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Melatonin Suppresses Astrocyte Reactivity and Mitigates Cognitive Deficits Induced by a High-Carbohydrate, High-Fat Diet in Rats

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Abstract

Diets high in carbohydrates and fat (HCHF) have been increasingly associated with cognitive impairment, which may be partly related to astrocyte activation and subsequent neuroinflammatory and oxidative responses. In this study, we investigated whether melatonin supplementation could improve HCHF diet-induced memory impairment and astrocyte reactivity in a rat model of diet-induced metabolic syndrome. Adult male Wistar rats were fed an HCHF diet for eight weeks to induce obesity and metabolic dysfunction, followed by daily oral treatment with melatonin (5, 10, or 30 mg/kg) for an additional four weeks while continuing the HCHF diet. Working memory was evaluated using the Y-maze spontaneous alternation test. Hippocampal and hypothalamic tissues were also examined for proinflammatory cytokines, oxidative stress markers, and glial fibrillary acidic protein (GFAP) expression as an indicator of astrogliosis.

HCHF feeding caused weight gain, dyslipidemia, glucose intolerance, impaired working memory, increased neuroinflammation and oxidative damage, and marked astrogliosis in both brain regions. Melatonin did not improve glucose tolerance or lipid profiles at any of the doses tested. However, working memory in melatonin-treated HCHF rats was comparable to that of the control group at all doses. Melatonin also reduced GFAP-positive astrocyte numbers, proinflammatory cytokine levels, and lipid peroxidation in a dose-dependent manner, while increasing superoxide dismutase activity. Interestingly, the improvement in working memory did not show a clear dose-dependent pattern. These findings suggest that the protective effect of melatonin against HCHF diet-induced brain injury may occur largely independently of its effects on systemic metabolic abnormalities and may instead involve reduced astrocyte activation and suppression of the associated inflammatory and oxidative processes.

Highlights

- Melatonin reversed HCHF diet-induced working memory impairment in rats at all doses tested
- Melatonin dose-dependently reduced hippocampal astrocyte reactivity (GFAP) and cytokines
- Cognitive and antigliotic effects occurred without correcting glucose or lipid metabolism

1. Introduction

Over the past decade, astrocyte biology has moved to the center of neurodegenerative disease research, with particular attention now paid to the earliest, potentially reversible stages of glial involvement. A large body of work now implicates non-neuronal glial cells, astrocytes chief among them, in both the pathogenesis and possible treatment of conditions such as amyotrophic lateral sclerosis, frontotemporal dementia, Alzheimer's disease, Parkinson's disease, epilepsy, and multiple sclerosis (1–3). Beyond their classical housekeeping roles in synaptic regulation, neurovascular coupling, and ionic homeostasis, astrocytes participate directly in immune signaling, cytokine release, and neurotrophic support, positioning them as active players in disease rather than passive bystanders (4).

Astrocytes respond readily to metabolic and proteotoxic disturbances, reacting to a wide range of pathological triggers, including energy imbalance, ionic disturbance, and molecular damage (5). Acute injury or inflammation elicits a rapid, transient astrocytic response marked by upregulation of reactive markers and release of inflammatory mediators aimed at containing damage and promoting repair (5). In chronic disease states, by contrast, astrocytes undergo sustained remodeling of gene expression, morphology, and function, changes that can be either protective or deleterious depending on context and disease stage (6).

A growing literature connects dietary pattern to this astrocytic response. Diets rich in refined carbohydrates and saturated fat — often termed 'Western' diets — are associated with obesity, insulin resistance, and dyslipidemia, and there is accumulating evidence that these same diets can drive astrocyte activation through inflammatory, oxidative, and glucometabolic pathways (7, 8). Preclinical work has shown that chronic exposure to a high-carbohydrate, high-fat (HCHF) diet produces reactive astrogliosis in the frontal cortex, hippocampus, and hypothalamus of rats, accompanied by neuronal injury and impaired recognition memory (9).

The association between diet-induced metabolic changes and astrocyte activation has increasingly been linked to impaired brain insulin signaling and changes in synaptic structure. In a rat model exposed to a short-term high-fat, high-fructose diet, Calvo-Ochoa et al. reported that only seven days of feeding was sufficient to induce hippocampal insulin resistance, reduced dendritic arborization, decreased synaptic protein levels, and reactive astrocyte changes in the CA1 region (10). These findings are consistent with the astrogliosis observed in the present study, despite the shorter duration of dietary exposure. Similarly, chronic consumption of a high-fat diet in mice has been reported to reduce astrocytic process arborization and decrease the expression of glial glutamate transporters in the hippocampus, together with systemic insulin resistance and dyslipidemia (11). These changes suggest that impaired glutamate clearance may be one of the mechanisms linking metabolic dysfunction to astrocyte alterations and subsequent synaptic and cognitive impairment. Therefore, astrocytes may act as an active link between systemic insulin resistance and hippocampal dysfunction rather than simply responding to peripheral metabolic changes. This also supports the potential value of directly targeting astrocyte reactivity rather than focusing only on the correction of systemic metabolic abnormalities.

Melatonin, the pineal neurohormone best known for its role in circadian regulation and sleep, has attracted growing interest as a candidate neuroprotective agent. Its antioxidant and anti-inflammatory properties, together with its capacity to promote neurotrophic signaling, have been linked to benefit across several models of neurodegeneration (12–14). Recent work has extended these observations to astrocyte-specific outcomes: melatonin has been shown to limit reactive astrogliosis and glial scar formation after cerebral ischemia-reperfusion injury (15), to attenuate astrocyte and microglial activation in demyelinating models (16), and to modulate astrocytic inflammatory signaling through pathways such as SIRT1/NF-κB following ischemic stroke (17).

Evidence from other models of brain injury suggests that the cognitive effects of melatonin may occur even without correcting the underlying metabolic or physiological abnormalities. In a rat model of chronic paradoxical sleep deprivation, which is associated with both cognitive impairment and metabolic disturbances, melatonin treatment improved memory performance and was accompanied by reduced serum cortisol, inflammatory cytokines, and tissue injury (18). A dose-dependent effect has also been reported for the cognitive and antigliotic effects of melatonin. In a streptozotocin-induced rat model of sporadic Alzheimer's disease, melatonin reduced the number of GFAP-positive astrocytes in the CA1 region to levels comparable with controls, whereas the extent of cognitive improvement differed according to the dose and the memory task evaluated (19). These findings suggest that the dose-response pattern of melatonin may vary across different cognitive functions, even when its effects on astrogliosis are more consistent. However, whether a similar difference between dose-dependent suppression of astrocyte activation and relatively dose-independent preservation of memory occurs in diet-induced metabolic syndrome has not been previously investigated.

Despite this expanding evidence base in ischemic and demyelinating contexts, melatonin's effect on astrocyte activation specifically within the setting of diet-induced metabolic syndrome remains largely unexplored. The present study was therefore designed to test whether melatonin treatment can modulate HCHF diet-induced astrogliosis, neuroinflammation, oxidative stress, and cognitive impairment in the rat brain.

2. Materials and Methods

2.1. Animals

Male Wistar rats, ten weeks of age and weighing 180–200 g at study onset, were obtained from the Animal House of Kerman University of Medical Sciences, Iran, and housed in polypropylene cages under a 12:12-hour light-dark cycle, 22–24°C, and 35–45% relative humidity, with free access to food and water throughout the study. All procedures were approved by the Ethics Committee of Kerman University of Medical Sciences (permit code IR.KMU.AH.REC.1400.143).

2.2. Diets and Treatment Protocol

Following one week of acclimatization, rats were randomly assigned to a normal-diet group (n = 24) or an HCHF-diet group (n = 48). The normal diet consisted of standard pelleted rodent chow (Behparvar Co., Iran). The HCHF diet was prepared according to Wong et al. (20) and contained 20% ghee, 39.5% sweetened condensed milk, 17.5% fructose, 15.5% powdered standard chow, 2.5% Hubbel-Mendel-Wakeman salt mixture, and 5% water. Drinking water was supplemented with 25% fructose. Previous studies using this feeding regimen have reported obesity, hypertension, glucose intolerance, dyslipidemia, hepatic steatosis, and increased inflammatory and oxidative stress markers in the hypothalamus, hippocampus, and frontal cortex after 8 weeks or longer of exposure (9, 21).

After 8 weeks, HCHF-fed rats were divided into four groups (n = 12 each) and received daily intragastric melatonin at 5, 10, or 30 mg/kg, or saline vehicle, for an additional 4 weeks while continuing the HCHF diet. Normal-diet rats were similarly divided into a saline-treated control group (n = 12) and a group receiving melatonin at 30 mg/kg (Control+Mel30, n = 12) for 4 weeks. Melatonin was administered one hour before lights-off to coincide with peak melatonin receptor sensitivity (22). Melatonin was purchased from Sigma (CAS 73-31-4, USA).

2.3. Metabolic Assessments

Body weight and food intake were recorded twice weekly. Insulin sensitivity was assessed at week 11 via intraperitoneal glucose tolerance testing (2 g/kg glucose) in six rats per group following a 12-hour fast, with tail-vein glucose sampled at baseline and 30, 60, 90, and 120 minutes post-injection (Accu-Chek Active glucometer); the area under the glucose curve was calculated for the full 120-minute window.

2.4. Y-Maze Working Memory Test

At week 12, spontaneous alternation behavior was assessed in a three-arm Y-maze (50 × 10 × 20 cm per arm, 120° angles) over an 8-minute session using the ANY-maze tracking system (v5.2). Percentage alternation was calculated as (number of alternations) / (total arm entries − 2) × 100.

2.5. Serum Biochemistry, Tissue Collection, and Sample Preparation

After a 12-hour fast, rats were anesthetized with intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg). Cardiac blood was collected and centrifuged (2500 g, 15 min, 4°C); serum total cholesterol and LDL were measured by enzymatic colorimetric assay (Riton, Iran). For histology, six rats per group were transcardially perfused with cold PBS followed by 4% paraformaldehyde and brains postfixed for 48 hours at 4°C. The remaining six rats per group were perfused with cold saline only; hippocampi and hypothalami were dissected on ice per the Spijker guideline (20), homogenized in cold PBS, centrifuged (2500 g, 4°C, 20 min), and supernatants stored at − 80°C.

2.6. Oxidative Stress and Cytokine Assays

Lipid peroxidation was quantified as malondialdehyde (MDA) via the thiobarbituric acid reactive substances assay, read at 532 nm and expressed as nmol/mg protein. Superoxide dismutase (SOD) activity was measured using a WST-1-based inhibition assay (Navand Salamat, Iran) read at 405 nm and expressed as U/mg protein. TNF-α and IL-1β concentrations in hippocampal and hypothalamic homogenates were measured by sandwich ELISA (Karmania-pars-gene, Iran; Eastbiopharm, China, respectively).

2.7. Immunohistochemistry for GFAP

GFAP immunoreactivity, an established marker of astroglial reactivity, was assessed in 5 μm paraffin sections of hippocampus and hypothalamus following standard deparaffinization, citrate-buffer antigen retrieval, and blocking with 10% goat serum. Sections were incubated overnight at 4°C with rabbit anti-

GFAP primary antibody (Boster PA1239, 1:400), followed by HRP-conjugated goat anti-rabbit secondary antibody (Abcam ab7090, 1:300) and DAB visualization. GFAP-positive cell counts were determined across two-to-four microscopic fields per region using a light microscope (Euromex CMEX-10) with a mounted digital camera (9).

2.8. Statistical Analysis

Data are presented as mean ± SD and were analyzed in GraphPad Prism 8.4.3. Normality was assessed with the Shapiro-Wilk test; group comparisons used one-way ANOVA with Tukey's post hoc test. $P < 0.05$ was considered significant.

3. Results

3.1. Energy Intake and Body Weight

HCHF-fed rats consumed significantly more energy than controls throughout the four-week melatonin treatment period (weeks 8–12; $p < 0.001$), Table 1. Melatonin at all doses reduced energy intake relative to the untreated HCHF group, without a clear dose-response relationship, though intake remained above control levels even with treatment ($p < 0.001$). Body weight was already significantly elevated in HCHF rats by week 8, prior to melatonin treatment. By week 12, melatonin at 10 and 30 mg/kg had significantly attenuated further weight gain relative to the untreated HCHF group ($p < 0.05$ and $p < 0.01$ vs. HCHF, respectively), although body weight in these groups remained significantly above control values ($p < 0.05$ and $p < 0.01$ vs. control, respectively). Melatonin at the highest dose (30 mg/kg) had no significant effect on energy intake or body weight in normal-diet rats (Control + Mel30 vs. Control, Table 1)

Table 1
Effects of melatonin treatment on total energy intake, body weight, and plasma lipids

Control	Energy intake (KJ/day/rat)	Body weight, week 8 (g)	Body weight, week 12 (g)	TC (mg/dL)	LDL (mg/dL)
	333.16 ± 12.00	**326.80 ± 7.30**	**335.5 ± 4.23**	**53.5 ± 7.1**	**12.5 ± 1.7**
C+Mel30	353.16 ± 12.33	327.03 ± 7.72	336.7 ± 12.11	60.0 ± 7.2	15.6 ± 1.9
HCHF	741.00 ± 31.89***	371.03 ± 11.15***	402.7 ± 10.93***	93.8 ± 12.9***	29.1 ± 2.9***
HCHF+Mel5	596.66 ± 60.55***, ###	367.22 ± 9.57***	406.2 ± 9.76***	81.5 ± 5.0***	24.1 ± 2.1***
HCHF+Mel10	650.83 ± 45.21***, ###	369.80 ± 10.61***	376.8 ± 9.23***, #	85.1 ± 11.9***	28.6 ± 3.5***
HCHF+Mel30	641.50 ± 24.31***, ###	368.71 ± 8.86***	362.2 ± 21.48**, ##	97.6 ± 19.0***	33.0 ± 5.8***

*TC, total cholesterol; LDL, low-density lipoprotein; C+Mel30, control-diet rats treated with 30 mg/kg melatonin; HCHF, high-carbohydrate high-fat diet-fed rats treated with saline; HCHF+Mel5/Mel10/Mel30, HCHF-fed rats treated with 5, 10, or 30 mg/kg melatonin, respectively. Values are mean ± SD. *, **, *** denote $p < 0.05$, $p < 0.01$, and $p < 0.001$ vs. control; #, ##, ### denote $p < 0.05$, $p < 0.01$, and $p < 0.001$ vs. HCHF.*

3.2. Lipid Profile

HCHF feeding significantly raised serum total cholesterol and LDL relative to controls. None of the three melatonin doses reduced these parameters in HCHF rats, and melatonin had no effect on lipid values in normal-diet rats.

3.3. Glucose Tolerance

HCHF rats displayed elevated blood glucose at all sampled time points during the glucose tolerance test relative to controls, with a correspondingly greater area under the glucose curve (AUC; Fig. 2A, B). Melatonin, at any dose, did not reduce blood glucose at any time point or lower the AUC relative to the untreated HCHF group.

3.4. Working Memory

HCHF feeding significantly reduced spontaneous alternation in the Y-maze relative to control rats ($p < 0.001$), consistent with impaired working memory (Fig. 2C). Alternation performance in rats treated with melatonin, at all three doses, no longer differed significantly from control values, with no differences among doses.

3.5. Oxidative Stress Markers

SOD activity was significantly reduced in both hippocampus and hypothalamus of HCHF rats relative to controls ($p < 0.001$ both regions). Melatonin increased SOD activity in a dose-dependent manner, with the

30 mg/kg dose normalizing hippocampal SOD to control levels. HCHF feeding also significantly raised hippocampal and hypothalamic MDA. In the hippocampus, melatonin at 10 and 30 mg/kg, but not at 5 mg/kg, normalized MDA to control levels. In the hypothalamus, all three doses normalized MDA. (Fig. 3(

3.6. Proinflammatory Cytokines

TNF-α and IL-1β were significantly elevated in both regions of HCHF rats relative to controls ($p < 0.001$). Melatonin, at all doses, significantly reduced TNF-α in both regions relative to the untreated HCHF group ($p < 0.001$); however, TNF-α was not fully normalized to control levels at any dose. The dose-response was more pronounced in the hypothalamus, where the 30 mg/kg dose produced a significantly greater reduction than the 5 mg/kg dose (Fig. 4).

3.7. Astrocyte Reactivity (GFAP Expression)

Representative photomicrographs of GFAP immunoreactivity are shown in Fig. 3. HCHF feeding significantly increased the number of GFAP-positive astrocytes across all examined hippocampal subfields (CA1, CA3, dentate gyrus) and in the hypothalamic arcuate nucleus, relative to controls ($p < 0.001$ all comparisons). Melatonin reduced GFAP-positive cell counts at all doses in CA1 and CA3 relative to HCHF, with the 10 and 30 mg/kg doses producing a significantly greater reduction than 5 mg/kg in CA3 (Fig. 5), and with full normalization to control levels achieved only at 10 and 30 mg/kg. In the dentate gyrus, 5 mg/kg was insufficient to reduce GFAP-positive cell counts, whereas 10 and 30 mg/kg reduced and normalized counts. In the hypothalamus, melatonin significantly reduced GFAP-positive cell counts at all three doses relative to HCHF, with no significant difference among the doses themselves — in contrast to the dose-dependent pattern observed in CA1, CA3, and the dentate gyrus.

4. Discussion

Our findings show that four weeks of melatonin treatment reversed HCHF diet-induced working memory impairment and astrocyte reactivity, despite leaving the underlying metabolic phenotype largely unchanged. This dissociation is the central finding of the present study and points to a brain-specific, rather than metabolic, route of action. A substantial literature now links metabolic syndrome, and HCHF diets specifically, to elevated risk of neurodegenerative disease, with chronic inflammation, oxidative stress, and insulin resistance proposed as shared mechanistic threads. Astrocyte activation has more recently emerged as a candidate mediator linking metabolic dysfunction to neuronal injury;

Fuentes and colleagues reported a comparable phenotype after twelve weeks of HCHF feeding, including memory impairment, oxidative stress, and astrocyte activation across the hippocampus, hypothalamus, and cortex (9). Our findings, obtained after a shorter eight-week feeding period, indicate that this phenotype is already established well before the twelve-week timepoint used in that study, and further show that it remains reversible at the level of astrocyte reactivity and cognition even without discontinuing the diet.

Our findings replicate the expected metabolic phenotype of HCHF feeding — weight gain, dyslipidemia, and glucose intolerance — consistent with prior characterizations of this dietary model. Four weeks of melatonin treatment produced a modest, calorie-intake-mediated reduction in weight gain but did not improve lipid profile or glucose tolerance at any dose. This null metabolic effect sits within a genuinely mixed literature: some rodent studies report that melatonin lowers blood lipids and glucose in models of diet-induced obesity, while others — including studies in both animals and humans — report no such effect. Differences in diet composition, treatment duration, dosing schedule, and whether treatment begins before or after metabolic disease is established likely all contribute to this heterogeneity, and most positive reports come from studies initiating melatonin prior to, rather than after, the establishment of obesity — a design that limits their relevance to an already-obese phenotype such as ours.

Despite this lack of metabolic benefit, working memory in melatonin-treated HCHF rats no longer differed from control levels at any dose tested. HCHF and comparable high-fat diets are consistently associated with hippocampal-dependent memory deficits in rodent models, an effect attributed to several convergent mechanisms: disrupted brain insulin signaling and neuronal plasticity, chronic low-grade neuroinflammation, oxidative damage to neurons and synapses, and, increasingly, diet-driven alterations in gut microbiota that affect the gut-brain axis. Our data speak most directly to the second and third of these mechanisms: HCHF feeding produced marked increases in hippocampal and hypothalamic TNF-α, IL-1β, and lipid peroxidation alongside reduced antioxidant capacity, and melatonin substantially reversed each of these changes even while leaving glucose and lipid metabolism unaffected. This dissociation suggests that melatonin's cognitive benefit in this model is mediated principally through its antioxidant and anti-inflammatory actions in the brain rather than through any systemic metabolic improvement — a pattern consistent with reports that melatonin preserves spatial memory in diabetic rats without correcting hyperglycemia, again pointing to a central, rather than peripheral, site of action.

The relationship between astrocyte reactivity, neuroinflammation, and oxidative stress in neurodegenerative disease is now understood to be substantially bidirectional and self-reinforcing: reactive astrocytes release inflammatory mediators that both damage neurons directly and promote further oxidative injury, which in turn sustains astrocyte activation.

We found that HCHF feeding markedly increased GFAP expression in both hippocampus and hypothalamus, and that melatonin attenuated this increase in a dose-dependent fashion, with the highest doses normalizing GFAP-positive cell counts in most regions examined.

To our knowledge, this is among the first reports characterizing melatonin's effect on astrogliosis specifically in the context of diet-induced metabolic syndrome, though the direction of effect is consistent with melatonin's antigliotic actions documented in other injury contexts. In models of traumatic brain injury, diabetic encephalopathy, and lipopolysaccharide-induced neuroinflammation, melatonin has similarly been shown to reduce hippocampal GFAP expression and associated glial markers. More recent work extends this pattern to cerebral ischemia-reperfusion injury, where melatonin

limits reactive astrogliosis and glial scar formation via GSK-3β- and RIP1K-dependent mechanisms, and to demyelinating disease models, where melatonin restrains both astrogliosis and microgliosis in a manner dependent on its immunomodulatory activity.

A possible mechanism linking the antioxidant and anti-inflammatory effects of melatonin to its reduction of astrogliosis is the SIRT1/Nrf2 signaling pathway. Melatonin can increase the activity of SIRT1, a NAD-dependent deacetylase, which promotes Nrf2 activation and subsequently limits NLRP3 inflammasome assembly. This process reduces caspase-1 activation and IL-1β maturation and may also suppress NF-κB-mediated expression of proinflammatory genes such as TNF-α (23). This pathway has also been demonstrated in astrocytes, where melatonin regulates inflammatory responses through Nrf2/SIRT1 signaling in rat cortical cultures without causing cytotoxicity at the tested concentrations. This mechanism may partly explain our findings, in which melatonin reduced IL-1β to control levels at most doses and in both brain regions, whereas TNF-α was only partially reduced. The increase in SOD activity is also consistent with Nrf2 activation and the subsequent induction of antioxidant enzymes. However, whether SIRT1/Nrf2 signaling is specifically activated in astrocytes under HCHF-induced metabolic stress remains unclear, as previous evidence has mainly been obtained from LPS- or MPTP-induced models. Further studies are therefore needed to confirm the involvement of this pathway in the present model (24, 25).

The difference we observed between the antigliotic effect of melatonin and its lack of metabolic effects differs from findings in a combined high-fat diet and streptozotocin rat model, where the reduction in hippocampal GFAP was mainly linked to improved insulin receptor and downstream ERK signaling together with better glycemic control (26). In our diet-induced model without streptozotocin, the reduction in astrocyte reactivity was observed without improvement in systemic glucose metabolism, suggesting that melatonin may act more directly on brain-related pathways. The dose-dependent reduction in GFAP observed at 5, 10, and 30 mg/kg also extends previous findings in streptozotocin-diabetic rats, where a single dose of 10 mg/kg reduced GFAP expression in the hippocampus, cortex, and cerebellum without assessment of a dose-response relationship (27). Our findings suggest that the antigliotic effect of melatonin increases with dose even in the absence of diabetes, with the two higher doses producing near-complete normalization of GFAP in most regions examined.

This study has several limitations. Melatonin treatment was initiated only after obesity and metabolic syndrome were already established, which may account for its lack of effect on glucose and lipid parameters and does not address whether earlier intervention would yield different metabolic outcomes. The mechanistic link between astrocyte suppression and cognitive improvement remains correlational in this design; astrocyte-specific loss-of-function or gain-of-function approaches would be needed to establish causality. Finally, the SIRT1/Nrf2–NLRP3 axis discussed above as a candidate mechanism was not directly tested in this model; receptor-dependent signaling through MT1/MT2 and its downstream targets remain to be examined directly in future work using this dietary paradigm.

5. Conclusion

Taken together, these findings show that melatonin preserves working memory under chronic HCHF feeding, and it does so without correcting glucose or lipid metabolism, pointing to a brain-centered rather than a metabolic route of action. The dose-dependent dampening of astrocyte activation, cytokine output, and oxidative damage in the hippocampus, together with a comparable though non-dose-dependent reduction in the hypothalamus, points to astrogliosis as a mechanistic target worth pursuing directly in future work on diet-related cognitive decline.

Declarations

Editorial Note on Prior Publication

A previous version of this manuscript was published and subsequently retracted from its original journal. The retraction was issued because two photomicrographs presented in the original Figure 3 — representing the CA3 hippocampal subfield of the C+Mel30 and HCHF+Mel30 groups — were inadvertently uploaded as duplicates of a single image during figure assembly. The quantitative data underlying Figure 4 were derived independently from the original microscopy files and were not affected by this error. In the present version, the correct and distinct CA3 photomicrographs for both groups have been substituted, and the manuscript text, discussion, and reference list have been revised and updated accordingly. This note is intended for inclusion in the cover letter to the receiving journal and, at the editor's discretion, as a footnote in the manuscript itself.

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Conflicts of Interest: The authors declare no conflicts of interest.

Availability of Data and Materials: The datasets used in the current study are available from the corresponding author upon reasonable request.

Authors' Contributions: M.B. and D.D. conceived and designed the study. D.D. and I.Z. performed the research and contributed to drafting the manuscript. M.P. and K.N. analyzed the data and prepared the manuscript draft. All authors read and approved the final manuscript.

Ethics Approval: All animal procedures were approved by the Kerman University of Medical Sciences Ethics Committee (permit code IR.KMU.AH.REC.1400.143) and conducted in accordance with the Declaration of Helsinki and comparable ethical standards.

Declaration of AI Use

During the preparation of this manuscript, the authors used Claude/ChatGPT to assist with language

editing, paraphrasing, and improving the clarity and grammatical accuracy of the English text. The tool was not used to generate scientific content, data, analysis, or interpretations. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

References

1. Acioglu C, Li L, Elkabes S (2021) Contribution of astrocytes to neuropathology of neurodegenerative diseases. Brain Res 1758:147291
2. Brandebura AN, Paumier A, Onur TS, Allen NJ (2023) Astrocyte contribution to dysfunction, risk and progression in neurodegenerative disorders. Nat Rev Neurosci 24(1):23–39
3. Huang J, Li C, Shang H (2022) Astrocytes in neurodegeneration: inspiration from genetics. Front NeuroSci 16:882316
4. Liu X, Ying J, Wang X et al (2021) Astrocytes in neural circuits: key factors in synaptic regulation and potential targets for neurodevelopmental disorders. Front Mol Neurosci 14:729273
5. Michinaga S, Koyama Y (2021) Pathophysiological responses and roles of astrocytes in traumatic brain injury. Int J Mol Sci 22(12):6418
6. Wang P, Ye Y (2021) Astrocytes in neurodegenerative diseases: a perspective from tauopathy and α-synucleinopathy. Life 11(9):938
7. Hahm JR, Jo MH, Ullah R, Kim MW, Kim MO (2020) Metabolic stress alters antioxidant systems, suppresses the adiponectin receptor 1 and induces Alzheimer's-like pathology in mice brain. Cells 9(1):249
8. Więckowska-Gacek A, Mietelska-Porowska A, Wydrych M, Wojda U (2021) Western diet as a trigger of Alzheimer's disease: from metabolic syndrome and systemic inflammation to neuroinflammation and neurodegeneration. Ageing Res Rev 70:101397
9. Fuentes E, Venegas B, Muñoz-Arenas G et al (2023) High-carbohydrate and fat diet consumption causes metabolic deterioration, neuronal damage, and loss of recognition memory in rats. J Chem Neuroanat 129:102237
10. Calvo-Ochoa E, Hernández-Ortega K, Ferrera P, Morimoto S, Arias C (2014) Short-term high-fat-and-fructose feeding produces insulin signaling alterations accompanied by neurite and synaptic reduction and astroglial activation in the rat hippocampus. J Cereb Blood Flow Metabolism 34(6):1001–1008
11. Tsai SF, Wu HT, Chen PC, Chen YW, Yu M, Wang TF et al (2018) High-fat diet suppresses the astrocytic process arborization and downregulates the glial glutamate transporters in the hippocampus of mice. Brain Res 1700:66–77 eng. [PubMed ID:30009766]. https://doi.org/10.1016/j.brainres.2018.07.017

12. Cardinali DP (2019) Melatonin: clinical perspectives in neurodegeneration. Front Endocrinol 10:480
13. Martín Giménez VM, de las Heras N, Lahera V, Tresguerres JAF, Reiter RJ, Manucha W (2022) Melatonin as an anti-aging therapy for age-related cardiovascular and neurodegenerative diseases. Front Aging Neurosci 14:888292
14. Miranda-Riestra A, Estrada-Reyes R, Torres-Sanchez ED et al (2022) Melatonin: a neurotrophic factor? Molecules 27(22):7742
15. Kittipoom K et al (2022) Melatonin attenuates reactive astrogliosis and glial scar formation following cerebral ischemia and reperfusion injury mediated by GSK-3β and RIP1K. J Cell Physiol 237(4):2151–2164
16. Shabani M, Ragerdi Kashani I et al (2025) Melatonin ameliorates astrogliosis and microgliosis in a cuprizone demyelinating mouse model. Biochem Biophys Rep 41:101936
17. Li XW, Yi BJ, Wang ZY et al (2025) Melatonin attenuates the inflammatory response of astrocytes following ischemic stroke through the SIRT1/NF-κB signaling pathway. Mol Neurobiol 62:6021–6035
18. Ahmed OG, Mahmoud GS, Samy SS, Sayed SA (2023) The protective effect of melatonin on chronic paradoxical sleep deprivation induced metabolic and memory deficit in rats. Int J Physiol Pathophysiology Pharmacol 15(3):56–74
19. Gáll Z, Boros B, Kelemen K, Urkon M, Zolcseak I, Márton K et al (2024) Melatonin improves cognitive dysfunction and decreases gliosis in the streptozotocin-induced rat model of sporadic Alzheimer's disease. Front Pharmacol 15–2024. https://doi.org/10.3389/fphar.2024.1447757. English
20. Wong SK, Chin KY, Suhaimi FH, Ahmad F, Ima-Nirwana S (2018) The effects of a modified high-carbohydrate high-fat diet on metabolic syndrome parameters in male rats. Exp Clin Endocrinol Diabetes 126(4):205–212
21. Panchal SK, Poudyal H, Iyer A et al (2011) High-carbohydrate, high-fat diet-induced metabolic syndrome and cardiovascular remodeling in rats. J Cardiovasc Pharmacol 57(5):611–624
22. Morgan PJ, Barrett P, Howell HE, Helliwell R (1994) Melatonin receptors: localization, molecular pharmacology and physiological significance. Neurochem Int 24(2):101–146
23. Arioz BI, Tastan B, Tarakcioglu E, Tufekci KU, Olcum M, Ersoy N et al (2019) Melatonin attenuates LPS-induced acute depressive-like behaviors and microglial NLRP3 inflammasome activation through the SIRT1/Nrf2 pathway. Front Immunol 10:1511
24. Rezena E, Cioccari MS, de Moraes ADM, de Oliveira GT, Da Silva VF, Schmitz I et al (2025) Melatonin modulates astrocyte inflammatory response and Nrf2/SIRT1 signaling pathways in adult rat cortical cultures. Biomedicines 13(12):2967
25. Kauppinen A, Suuronen T, Ojala J, Kaarniranta K, Salminen A (2013) Antagonistic crosstalk between NF-κB and SIRT1 in the regulation of inflammation and metabolic disorders. Cell Signal 25(10):1939–1948 eng. [PubMed ID:23770291]. https://doi.org/10.1016/j.cellsig.2013.06.007
26. Wongchitrat P, Lansubsakul N, Kamsrijai U, Sae-Ung K, Mukda S, Govitrapong P (2016) Melatonin attenuates the high-fat diet and streptozotocin-induced reduction in rat hippocampal neurogenesis.

Neurochem Int 100:97–109

27. Baydas G, Reiter RJ, Yasar A, Tuzcu M, Akdemir I, Nedzvetskii VS (2003) Melatonin reduces glial reactivity in the hippocampus, cortex, and cerebellum of streptozotocin-induced diabetic rats. Free Radic Biol Med 35(7):797–804

Figures

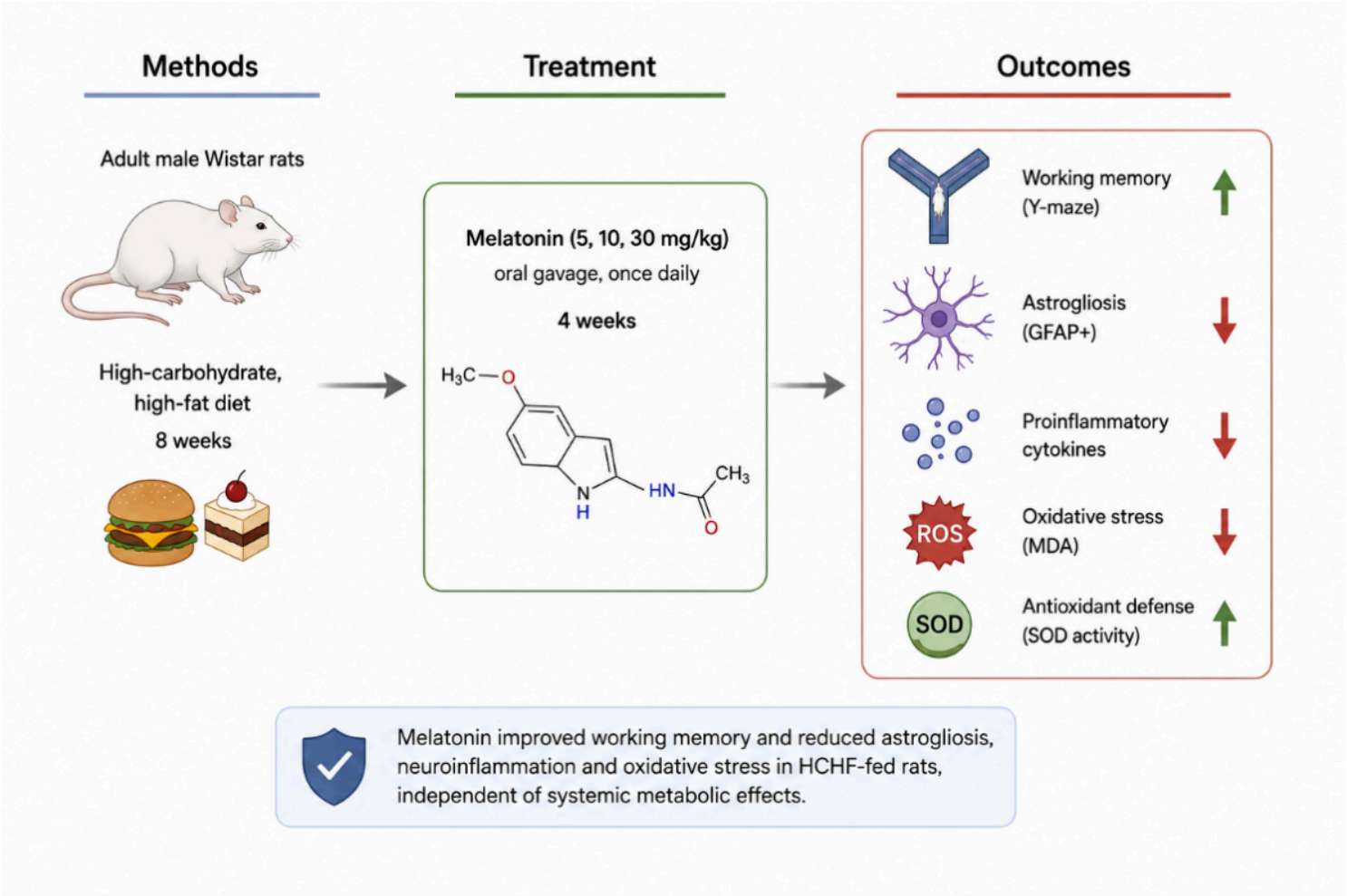


Figure 1

Graphical Abstract

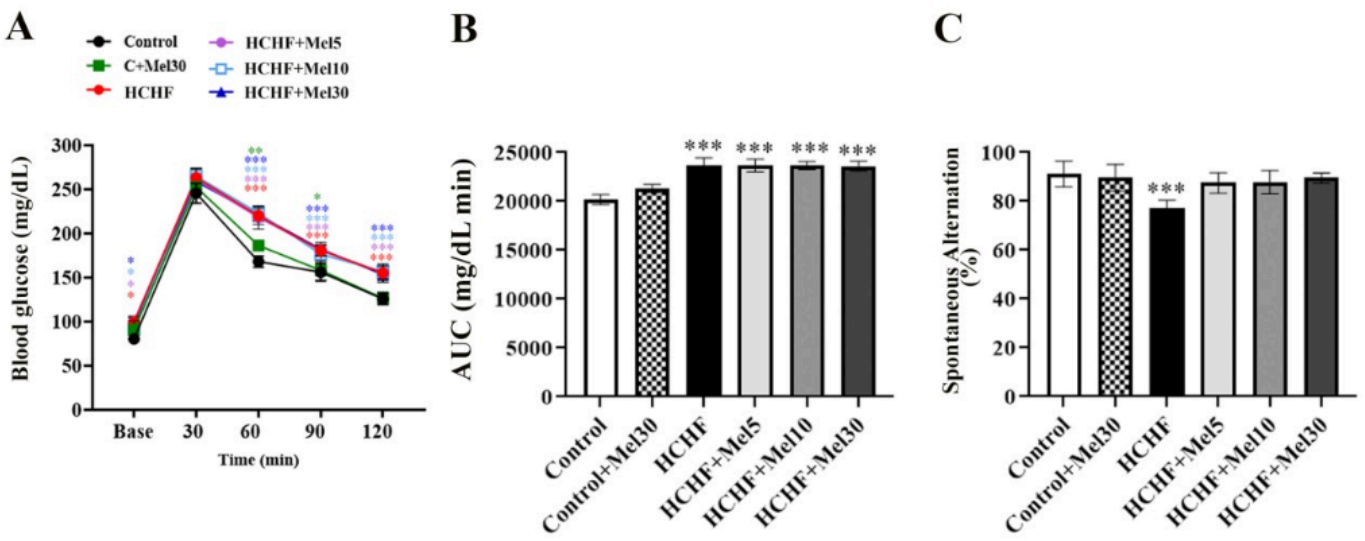


Figure 2

*Effect of melatonin on glucose tolerance and working memory in HCHF diet-fed rats. (A) Blood glucose curves during the intraperitoneal glucose tolerance test (IPGTT). (B) Area under the IPGTT curve, reflecting overall glucose clearance. (C) Percentage spontaneous alternation in the Y-maze, reflecting working memory performance. HCHF-fed rats received saline (HCHF) or melatonin at 5, 10, or 30 mg/kg (HCHF+Mel5/10/30); control-diet rats received saline (Control) or the highest melatonin dose (C+Mel30). *, **, *** p<0.05, p<0.01, p<0.001 vs. control; #, ##, ### p<0.05, p<0.01, p<0.001 vs. HCHF.*

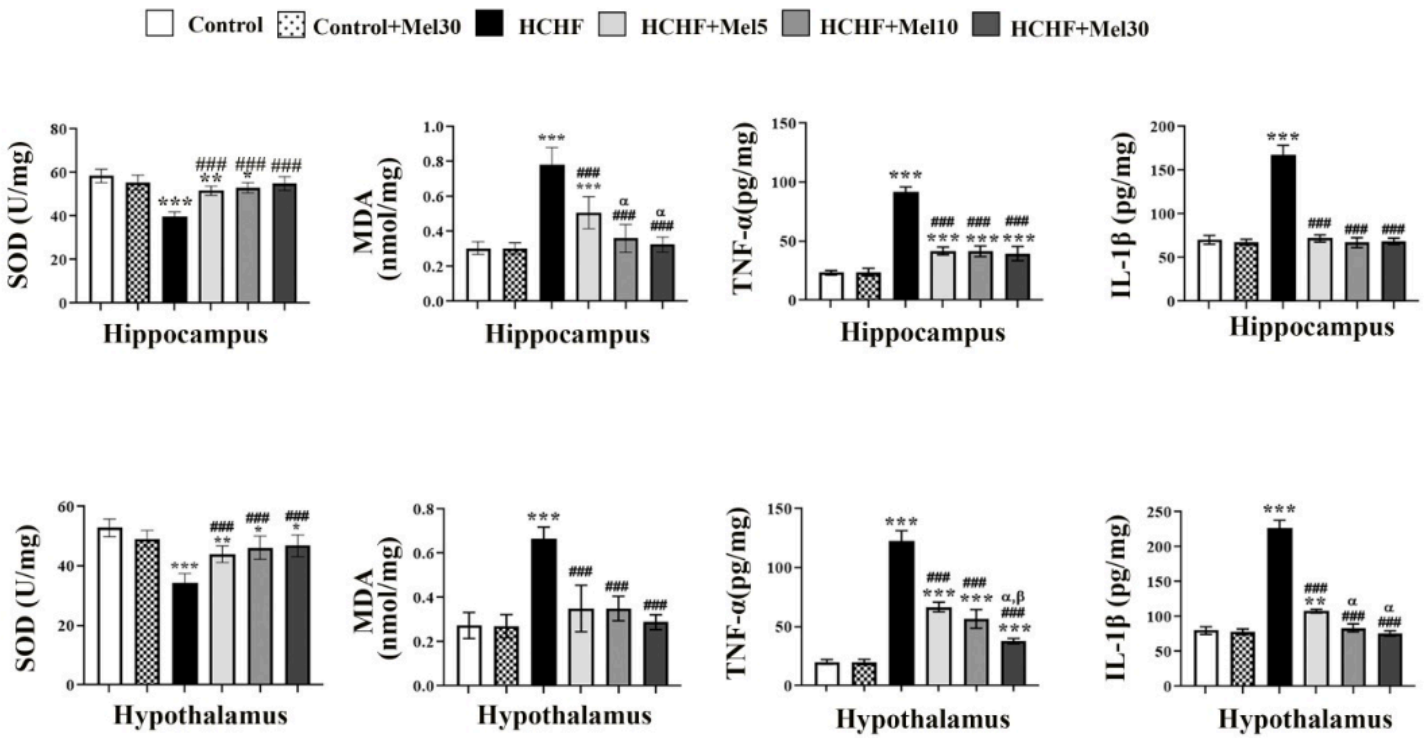


Figure 3

*Effect of melatonin on oxidative stress markers and proinflammatory cytokines in the hippocampus and hypothalamus of HCHF diet-fed rats. SOD, superoxide dismutase; MDA, malondialdehyde (lipid peroxidation index). Group labels as in Fig. 1. *, **, *** p<0.05, p<0.01, p<0.001 vs. control; #, ##, ### p<0.05, p<0.01, p<0.001 vs. HCHF; α p<0.05 vs. HCHF+Mel5; β p<0.05 vs. HCHF+Mel10.*

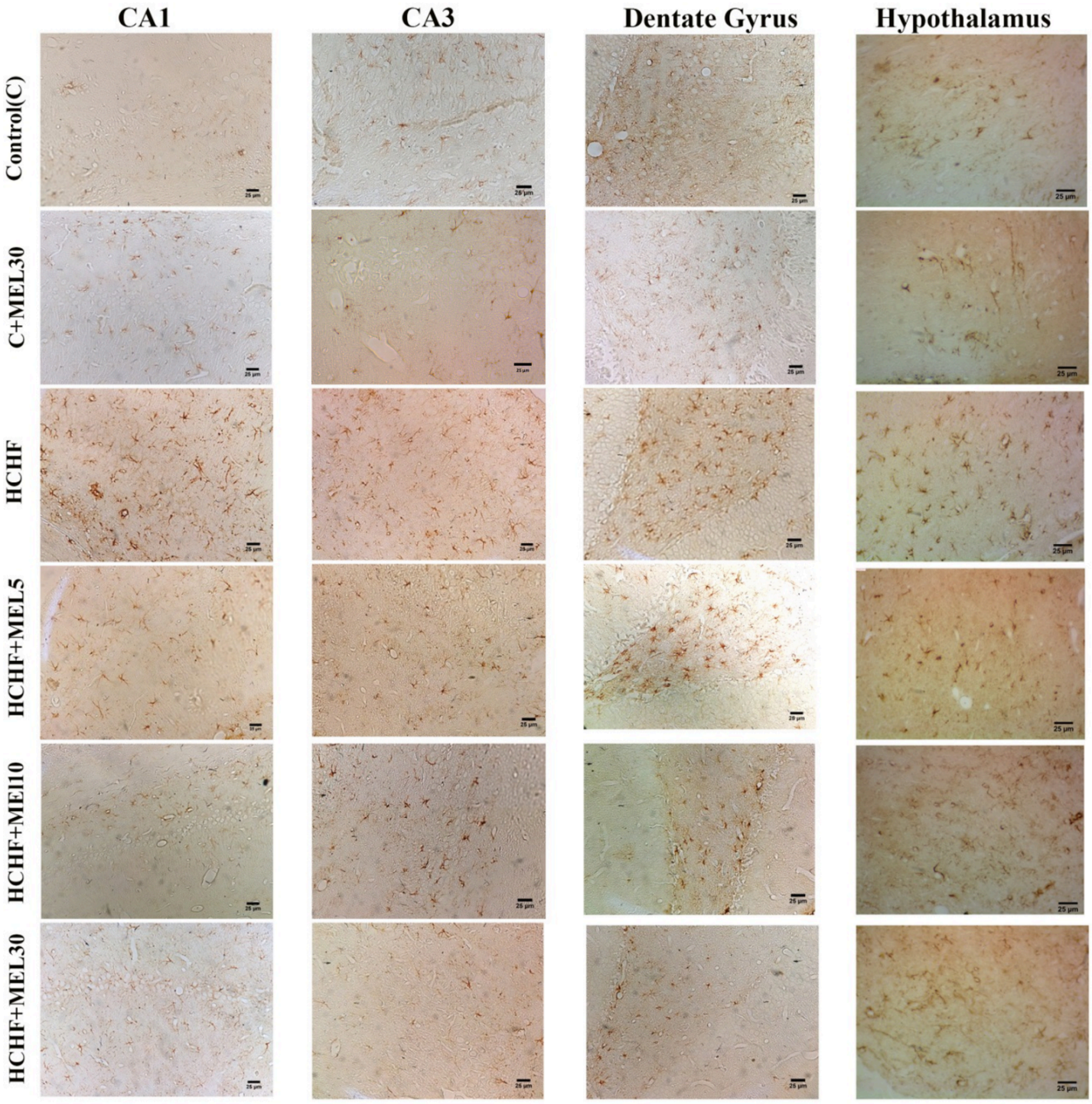


Figure 4

Photomicrographs showing the impact of melatonin therapy on GFAP (glial fibrillary acidic protein) immunoreactivity in the hippocampus (CA1, CA3, and dentate gyrus) and hypothalamus (arcuate nucleus)

of high-carbohydrate, high-fat (HCHF) diet-treated rats. HCHF-fed rats were orally treated with saline (HCHF) or melatonin at 5 mg/kg (HCHF+Mel5), 10 mg/kg (HCHF+Mel10), or 30 mg/kg (HCHF+Mel30). Control diet-treated groups received normal saline (Control) or the highest dose of melatonin (C+Mel30). 400X magnification, scale bar = 25 μm. Note: the CA3 photomicrographs for the C+Mel30 and HCHF+Mel30 groups have been corrected in this version; see Editorial Note above.

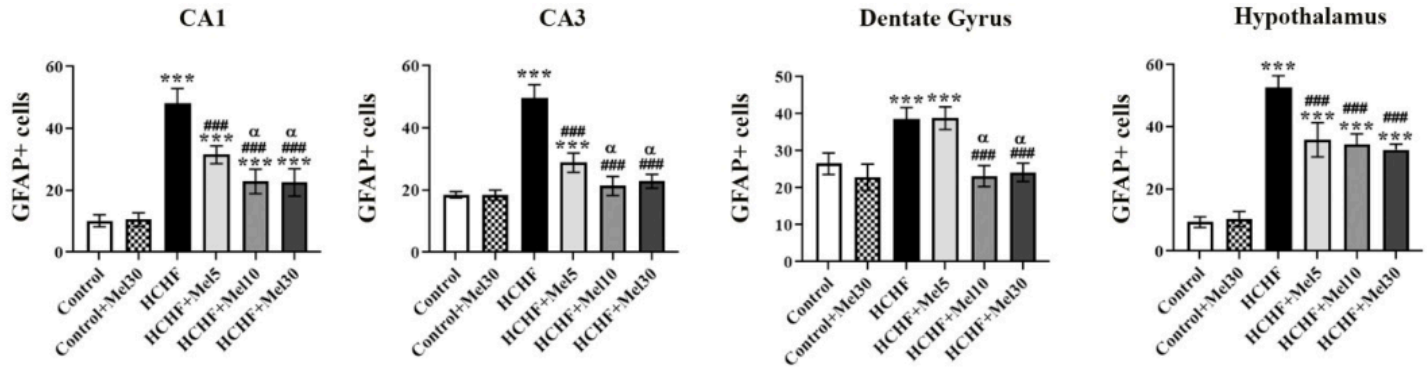


Figure 5

*Quantification of GFAP-positive cell counts in the hippocampus (CA1, CA3, and dentate gyrus) and hypothalamus (arcuate nucleus) of HCHF diet-fed rats treated with melatonin. Group labels as in Fig. 1. Values are mean ± SD. *** p<0.001 vs. control; ### p<0.001 vs. HCHF; α p<0.05 vs. HCHF+Mel5.*