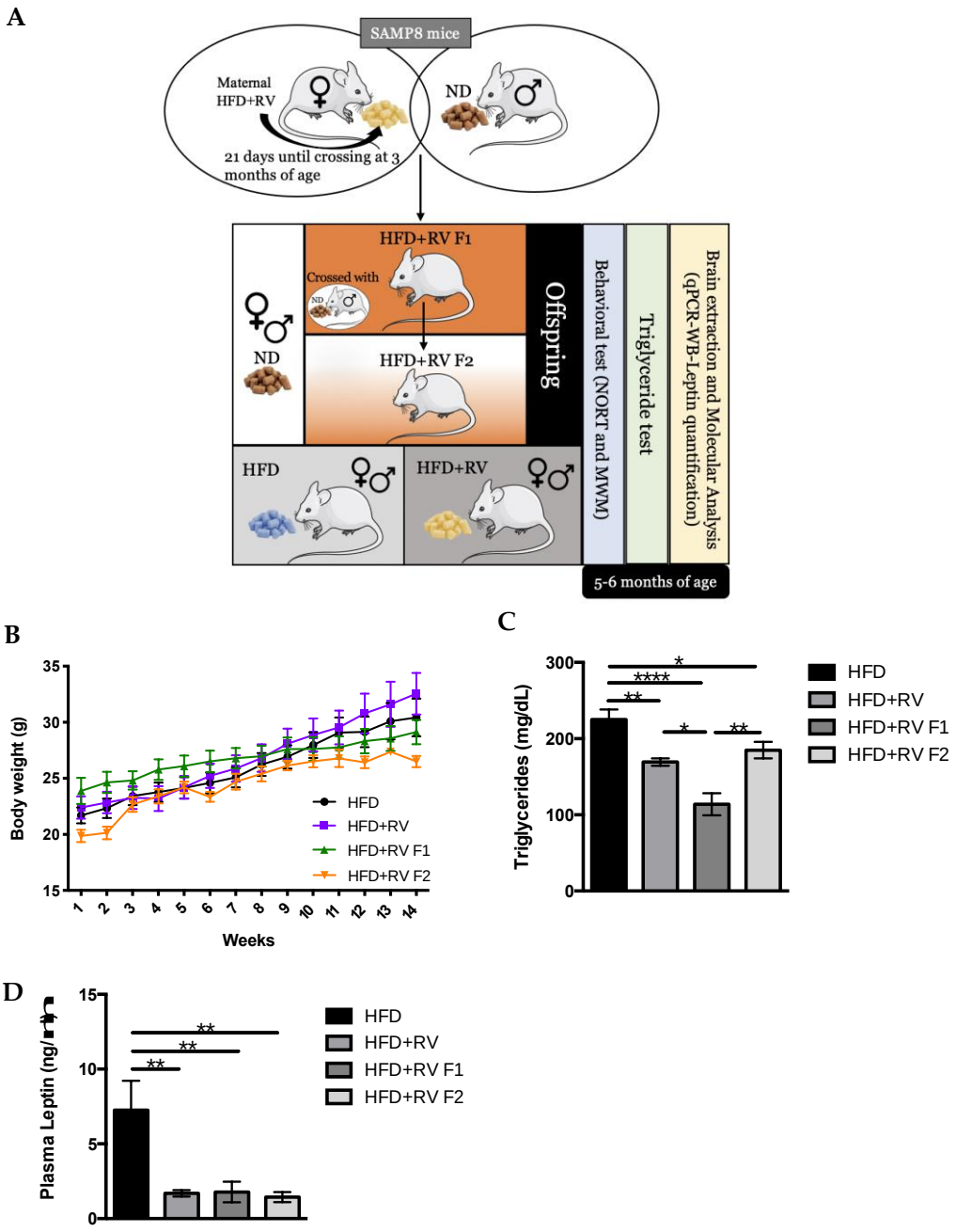

Figure 1. Overview of experimental design and procedure to obtain the offspring of HFD+RV (direct exposed effect), HFD+RV F1 (intergenerational inheritance), HFD+RV F2 (transgenerational inheritance). Mice performed the behavioral test that measured memory state, later euthanized, and brain extraction was performed for the subsequent molecular analysis at 6 months of age (A). Results

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of body weight from each week (B). Results of TG (mg/dL) in blood (C). Results of plasma leptin levels (ng/mL) (D). Values represented are mean ± Standard error of the mean (SEM); n = 60 (HFD n = 14, HFD+RV n = 15, HFD+RV F1 n = 14, HFD+RV F2 n = 15; for each group). Statistics: *p<0.05; **p<0.01; **p<0.01; ****p<0.0001.

2.2. RV prevented cognitive impairment induced by HFD in SAMP8 mice across generations.

Mice cognitive state was evaluated by the novel object recognition test (NORT) and morris water maze (MWM). The HFD group presented impaired short-term memory in comparison with HFD+RV and HFD+RV F1 groups, as well as a clear tendency was found between HFD and HFD+RV F2 group, demonstrating the efficacy of the RV dietary supplementation, including the next generations (Figure 2A). Furthermore, a clear improvement in the long-term memory in all HDF+RV groups was found, being only a significantly increased in the HDF+RV directly exposed group (Figure 2B). We also found an improvement in cognition regarding the spatial memory tested by MWM. Namely, every mouse reduced the latency to platform the test day in comparison with the first day of learning (Figure 2C). Furthermore, we observed that the HFD+RV F1 group showed a significant decrease in the latency to target compared to the other groups (Figure 2D). Likewise, the HFD+RV F1 was the unique group that had a significant decrease in distance to the target, confirming the inheritance effect of RV regarding the cognition (Figure 2E).

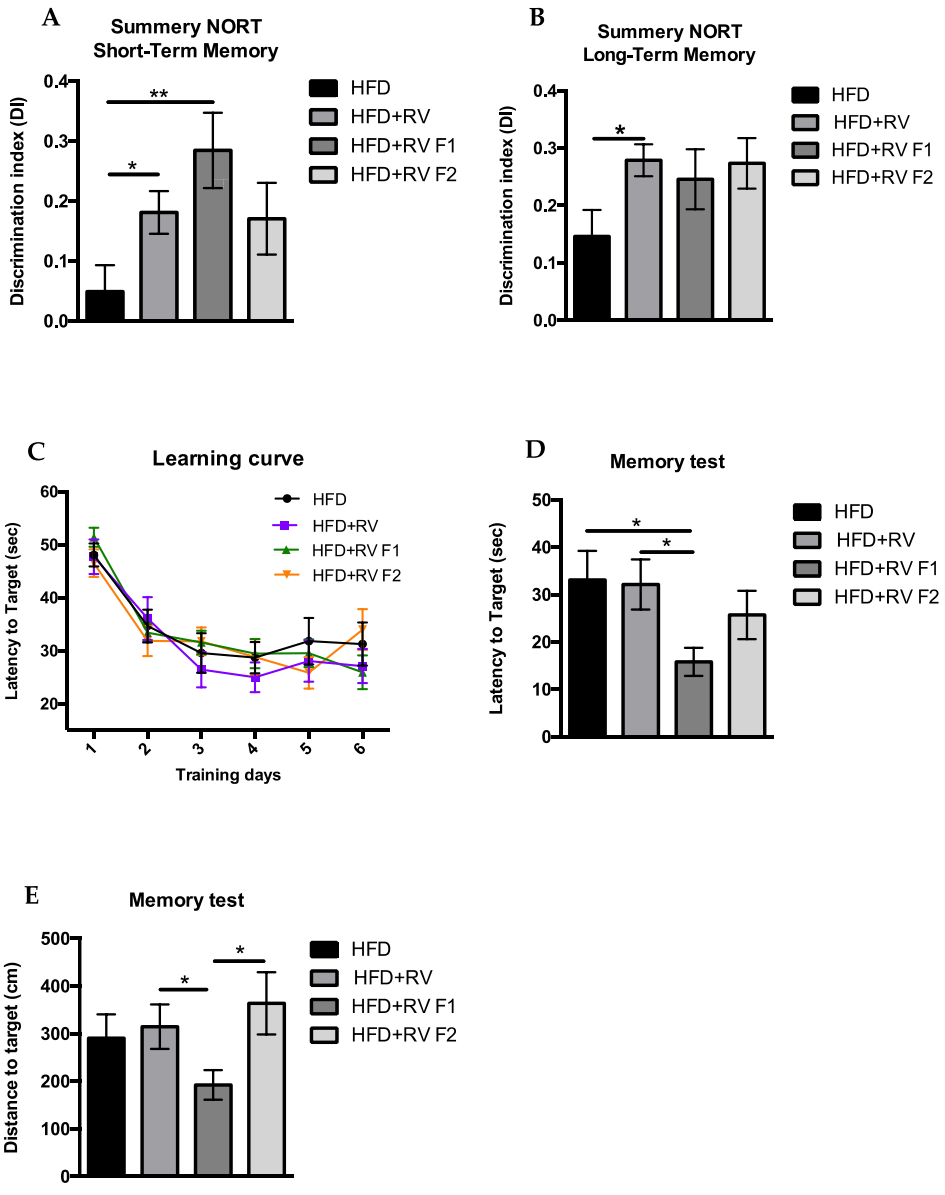

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Figure 2. Results of Discrimination index (DI) of short-term memory (A), and long-term memory (B) from NORT of SAMP8 at 6 months of age for all). Results of Morris Water Maze (MWM) in SAMP8 mice at 6 months of age for all groups. The learning curve of the memory test for 6 days (C). Latency to the target in time at day 7 (spatial memory test) (D), and distance travelled at day 7 (spatial memory test) (E). Values represented are mean ± Standard error of the mean (SEM); n = 60 (HFD n = 14, HFD+RV n = 17, HFD+RV F1 n = 14, HFD+RV F2 n = 15; for each group, same n of females and males). Statistics: *p<0.05; **p<0.01; ***p<0.001.

2.3. Adult offspring of SAMP8 mice shown global changes in methylation patterns and its machinery after maternal HFD+RV diet.

We studied the 5-mC and m^{6}A levels in the hippocampus of SAMP8 mice at 6 months of age after the intervention. In one hand, we only found that 5-mC levels were significantly increased in HFD+RV F1 mice in comparison with HFD group (Figure 3A). Likewise, the HFD+RV and HFD+RV F2 groups showed a tendency to maintain this increase (Figure 3A). In the same way, we studied the gene expression of several methyltransferases, which modulate this epigenetic mark (Figure 3B). Results showed a significantly increased gene expression of *DNA methyltransferase 1 (Dnmt1)* and *DNA methyltransferase 3 alpha (Dnmt3a)* in the HFD+RV F1 group in comparison with the other groups, confirming the epigenetic inheritance in the next generation directly exposed to RV and losing the pattern maintenance in the F2 generation the indirectly exposed (Figure 3B). Then, the m^{6}A levels were measured. We found that the HFD+RV F1 group showed significantly increased levels in comparison to the other groups (Figure 3C). In parallel, there were a significantly reduced gene expression of the enzymes *Methyltransferase like 3 (Mettl3)* and *FTO alpha-ketoglutarate dependent dioxygenase (Fto)* in HFD+RV and HFD+RV F1 compared to the HFD group (Figure 3D), as well as increased m^{6}A levels in the HFD+RV F2 group in comparison with the HFD+RV F1. By last, we only found a significantly increased gene expression of *Fto* in the HFD+RV F2 group (Figure 3D).

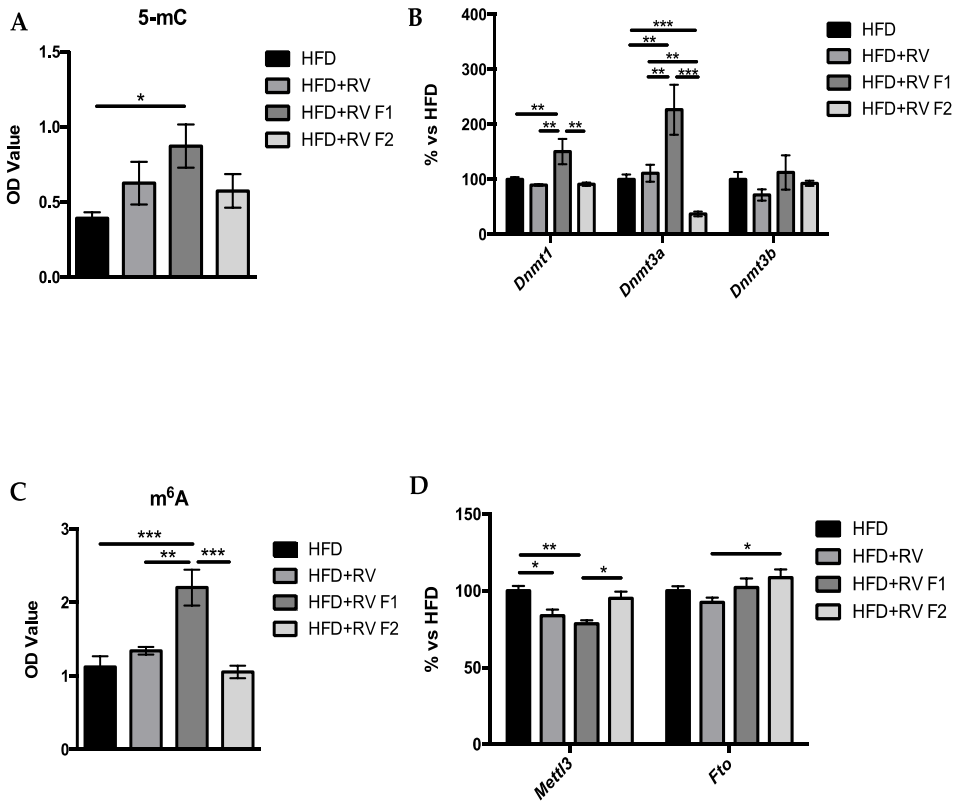

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Figure 3. 5-mC levels in the hippocampus of SAMP8 mice at 6 months of age for all groups (A). Relative gene expression of *Dnmt1*, *Dnmt3a*, and *Dnmt3b* (B). m^6A levels from the hippocampus of SAMP8 mice at 6 months of age for all groups (C). Relative gene expression for *Mettl3* and *Fto* (D). Gene expression levels were measured by real-time PCR from hippocampal tissue. Mean ± Standard error of the mean (SEM) in bar graphs are adjusted to 100% for each gene of HFD; n = 16-24 (HFD n = 4-6, HFD+RV n = 4-6, HFD+RV F1 n = 4-6, HFD+RV F2 n = 4-6; for each group, females n= 3-4, males n = 3-4). Statistics: *p<0.05; **p<0.01; ***p<0.001.

2.4. RV diet modified the inflammatory markers in the hippocampus of SAMP8 and its offspring under HFD.

One of the main effects of RV is its anti-inflammatory effect. For this reason, we studied the gene expression of some pro-inflammatory markers in the hippocampus of SAMP8 mice. Firstly, we found a significantly decreased gene expression for *Il1-ß, Il-6, C-X-C motif chemokine ligand 10 (Cxcl-10), the pro-inflammatory factors monocyte chemoattractant protein 1 (Mcp-1), Transforming growth factor beta 1 (Tgf-ß1)* and a tendency to decrease for *Tnf-α* in the HFD+RV compared to the HFD group (Figure 4A-F). Moreover, the HFD+RV F1 and HFD+RV F2 groups showed mainly the same gene expression pattern for every pro-inflammatory marker, being only statistically different for *Cxcl-10* in the HFD+RV F2 group, suggesting the multigenerational effects of RV (Figure 4C).

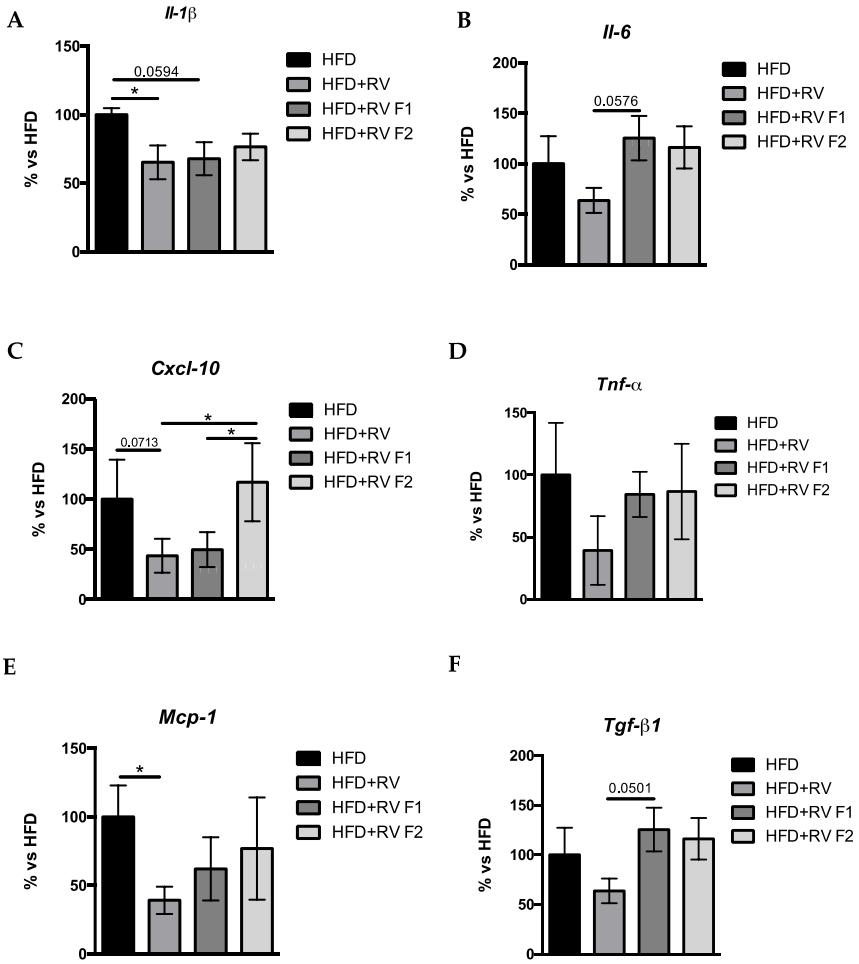

Figure 4. Results of gene expression of inflammatory markers for *Il1-ß* (A), *Il-6* (B), *Cxcl-10* (C), *Tnf-α* (D), *Mcp-1* (E), and *Tgf-ß1* (F) in the hippocampus of SAMP8 mice at 6 months of age. Gene expression levels were measured by real-time PCR from hippocampal mRNA. Values are the Mean ± Standard error of the mean (SEM) in bar graphs are adjusted to 100% for each gene of HFD; n = 16-

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24 (HFD n = 4-6, HFD+RV n = 4-6, HFD+RV F1 n = 4-6, HFD+RV F2 n = 4-6; for each group, females n= 3-4, males n = 3-4). Statistics: *p<0.05.

2.5. RV diet rescued synaptic dysfunction, reverting the effects of HFD in the hippocampus of SAMP8 mice and its offspring.

To evaluate the synaptic dysfunction, we investigated several neurotrophins that modulate this process. Our findings were that the HFD+RV group showed a significant statistically increase in neurotrophins such as *Nerve growth factor (Ngf)* and *Nt3* gene expression in comparison with the other groups (Figure 5A, 5B). Likewise, we evaluated the gene expression levels of its receptors, and we found that the *Neurotrophic receptor tyrosine kinase 2 (TrkB)* had a significant increase in the HFD+RV F1 group in comparison with the other groups (Figure 5C). However, we obtained a significant decreased in gene expression of *Neurotrophic receptor tyrosine kinase 1 (TrkA)* (Figure 5C) despite the *Ngf* expression levels had been increased. Next, the WB analysis revealed a significant increase in BDNF protein levels in the HFD+RV F1, but no changes were found in the HFD+RV (directly exposed) and HFD+RV F2 (indirectly exposed) groups compared to the HFD group (Figure 5D). Moreover, the ratio of phosphorylated protein kinase B (p-Akt) and total protein levels (p-Akt/Akt total) was measured, and we found a significant increase in HFD+RV group in comparison with HFD as well as a tendency to maintain the same pattern protein levels of p-Akt in the offspring, suggesting the multigenerational inheritance of RV (Figure 5E).

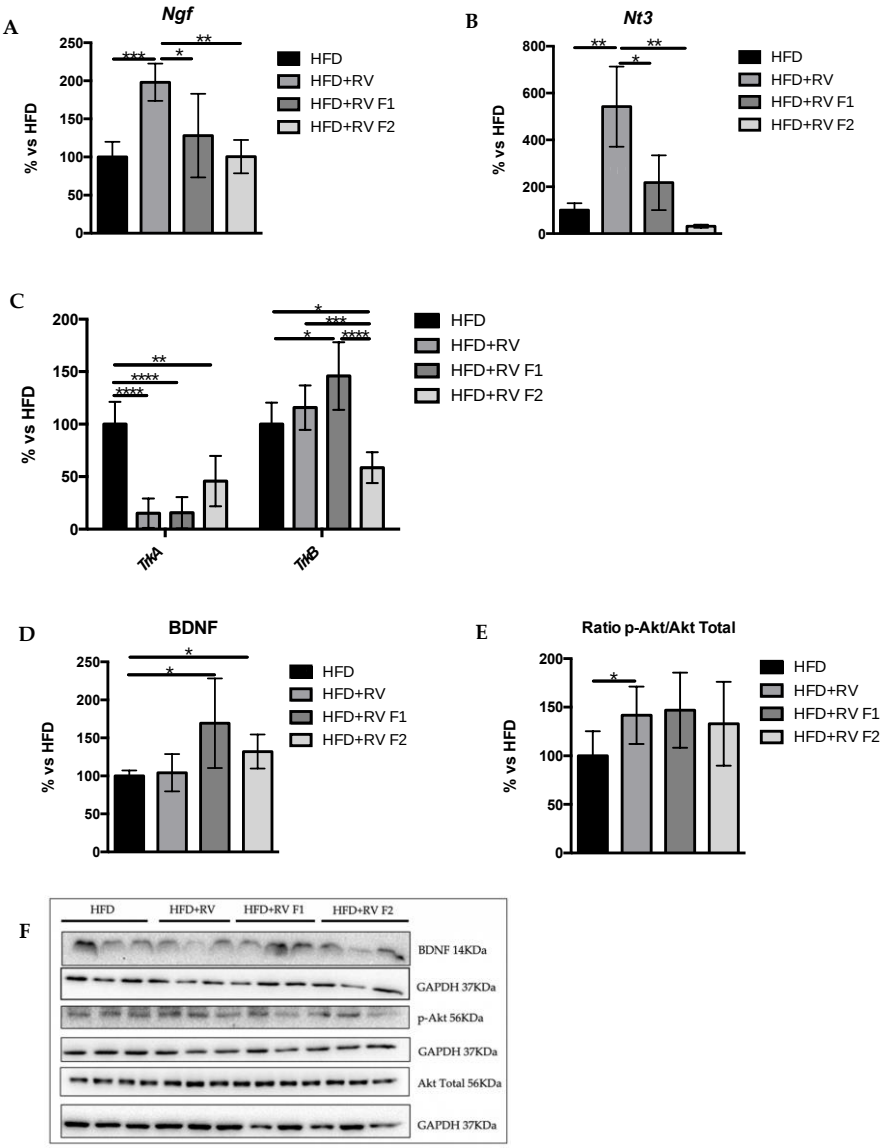

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Figure 5. Synaptic plasticity markers in the hippocampus of SAMP8 mice at 6 months of age. Results of gene expression of *Ngf* (A), *Nt3* (B) and its receptors *TrkA* and *TrkB* (C). Representative results by WB of BDNF (D) and p-Akt (E) and quantifications (F). For WB values are adjusted to 100% for each protein level of HFD group. Percentage of gene expression was determined by real-time PCR. Mean ± Standard error of the mean (SEM) in bar graphs are adjusted to 100% for each gene of HFD group; n = 16-24 (HFD n = 4-6, HFD+RV n = 4-6, HFD+RV F1 n = 4-6, HFD+RV F2 n = 4-6; for each group, females n= 3-4, males n = 3-4). Statistics: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

4. Discussion

The global increase in life expectancy can be associated with cognitive impairment and AD through changes in the lifestyle [41]. Therefore, it is clear a connection or contribution of nutrition as the main factor that can trigger the cognitive decline [42]. Indeed, animal and human studies have highlighted the link between alterations in life environment and increased risk of cognitive decline in later life, including effects on the offspring [43,44]. A growing body of evidence demonstrates that HFD promotes MeS associated with impaired glucose tolerance, hypertriglyceridemia, increased leptin levels, among others, might be relevant in the progression of age-related cognitive decline and AD [45,46]. However, although some knowledge exists the mechanisms by which environmental insults can have long-term effects on offspring are relatively unclear. To date, no definitive mechanisms have been demonstrated that could explain multigenerational inheritance after diet or supplementation exposure, but there have been reports of epigenetic modification following HFD intervention within the next generation, including RV interventions. Thus, in the present study, we showed that maternal RV supplementation not only recovers some of the key events presented in neurodegeneration such as epigenetic modifications, neuroinflammation, synaptic dysfunction as well as cognitive impairment, but we also demonstrated the maintenance on the offspring. Our previous data demonstrated that RV intake supplementation promoted changes in the cognitive state and molecular pathways in the hippocampus of SAMP8 transmitted to the offspring [32].

By one hand, as mentioned, HFD is widely related to the onset of MeS in animal models. Importantly, SAMP8 mice have a particular response to HFD. For example, weight gain or glucose tolerance that was not impaired after HFD, probably because SAMP8 demonstrates increased resistance to insulin in normal diet conditions [47]. On the other hand, it is well described that maternal HFD sensitizes offspring to metabolic dysregulation and that RV dietary supplementation improved most of the altered metabolic dysregulation [48]. Here, we reported that body weight is unchanged among groups, suggesting the same effect of the HFD across generations, and confirming the previous studies in which we did not observe changes in body weight and caloric intake in SAMP8 fed with HFD or HFD+RV [49,50]. Interestingly, we found that RV alleviates some MeS traits induced by HFD by reducing triglycerides and leptin plasma levels in all HFD+RV groups compared to the HFD group, suggesting that RV can promote peripheral effects regarding the hypertriglyceridemia and leptin resistance induced by HFD . In the same line, a recent report described that RV diet after weaning could alleviate leptin resistance induced by HFD [51].

Considering the well-demonstrated beneficial effects of RV regarding the cognition [32], we examined the cognition by NORT and MWM. Results revealed that maternal RV supplementation displayed better cognitive performance under HFD, showing higher DI in short- and long-term memories for all SAMP8 groups. The novelty was identified for F1 and F2 mice after 24 h of the first trial, indicating a beneficial effect of maternal RV in offspring, albeit not direct RV contact with individuals. In spatial memory analysis, some discrepancies were found among HFD+RV groups, although a better spatial memory was determined in the F1 generation, these improvements disappear in the F2 generation.

As mentioned, RV has pleiotropic actions in the organism modifying several molecular pathways starting with its antioxidant action or anti-inflammatory, furthermore to activation of NAD-dependent protein deacetylase sirtuin-1 (SIRT1), then RV can affect epigenetic marks [52–56].

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Regarding the intervention with HFD, RV protective role against HFD has been described [57,58] as well as its neuroprotective effects can be transmitted across generations by epigenetic modifications in parents [59,60]. In this regard, in addition to improvements in cognition, changes in epigenetic patterns induced by RV have been found, including on the offspring. 5-mC has been demonstrated as essential for learning and memory, cognitive function [28], age-related alterations, as well as synaptic plasticity [61]. Remarkably, we found a higher degree of 5-mC levels in all HFD+RV groups, being statistically significant in the F1 generation. Accordingly, we showed changes in the methylation enzymatic machinery (*Dnmt1* and *Dnmt3a*) gene expression profile in HFD+RV F1 group, suggesting the requirement of these two enzymes to promote changes in 5-mC pattern modifications. Similarly, several reports have been shown the modification of 5-mC patterns in the offspring after maternal supplementation of other compounds, such as folate [62], methionine [63], choline [64] or restrictive diet [65]. Those reports and our results pointed out the 5-mC as a potential epigenetic target for cognitive decline in ageing and AD onset. Next, we evaluated the m^6A an epigenetic mark, the most abundant modification in eukaryotic RNA. m^6A RNA methylation and changes in gene expression are determined in development and related to AD onset [66,67]. Nevertheless, few studies focused on the association between m^6A and cognitive impairment and, until now, no *in vivo* study has demonstrated the participation of the m^6A in cognitive decline in aging and AD. In this regard abnormalities in m^6A levels, including the *Mettl3* and *Fto* gene expression, was reported in the cortex and hippocampus of double transgenic mice of amyloid precursor protein and presenilin 1 (APP/PS1) mice, a model of AD, in comparison with the healthy control [66]. Thus, our results provide the first evidence in which the consumption of RV induced changes in the hippocampus of SAMP8, a model of accelerated senescence and early onset AD, in the offspring for this epigenetic marker. Strikingly, the highest levels of m^6A appear in the HFD+RV F1 group, but this epigenetic mark was lost in F2. These results can be explained in part by underlying germline effects promoted by RV, which were not found in the F2 generation (indirectly RV exposed group). In parallel, the expression of *Mettl3* in the hippocampus of HFD+RV in the F1 generation was lower than the HFD group and HFD+RV F2, suggesting the intergenerational inheritance, but not transgenerational effect. In the case of the *Fto* expression, we only found changes between the HFD+RV and HFD+RV F2 groups, confirming the loss of the inheritance effect promoted by RV in the hippocampus of SAMP8 for this epigenetic mark.

Neuroinflammation has been linked with the risk of developing cognitive impairment [68,69]. At the same time, it is well established the anti-inflammatory effect of RV by different mechanisms such as SIRT1 activation, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kß) down-regulation, including the modulation of microglial activation, thereby promoting a reduction of pro-inflammatory cytokines already mentioned [70,71]. In the current work, a diminished gene expression of several pro-inflammatory cytokines in HFD+RV groups was found, demonstrating the transmission across generations of the anti-inflammatory effect. However, the anti-inflammatory pattern in the HFD+RV F2 group was bizarre (inconsistent), suggesting the low transmission across generations (transgenerational epigenetic inheritance), losing beneficial RV effect when undirect action was evaluated. Indeed, previous findings which demonstrate the anti-inflammatory effects induced by maternal RV supplementation showed the same discrepancies regarding its transgenerational effect [32]. This fact can be explained, in part, because of the inflammatory process is activated by different molecular pathways like oxidative stress (OS) [72–74].

Given the synaptic disruption is the higher correlate event of cognitive loss, alterations in synaptic plasticity process are considered one of the most critical mechanisms of age-related cognitive decline [75] and AD [76]. Thus, it is well known the role of neurotrophins in the impaired memory formation and neuronal degeneration [77,78]. For instance, long term potentiation (LTP) is necessary for memory formation and is maintained by BDNF and NT3 at TrkB receptor [77], resulting in structural changes at the synapse [78]. Likewise, NGF and its neurotrophin receptor TrkA promote the same effect in the synaptic contact between neurons [79]. In this study, we found robust changes for some of neurotrophins and receptors in the HFD+RV groups. In line with our results, some studies

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have been described that RV induces the expression of glial cell-derived neurotrophic factor (GDNF), BDNF, and NT3 under different interventions in the brain, confirming our data [80–82]. Moreover, little has been published on transgenerational changes in neurotrophins after maternal RV supplementation. Here, we found subtle changes in HFD+RV F1 group and the HFD+RV F2 groups, demonstrating the reduced capacity of RV to modify epigenetic marks that are maintained in the indirectly exposed generation, and as a consequence facilitate changes in expression of neurotrophic pathways across generations, that are related to molecular neuroprotective effects observed in F1 and F2. In addition, the increased p-Akt ratio in HFD+RV groups in comparison with HFD group was found, confirming the neuroprotective effect of RV by promoting synaptic plasticity.

Taken together, the new data presented above demonstrated that maternal RV supplementation mediated several modifications under HFD through reduction of hypertriglyceridemia and leptin resistance, epigenetic alterations, neuroinflammation and synaptic dysfunction for maintaining better cognitive performance in SAMP8 and its offspring (Figure 6). Therefore, this study shows new important insights of the beneficial effects and mechanisms promoted by RV after maternal supplementation on inheritance, reinforcing the importance of life styles interventions in preventing cognitive decline and neurodegenerative diseases through strategies based on a healthy diet or food intake supplementation.

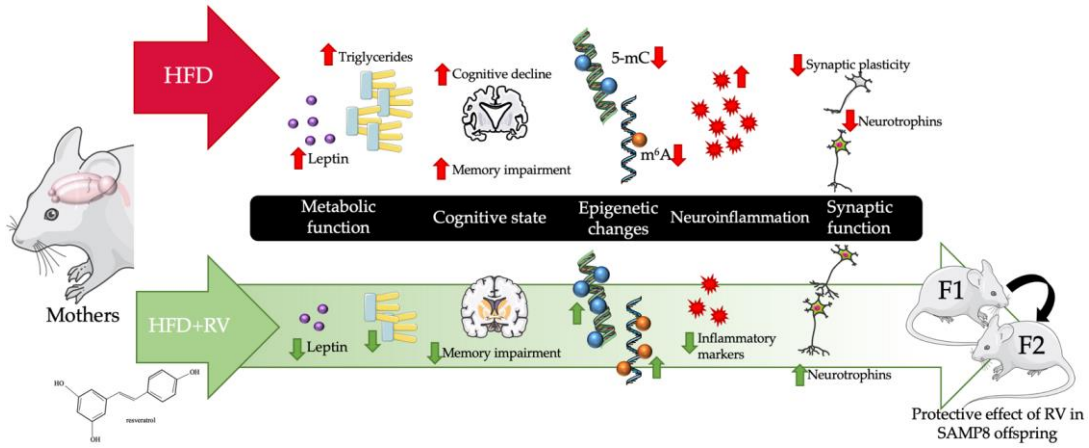

Figure 6. Schematic representation of the neuroprotective effect of maternal RV intake under HFD across generations.

4. Materials and Methods

4.1. Animals

SAMP8 offspring were generated from the high-fat diet (HFD) group (Females F0 (HFD F0, n=4) that had access to diet (AIN-93G) modified to provide 60% of Kcal from fat with carbohydrate/protein/fat ratio of 16:23:61 % of Kcal). Specifically, HFD+RV contains 1g/kg w/w of RV (Females HFD+RV F0, n=4). Mice were fed for 2 months and the supplementation diet was interrupted before crossing HFD+RV F0 females with CT males, which had access to standard chow with carbohydrate/protein/fat ratio of 64:19:17 % of Kcal to obtain the first generation (the intergenerational inheritance offspring (HFD+RV F1, n= 14; females n=4, males n=10) for accelerating the transgenerational effect one generation early (in F2 instead F3). Then, we crossed HFD+RV F1 females (Females HFD+RV F1, n=4) with CT males to generate the second generation (the transgenerational inheritance offspring (HFD+RV F2, n=15; females n=10, males n=5). HFD+RV F1 and HFD+RV F2 groups were fed standard diet (Figure 1A). Animals had free access to food, water and were kept under standard temperature conditions (22±2ºC) as well as 12h:12h light-dark cycles (300 lux/ 0 lux) until 6 months of age.

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Mice were treated according to European Community Council Directive 86/609/EEC and were approved by the Institutional Animal Care and Use Committee of the University of Barcelona (670/14/8102) and by Generalitat de Catalunya, Spain (10291). Every effort was made to minimize animal suffering and to reduce the number of animals.

4.2. Behavioural test

4.2.1. Novel object recognition test (NORT)

In summary, mice were placed in a 90º, two-arms, 25 cm-long, 20 cm-high, 5 cm-wide black mazes. Firstly, mice were individually habituated to the maze for 10 min for 3 days. The fourth day, the animals were exposed to a 10 min acquisition trial (first trial), during which they placed in the maze in the presence of two identical novel objects at the end of each arm. After 2h, the animal was exposed to two objects, in this time, one old object and one novel object. On the fifth day, the animal was exposed at 24h in the same conditions. The time that mice explored the Novel object (TN) and Time that mice explored the Old object (TO) were measured generating a Discrimination Index (DI) that we calculated as (TN – TO)/(TN + TO) in both performances evaluating short- and long-term memory of animals. To avoid object preference biases objects were counterbalanced. The maze, surface, and objects were cleaned with 70% ethanol between the animal's trials to eliminate olfactory cues.

4.2.2. Morris water maze (MWM)

To evaluate the spatial learning and memory of mice, we exposed the animals to an open circular pool (100 cm in diameter, 50 cm in height) filled with water. Water was painted white with latex in order to opaque it, and its temperature was 22±1 ºC. Two perpendicular axes were defined; thus, the water surface was divided into four quadrants (NE, SE, SW and NW) and five starting points were set (NE, E, SE, S and SW). For visual clues (N, S, E and W) were placed on the walls of the tank so that animal could orientate and fulfil the objective. MWM consisted of training mice to find a submerged platform (Learning phase), for 6 consecutive days, and assess whether the animal had learned and remembered where the platform was the last day (day 7) when it was removed (Spatial memory test). For 6 learning days, 5 trials were performed in 5 different points that we explained before. In each trial, the mouse was placed gently into the water, facing the wall of the pool, allowed to swim for 60 seconds, and there was not a resting time between trials. If the animal was not able to locate the platform, the investigator guided it to the platform and was allowed to rest and orientate for 30 seconds. The platform was placed approximately in the middle of one of the quadrants, 1.5 cm below the water level. Above the pool, there was a camera that recorded the animal's swimming paths with SMART® program ver.3.0 (Panlab). During the learning phase, a learning curve was drawn, in which is represented the latency to find the platform every training day. On the day test, more parameters were measured, such as the target crossings, the swum distance to target or time in the platform zone.

4.3. Immunodetection experiments

4.3.1. Brain processing

Mice were euthanized one day after behavioral test finished by cervical dislocation. Brains were immediately removed, and the hippocampus was isolated and frozen on powdered dry ice. They were kept at -80ºC until use.

4.3.2. Plasma isolated and leptin quantification

Briefly, blood was collected when the mice were euthanized using heparinized tubes (Micro tube 1,1 mL Z-Gel; Sarstedt AG & Co. KG). Plasma was isolated by centrifugation for 5 min at 10.000xg at

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20ºC and frozen at -20ºC until assay. We quantified Leptin levels in plasma samples with The Kit Mouse Leptin ELISA Kit (Cat. # EZML-82-K; Millipore). This kit to detect leptin in samples with specific antibodies that bind to leptin and to react in the presence of sufficient substrate. The enzyme activity was measured spectrophotometrically by colorimetric reaction at the absorbance of 450nm and corrected by 590nm. Therefore, the increase in absorbency is directly proportional to the amount of captured leptin in samples. Later, we quantified using a standard curve of known concentrations of mouse leptin (0,23-30 ng/mL).

4.3.3. Western Blotting (WB)

The hippocampus tissue from each animal was homogenized in lysis buffer (Tris HCl pH 7.4 mM, NaCl 150mM, EDTA 5mM and 1X-Triton X-100) containing phosphatase and protease inhibitors (Cocktail II, Sigma-Aldrich) to obtain total protein homogenates. The Bradford technique determined total protein concentration. For WB aliquots of 15 mg of total hippocampal protein was used and separated by sodium dodecyl sulfate-polyAcrylamide Gel Electrolysis (SDS-PAGE) (8-20%) and transferred into polyvinylidene difluoride (PVDF) membranes (Millipore) for 2h. The membranes were blocked in 5% non-fat milk in TRIS-buffered saline (TBS) containing 0.1% Tween 20 (TBS-T) for 1h at room temperature. And then, overnight incubation at 4ºC with primary antibodies listed in Table S1. on the next day, membranes were washed with TBS-T 3 times for 5 min and incubated with secondary antibodies for 1h at room temperature. Immunoreactive protein was viewed with a Chemiluminiscence-based detection kit, following the manufacturer's protocol (ECL Kit; Millipore), and digital images were acquired using a ChemiDoc XRS+ System (BioRad, California, USA). Semi-quantitative analyses were done using ImageLab Software (BioRad, California, USA), and results were expressed in Arbitrary Units (AU) considering control protein levels as 100%. Protein loading was routinely monitored by immunodetection of Glyceraldehyde-3-phosphate dehydrogenase (GADPH).

4.4. RNA extraction and Gene Expression Determination by q-PCR

Total RNA isolation was carried out using TRIsure™ reagent following the manufacturer's instructions (Bioline Reagent, London, UK). The yield, purity, and quality of RNA were determined spectrophotometrically with a NanoDrop™ ND-1000 (Thermo Scientific) apparatus and an Agilent 2100B Bioanalyzer (Agilent Technologies). RNAs with 260/280 ratios and RIN higher than 1.9 and 7.5, respectively, were selected. Reverse transcription-polymerase chain reaction (RT-PCR) was performed as follows: 2 μg of messenger RNA (mRNA) was reverse-transcribed using the High capacity cDNA reverse transcription Kit (Applied Biosystems). Real-time quantitative PCR (qPCR) was used to quantify mRNA expression of chromatin-modifying, inflammatory and synaptic plasticity genes listed in Table S2.

For SYBR® green real-time PCR was performed in a Step One Plus Detection System (Applied-Biosystems) employing SYBR® Green PCR Master Mix (Applied-Biosystems). Each reaction mixture contained 6.75 μL of complementary DNA (cDNA) (which concentration was 2 μg/μL), 0.75 μL of each primer (which concentration was 100 nM), and 6.75 μL of SYBR® Green PCR Master Mix (2X).

Data were analyzed utilizing the comparative Cycle threshold (Ct) method (ΔΔCt), where the housekeeping gene level was used to normalize differences in sample loading and preparation. Normalization of expression levels was performed with β-actin for SYBR® Green-based real-time PCR results. Each sample was analyzed in duplicate, and the results represent the n-fold difference of the transcript levels among different groups.

4.5. Global DNA methylation determination

Briefly, isolation of genomic DNA was conducted using the FitAmpTM Blood and Cultured Cell DNA Extraction Kit, according to the manufacturer's instructions. Then, Methylflash Methylated

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DNA Quantification Kit (Epigentek, Farmingdale, NY, USA) was used to detect methylated DNA. Specifically, this kit is based on the antibody detection of 5-mC residues that allows colorimetric quantification by reading absorbance at 450 nm using a Microplate Photometer. The absolute amount of methylated DNA (proportional to the Optical Density [OD] intensity) was measured and quantified using a standard curve plotting OD values vs. five serial dilutions of a control methylated DNA (0.5 – 10 ng).

4.6. m^{6}A RNA Methylation Quantification

To determine m^{6}A RNA methylation status, we used the EpiQuik™ m^{6}A Quantification Kit according to the manufacturer's instructions (Epigentek, Farmingdale, NY, USA) using total RNA isolated from mice. The kit is based on specific antibody detection of m^{6}A residues, which trigger an ELISA-like reaction that allows colorimetric quantification by reading absorbance at 450 nm using a Microplate Photometer. The absolute amount of m^{6}A (proportional to the Optical Density [OD] intensity) was measured and quantified using a standard curve plotting OD values vs. six serial dilutions of a control m^{6}A (0.01 – 0.5 ng).

4.7. Data analysis

The statistical analysis was conducted using GraphPad Prism ver. 8 statistical software. Data are expressed as the mean ± Standard Error of the Mean (SEM) of at least 7 samples per group for behavioral tests and 3-5 samples per group for molecular analysis. Means were compared by One-way Analysis of variance (ANOVA), followed by Tukey post-hoc analysis or two-tail Student's t-test when it was necessary. Statistical significance was considered when p-values were <0.05. The Statistical outliers were determined with Grubbs' test and subsequently removed from the analysis.

Supplementary Materials: Supplementary materials can be found at www.mdpi.com/xxx/s1. **Table S1.** Antibodies used in Western blot studies. **Table S2.** Primers used in qPCR.

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Abbreviations

AD	Alzheimer's disease
Akt	Protein kinase B
ANOVA	One-way analysis of variance
APP/PS1 mice	Double transgenic mice of amyloid precursor protein and presenilin 1
BDNF	Bran-derived neurotrophic factor
cDNA	Complementary DNA
CNS	Central nervous system
Ct	Cycle threshold
Cxcl-10	C-X-C motif chemokine ligand 10
DI	Discrimination index
DNA	Desoxyribonucleic acid
5-hmC	Hydroxymethylation
5-mC	DNA methylation
Dnmt1	DNA methyltransferase 1
Dnmt3a	DNA methyltransferase 3 alpha
Dnmt3b	DNA methyltransferase 3 beta

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Fto	Alpha-ketoglutarate dependent dioxygenase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GDNF	Glial cell-derived neurotrophic factor
HFD	High fat diet
HFD+RV	High fat diet with resveratrol
HFD+RV F1	High fat diet with resveratrol first generation
HFD+RV F2	High fat diet with resveratrol second generation
Il-6	Interleukin 6
Il-1β	Interleukin 1 beta
LTP	Long term potentiation
m^6A	N-6-methyladenosine
Mcp-1	Monocyte chemoattractant protein 1
MeS	Metabolic syndrome
Mettl3	Methyltransferase like 3
mRNA	Mature messenger RNA
MWM	Morris water maze
ND	Normal diet
NF-κβ	Nuclear factor NF-kappa-B
NGF	Nerve growth factor
NORT	Novel object recognition test
NT3	Neurotrophin-3
OD	Optical density
OS	Oxidative stress
p-Akt	Phosphorylated RAC-alpha serine/threonine-protein kinase
PCR	Polymerase chain reaction
PVDF	Polyvinylidene difluoride
qPCR	Real-time quantitative polymerase chain reaction
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptional-polymerase chain reaction
RV	Resveratrol
SAMP8	Senescence-accelerated mouse prone 8
SAMR1	Senescence-accelerated mouse resistant 1
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrolysis
SEM	Standard error of the mean
SIRT1	NAD-dependent protein deacetylase sirtuin-1
TBS	Tris-buffer saline
TBS-T	Tris-buffer saline tween 20
TG	Triglycerides
TGF-β1	Transforming growth factor beta 1
TN	Time with new object
TNF-α	Tumor necrosis factor-alpha
TO	Time with old object
TrkA	Neurotrophic receptor tyrosine kinase 1
TrkB	Neurotrophic receptor tyrosine kinase 2
WB	Western blot

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