Intermittent Fasting serves as a Potential Intervention to counteract the High Fat Diet induced Neuronal Changes in Dentate Gyrus of Rats

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Abstract

Background: High-fat diet (HFD) consumed by adolescent mice is known to impede learning and memory processes within the dentate gyrus (DG) and implies that adolescence is especially susceptible to the detrimental effects of a HFD on hippocampal function.

Objectives: This study aims to examine the consequences of intermittent fasting (IF) on neuron changes resulting from HFD in the rat DG.

Materials and methods: 60 male Wistar albino rats, each weighing 150 ± 20 grams, at 12 weeks of age. Four groups of rats were established with equal numbers: Group I was provided with unrestricted access to water and a standard diet of rat chow for 24 weeks. Group II was fed High fat diet(HFD). Group III contained rats following IF regimen. Group IV contained rats initially fed HFD for 24 weeks, followed by 8 weeks of IF regimen. At the end of the experiment, rats from all groups were scarified, the brain was isolated, fixed, sectioned and then stained with hematoxylin and eosin (H&E) , Gallocyainine and immunohistochemical stains for glial fibrillary acidic protein (GFAP), Nuclear factor erythroid 2–related factor 2 (Nrf2) and tyrosine hydroxylase (TH) for light microscope examination.

Results: Histological examination of group II showed disruption of DG blades, widening in the subgranular area and vacuolization of granule cells. Restoring of granular layer normal structure was observed in Group IV. Astrocytes number was found to be significantly high in group II. Nrf2 was significantly high among group III. The level of TH expression was significantly low among group II.

Conclusion: HFD caused significant neuronal degeneration, disrupted DG architecture, and alterations in astrocyte activity and molecular markers. IF showed protective and restorative effects on DG integrity and function.

Keywords: Dentate Gyrus; High Fat Diet; Intermittent Fasting; Neuronal Changes.

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Introduction

For several decades, due to their higher caloric density; dietary fats have been characterized as a key component of an "unhealthy" diet. The consumption of fat has been linked to the development of obesity and associated metabolic problems, as indicated by studies **(Mattson et al., 2003;Ribarič, 2012).**

Studies have found that consuming a high fat diet (HFD) a short period can lead to impairment of learning and memory processes dependent on the hippocampus in adolescent mice, with a notable impact on the dentate gyrus **(Kaczmarczyk et al., 2013).**

Inflammation development is an essential process in HFD-induced obesity. The activation of peripheral inflammation can send signals to the brain through multiple pathways of communication **(Zheng et al., 2014).** The substance can alleviate sickness symptoms, memory impairments and depression by influencing neurotransmitters that regulate behavioral functions **(Dantzer et al., 2008).** Many studies reported that early exposure to HFD in experimental animals causes pathological changes in dendritic spines, inflammation and impairment in DG plasticity **(Sobesky et al., 2014;Patton et al., 2018).**

Intermittent fasting (IF) typically involves alternating periods of unrestricted eating with periods of either complete or partial calorie restriction, ensuring that the animal still receives its necessary vitamins and minerals **(Mattson et al., 2003).** This process specifically results in the development of brain plasticity at both cellular and molecular levels, accompanied by improvements in behavior **(Naderi Ghalenoie et al., 2015).** Hippocampal neurons in rats receiving a maintained IF regimen showed greater resistance to chemically induced degeneration **(Bruce-Keller et al., 1999; Qiu et al., 2012 ;Murphy et al., 2014).**

A basic leucine transcription factor called nuclear factor erythroid 2-related factor 2 (Nrf2) is necessary to preserve cellular redox and metabolic balance by controlling levels of cellular antioxidants and reducing inflammatory stress **(Nguyen et al., 2003).** The link between obesity and both inflammation and oxidative stress makes the Nrf2 pathway's potential benefits particularly noteworthy **(Hurrle andHsu, 2017).** Investigating the possible effects of IF on HFD-induced neuronal changes in the rat dentate gyrus was the aim of this study.

Materials and methods

Animals

This experimental, prospective, analytical study involved 60 male Wistar albino rats weighing 150 ± 20 grams, at 12 weeks of age. The animals were placed in separate stainless-steel enclosures within a controlled environment featuring a 25, 12-hour day/night rotation and humidity of 50% ± 5 at South Valley University's animal facility. The research was conducted following the approval from the Ethical Committee of South Valley University Hospitals in South Valley, Egypt (approval code : SVU,MED, HIS002-4-25-3-11).

Experimental design:

Four equal groups of rats were established: Group I (control group) had unrestricted access to water and adhered to a standard 24-week diet consisting of rat chow. Group II (HFD group) composed of rats fed a 60% fat calorie diet comprised of 20% fresh soybean oil, 20% oxidized soybean oil, and 20% margarine for 24 weeks **(Dhibi et al., 2011).** Group III (IF group) consisted of rats fed an intermittent fasting regimen, 16.8 regimens, where the rats fasted for 16 hours and had free access to food and water for 8 hours daily for 24 weeks **(Belkacemi et al., 2012).** Group IV (HFD-IF group) initially had rats fed a high-fat diet for 24 weeks similar to

group II, then transitioned to an intermittent fasting regimen for a further 8 weeks.

At the end of the experiment, rats from all groups underwent anesthesia with chloroform and then the following procedures took place in each group. Five rats were fixed in 10% neutral buffered formaldehyde for light microscopy. The brain specimens were subsequently preserved in a 10% formalin solution that had been neutralized to a pH of 7.2, after which they underwent dehydration, clearing, and were then embedded in paraffin. Sections of coronal paraffin were prepared at a thickness of 4-6 micrometers and then stained with hematoxylin and eosin (H&E), gallocyanin as a special stain, and immunohistochemical stain for glial fibrillary acidic protein (GFAP), Nuclear factor erythroid 2–related factor 2 Nrf2 and tyrosine hydroxylase enzyme (TH).

. For GFAP polyclonal antibody (GFAP; 1:1000, Merck Millipore, MAB360)_ from Cosmo Bio company, USA used. For (Nrf2) slides were incubated with monoclonal antibody against Nrf2 1 : 100 (Nrf2 Santa Cruz Laboratory), catalogue number A0674 from Raybiotech Company_ California. Sections were then incubated with a Biotin-conjugated secondary antibody and Streptavidin-Enzyme Conjugate (LSAB System HRP, BIOCARE). The immune reaction resulted in the oxidation of the 3,3′-diaminobenzidine by peroxidase (Liquid DAB, DAKO Carpinteria, CA) into an insoluble brown precipitate. The reaction sites were visualized as a brown staining. Counterstaining with hematoxylin was performed after immunostaining. For tyrosine hydroxylase enzyme (TH) TH 1:1000, Merck Millipore, MilliporeSigma, Burlington, MA, USA, AB152).

The paraffin sections were placed on positive-charged glass slides. Complete deparaffinization in xylene and rehydration in descending grades of ethanol were performed. The sections were incubated for 10 minutes with 10% H2O2 to block endogenous peroxidase activity and unmasking of antigenic sites was carried out by transmitting sections into 0.01 mol/l citrate buffer (pH 6.0) for 10 minutes, and then boiling in a microwave for 5 minutes. Incubation with the primary antibody (anti- GFAP, 1:100), (anti-TH , 1:1000), (anti Nrf2,1:100) was carried out for 1 hour at a dilution of 1/100 and then washed and incubated with biotinylated secondary antibodies and then with the avidin–biotin complex. Finally, reactions were develop with 0.05%diaminobenzidine slides, and counterstained with Heamatoxylin, dehydrated, cleared, and mounted. Negative control sections were prepared using PBS without using the primary antibody. (Cattoretti et al., 1993).

Histomorphometric study and image analysis

Histomorphometric analysis was performed using the ImageJ software system. Spatial calibration with an object micrometer was conducted before each analysis. Five images were selected from each animal in each group. % GFAP intensity/surface area, % Nrf2 intensity/surface area, and % TH intensity/surface area were estimated.

Statistical analysis

SPSS v26 (IBM Inc., Chicago, IL, USA) was employed to conduct the statistical analysis. An ANOVA (F) test and a post hoc analysis (Tukey) were utilized to compare the quantitative variables, which were presented as mean and standard deviations among the four groups. The Chi-square test was utilized to analyse qualitative variables, which were expressed as frequency and percentage (%). In a two-tailed test, a p value of less than 0.05 was considered statistically significant.

Results

Hematoxylin and eosin stain (H&E):

Histological examination of H&E stained sections of control group (**Fig. 1a**) showed that the dentate gyrus (DG) is composed of outer and inner blades and CA4 region is enclosed in between. HFD group (**Fig.1b**) showed disruption of granular layer of both outer and inner blades. In IF group (**Fig.1c**), the two blades of DG were normal with CA4 region in between. On the other hand, section from HFD+IF group (**Fig. 1d**) revealed disruption of granular layer of outer and inner blades with wide subgranular space. DG of control group section on higher magnification (**Fig. 2a**) was formed of three layers; polymorphic layer, granular layer and molecular layer. The granular layer revealed aggregation of rounded granule cells with deeply stained nucleiand basophilic cytoplasm. The Molecular and Polymorphic layers had many blood capillaries and glial cells. In comparison, section from HFD group (**Fig.2, b**) showed mostly normal granule cells, but few cells had vacuolated cytoplasm and pyknotic nuclei. There was also widening in the sub granular area. In IF group (**Fig. 2c**) DG was formed of three layers; polymorphic layer, granular layer and molecular layer. The granular layer revealed aggregation of rounded granule cells with deeply stained nuclei. The molecular and polymorphic layers had many blood capillaries and glial cells. In HFD+IF group (**Fig.2, d**) there were restoration of the normal structure of granule neurons, while few neurons appeared dark and shrunken. The molecular and polymorphic layers had many blood capillaries and glial cells.

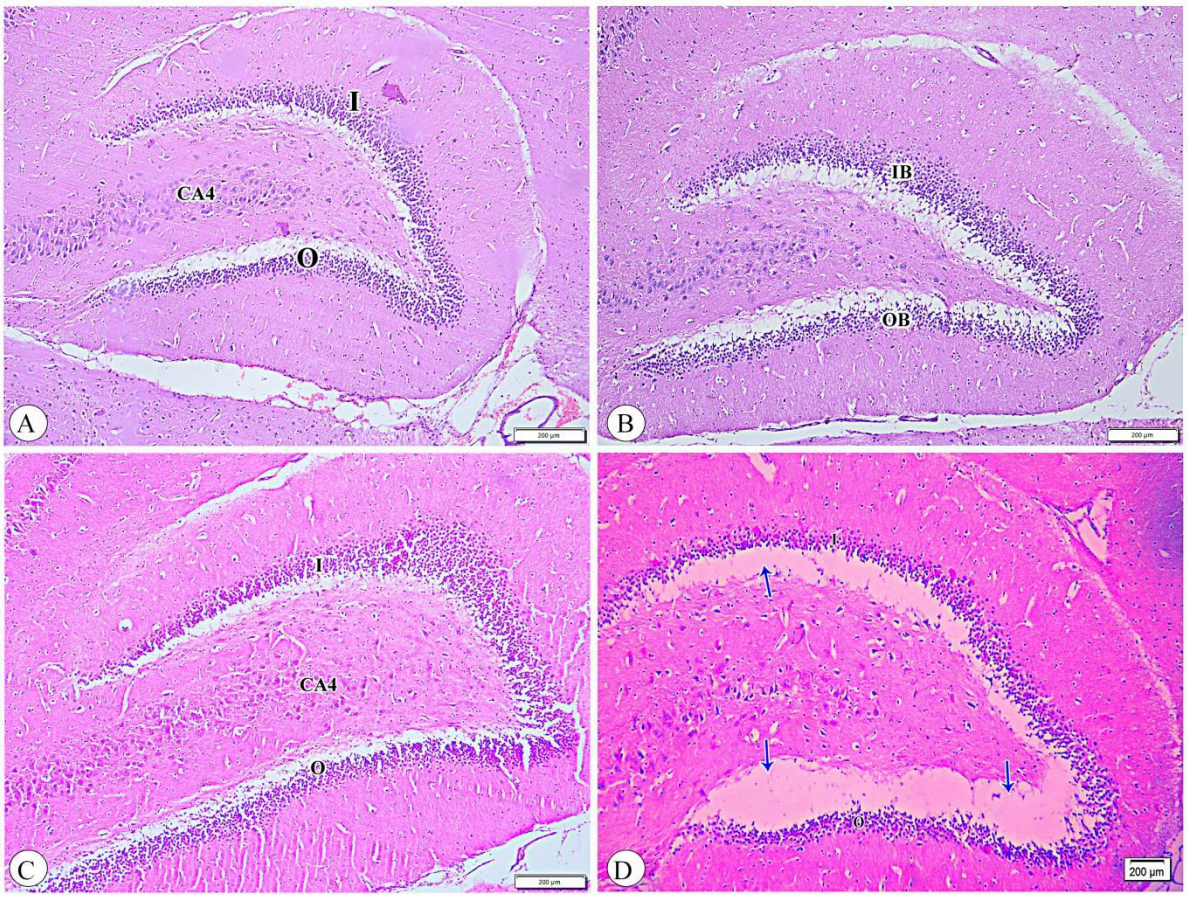

Fig.1. Photomicrographs of H&E stained sections of the dentate gyrus (DG) of all groups. (A) Control group showing the two blades of dentate gyrus; the outer (O) and the inner ones (I) with CA4in between. (B) HFD group showing disruption of granular layer of outer (OB) and inner (IB) blades., (C) IF group showing the two blades of

dentate gyrus; the outer (O) and the inner blades (I) with CA4 in between. (D) HFD+IF group showing disruption of granular layer of outer (O) and inner (I) blades with wide subgranular spaces (blue arrows). Scale par 200 µm

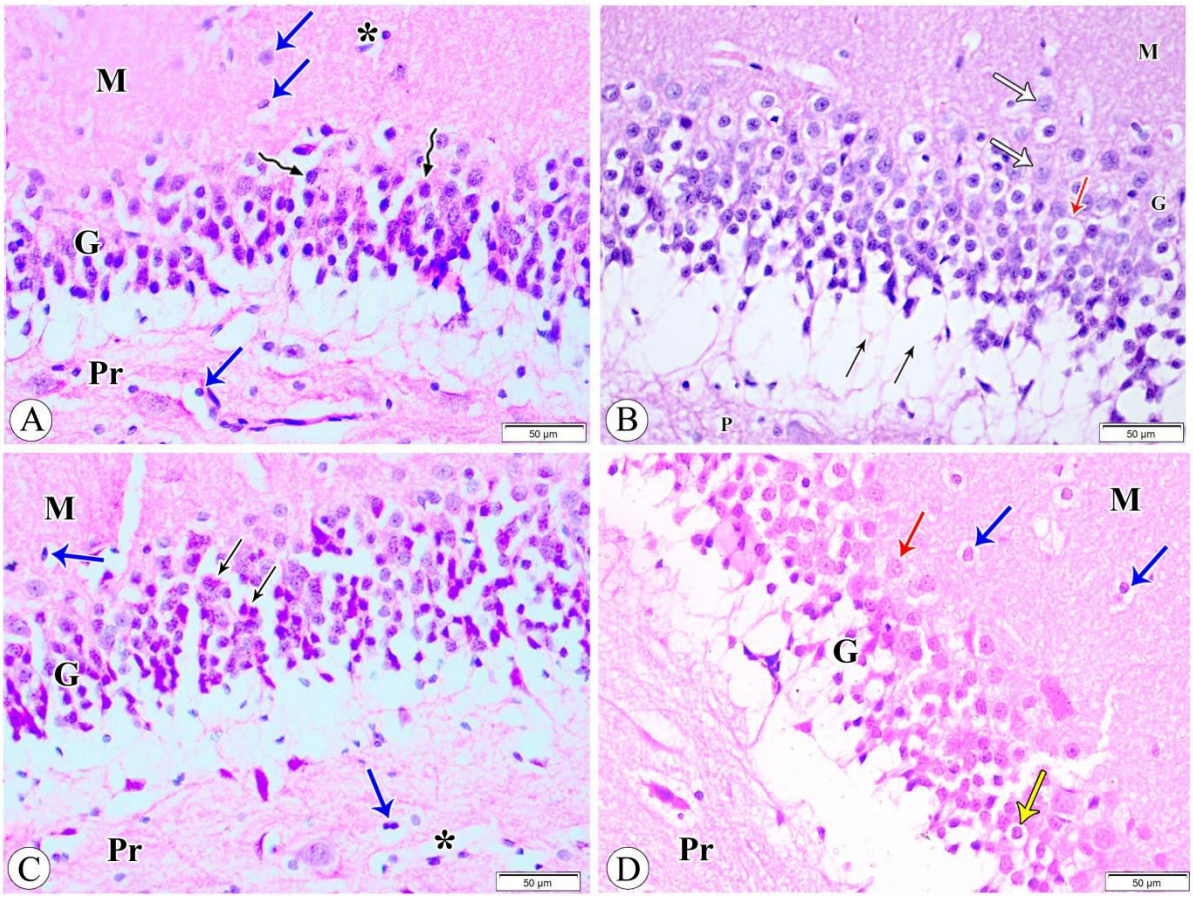

Fig.2. High magnification of the DG stained with Hx&E for all groups. (A) Control group showing well defined three layers; polymorphic layer (Pr), granular layer (G) and molecular layer (M). The G layer reveals aggregation of rounded granule cells with deeply stained nuclei (Zigzag arrows). The M and P layers have many blood capillaries (star) and glial cells (Blue arrow). (B) HFD group showing normal granule cells (White arrows) in granular layer (G). Many cells have vacuolated cytoplasm and pyknotic nuclei (Red arrow). Also there is widening in the sub granular area (Black arrows). (C) IF group shows well defined three layers; polymorphic layer (Pr), granular layer (G) and molecular layer (M). The G layer shows aggregation of rounded granule cells with deeply stained nuclei (Black arrows). The M and Pr layers have many blood capillaries (Star) and glial cells (Blue arrow). (D) HFD+IF group Showing restoration of the normal structure of granule neurons (Red arrow) in granular layer (G). Few neurons appear dark with shrunken perikaryons (Yellow arrows). The M and P layers have many glial cells (Blue arrow). Scale bar = 50µm.

Gallocyanin stain

Sections from control group stained with gallocyanine (**Fig. 3a**) revealed that the DG contained granular cells with rounded, vesicular nuclei, prominent nucleoli and basophilic cytoplasm. In HFD group (**Fig. 3b**) the DG showed that some of the granule cells were darkly stained, while others were lightly stained with vesicular nuclei and prominent nucleoli. In IF group (**Fig. 3c**), the neurons had rounded and vesicular nuclei with prominent nucleoli. Section from HFD+IF group (**Fig. 3d**) showed wide spaces between neurons of

granular layer. Some neurons were degenerated and showed chromatolysis; while others were normal and contained vesicular nuclei.

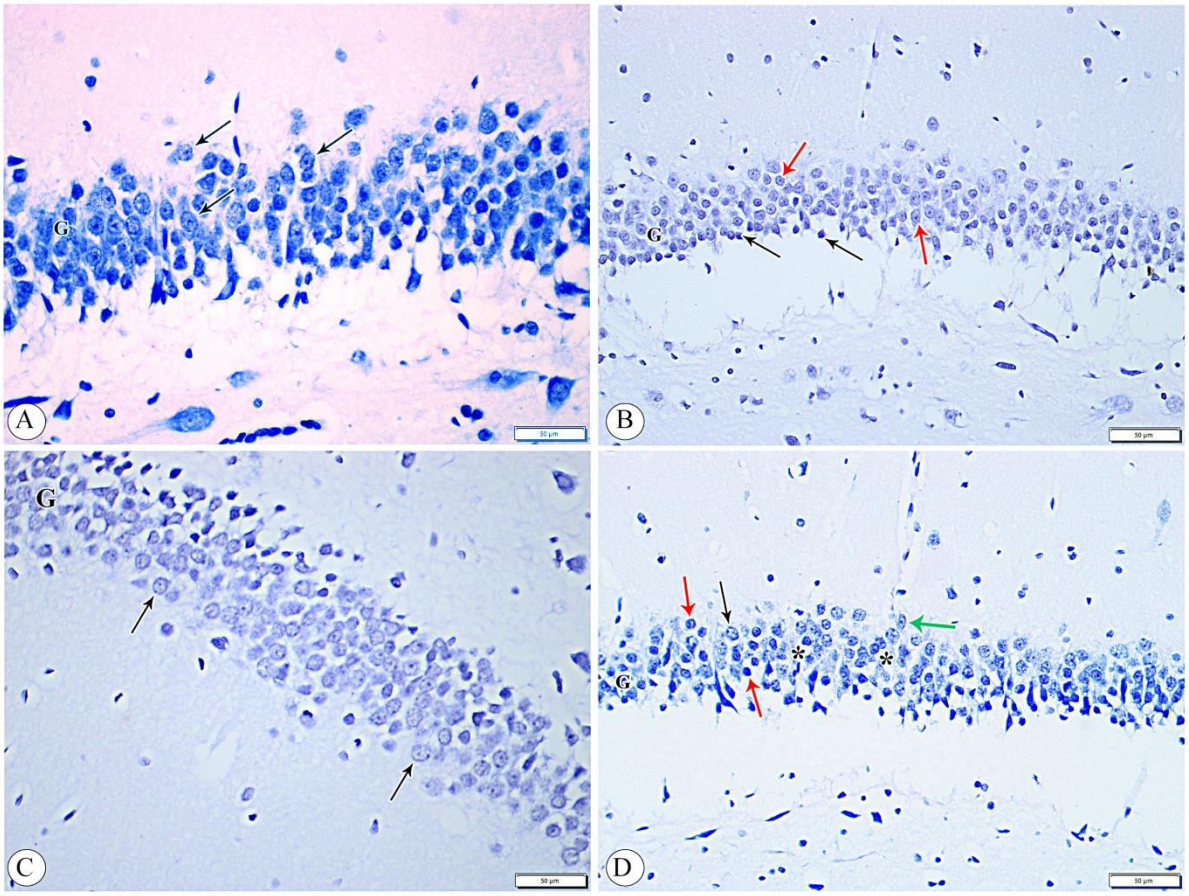

Fig.3. Gallocyanine stained sections of the DG of all groups. (A) Control group showing rounded, vesicular nuclei of of granule cells in granular layer (G) with prominent nucleoli (Black arrows). (B) HFD group showing cell bodies of neurons in granular layer (G). Some of these neurons is darkly stained (Black arrow); while others are lightly stained with vesicular nucleus and prominent nucleuoli (Red arrows). (C) IF group showing rounded, vesicular nuclei of neurons in granular layer (G) with prominent nucleoli (Black arrows). (D) HFD+IF group showing wide spaces (Star) between neurons of granular layer (G). Some neurons are degenerated (Red arrows) and showing chromatolysis (Black arrow); while others are normal and contain vesicular nuclei (Green arrow). Scale bar = 50µm.

Immunohistochemical results

A. **Glial fibrillary acidic protein (GFAP) :** Control group showed positive reaction of astrocytes for GFAP in DG (**Fig. 4a**). There was increased number of positive astrocytes in DG of HFD group (**Fig. 4b**). The astrocytes showed positive reaction in DG of IF group (**Fig. 4c**). Interestingly, HIF group showed decrease in the number of positive astrocytes in comparison with HFD group (**Fig. 4d**).

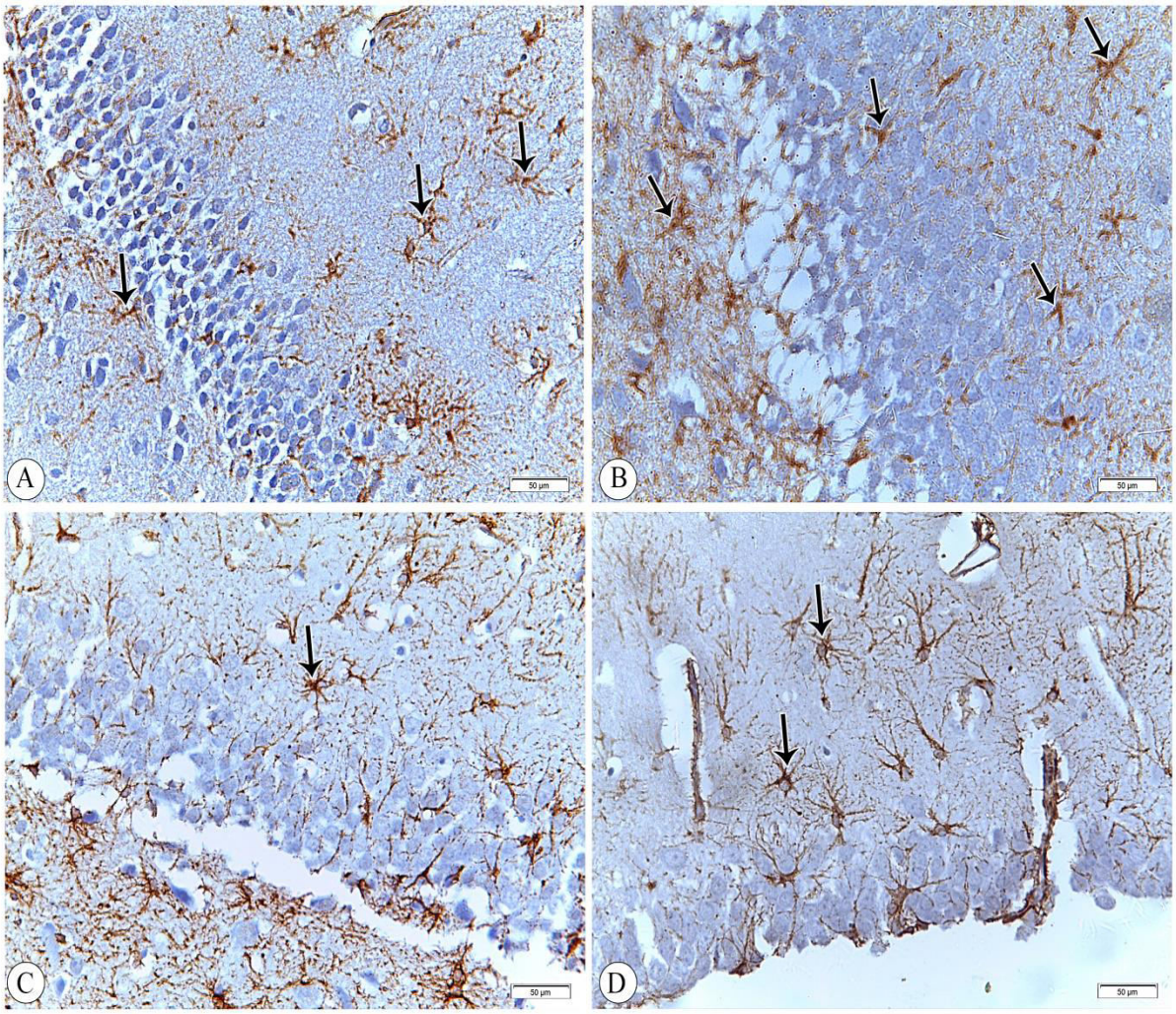

Fig.4. Immunohistochemical expression of GFAP in the DG of all groups (A) Control group showing a positive reaction in astrocytes (arrows). (B) HFD group showing increased the number of positively stained astrocytes (arrows). (C) IF group showing positive reaction of astrocytes (arrows). (D) HFD+IF group showing dcreased number of positively stained astrocytes as compared to HFD group. Scale bar = 50µm

B. Nuclear factor erythroid 2–related factor 2(Nrf2) : The immunohistochemical localization of Nrf2 protein in control group revealed moderate positive immunoreaction in DG (**Fig. 5a**). There was negative reaction in DG from HFD group (**Fig. 5b**). In IF, strong positive expression for Nrf2 was reported (**Fig. 5c**). HFD+IF showed weak positive expression for Nrf2 in DG (**Fig. 5d**).

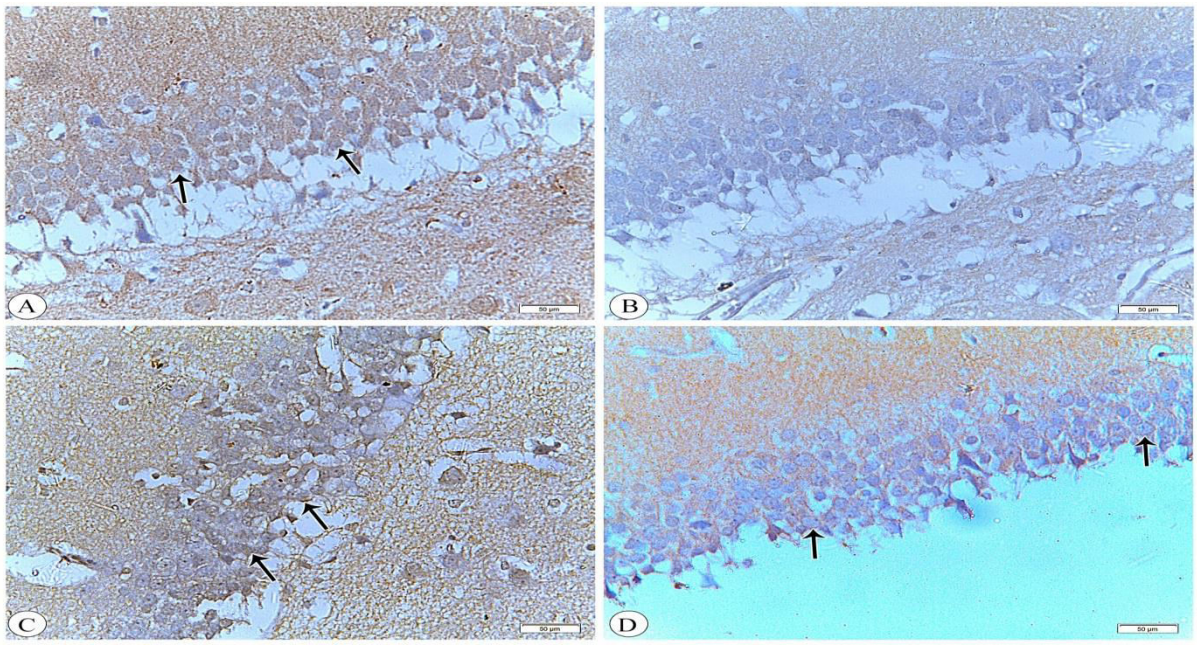

Fig.5. Immunohistochemical expression of Nrf2 protein in the DG of (A) control group showing moderate immunostaining in nerve cells (arrows). (B) HFD group showing negative reaction of the cytoplasm of granular cells. (C) IF group showing positive immunostaining of nerve cells (arrows). (D) HFD+IF group showing mild positive reaction in few neurons (arrows). Scale bar = 50µm

C. Tyrosine hydroxylase enzyme (TH) : Control group showed strong immune-expression of TH protein in the DG (**Fig. 6a**), while section from HFD group showed negative TH expression (**Fig. 6b**). Sections from IF group showed strong positive expression of TH (**Fig. 6c**). In HFD+IF group, TH protein expression showed mild positivity in DG (**Fig. 6d**).

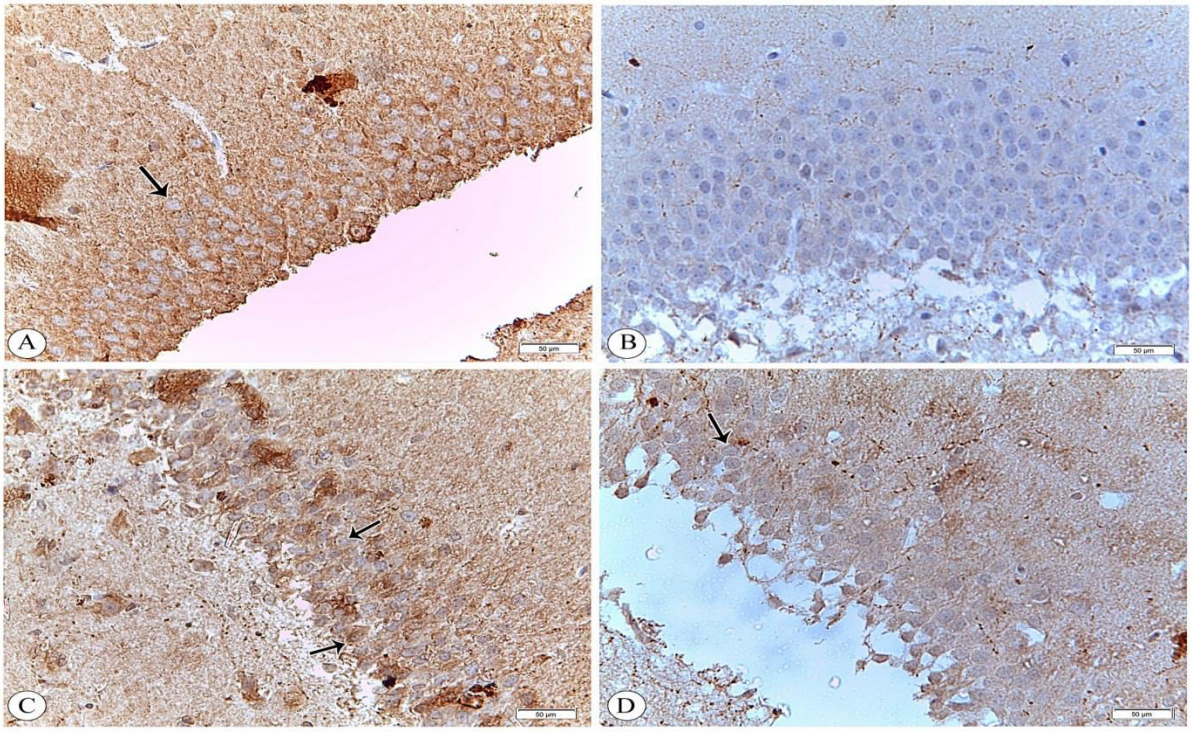

Fig.7. Immunohistochemical localization of TH protein in the DG of (A) Control group showing strong positive staining of nerve cells (arrows). (B) HFD group showing

negative staining of nerve cells. (C) IF group showing strong positive staining of nerve cells. (D) HFD+IF group showing moderate positive staining of nerve cells (arrows). Scale bar = 50µm

Statistical results

The number of astrocytes varied substantially between the examined groups, with the highest count found in the HFD group (*P<0.05*). TH levels were notably different among the groups, with the HFD group exhibiting the lowest TH expression (P<0.05). Nrf2 levels were also considerably different among the groups, with the IF groups displaying the highest Nrf2 expression (*P<0.05*). (**Table.1, Fig.8)**

Table 1. Astrocyte number, tyrosine hydroxylase and Nrf2 among the studied groups

	Control group (n=15)	HFD group (n=15)	IF group (n=15)	HDF+IF group (n=15)	P
Astrocyte number					
DG	9.00±0.65465	18.00±0.75593	6.00±0.75593	11.00±0.65465	**<0.001***
P1<0.001*, P2<0.001*, P3<0.001*, P4<0.001*, P5<0.001*, P6<0.001*					
Tyrosine hydroxylase					
DG	57.171±0.229	0.474±0.0354	51.401±0.1735	19.2794±0.266	**<0.001***
P1<0.001*, P2<0.001*, P3<0.001*, P4<0.001*, P5<0.001*, P6<0.001*					
Nrf2					
DG	11.430±0.3724	0.110±0.0006	22.129±0.1429	6.243±0.0611	**<0.001***
P1<0.001*, P2<0.001*, P3<0.001*, P4<0.001*, P5<0.001*, P6<0.001*					

Data is presented as mean ± SD. * Significant P value < 0.05. P1: Group1 vs. Group 2, P2: Group 1 vs. Group 3, P3: Group 1 vs. Group 4, P4: Group 2 vs. Group 3, P5: Group 2 vs. Group 4, P6: Group 3 vs. Group 4. HFD: High fat diet, IF: Intermittent fasting, DG: Dentate gyrus, TH: Tyrosine hydroxylase, Nrf2: nuclear factor erythroid related factor 2

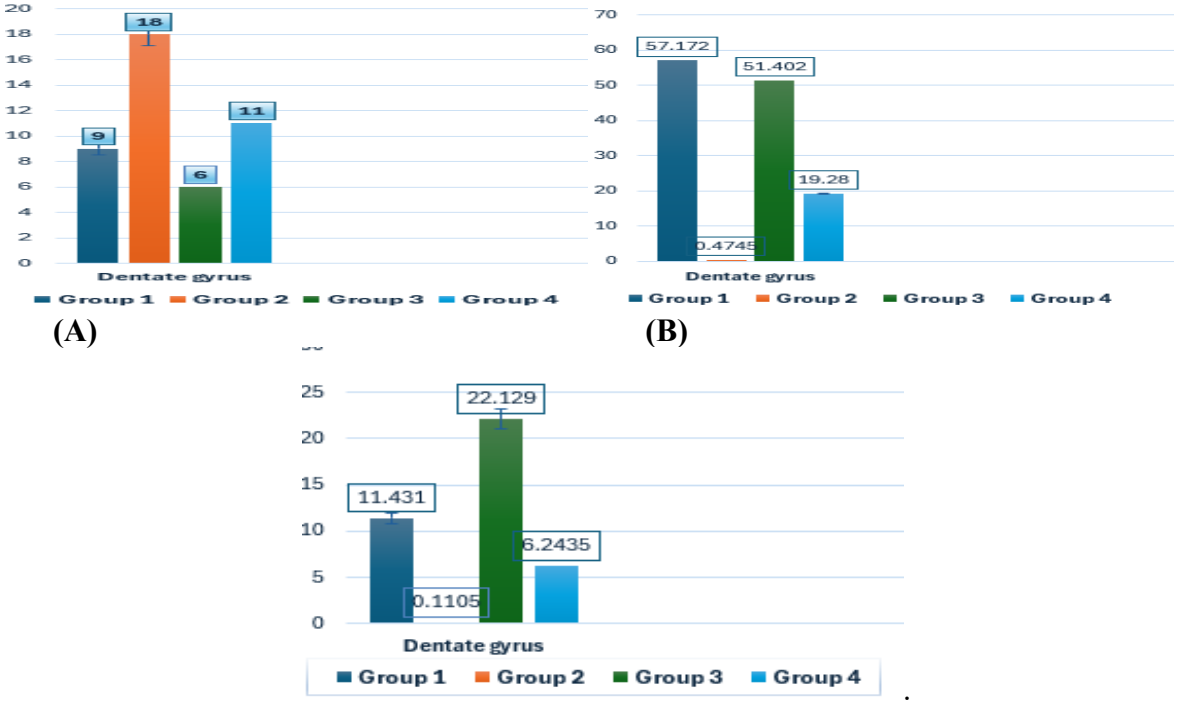

Fig.8. Mean of (A) astrocte number, (B) tyrosine hydroxylase area percentage and (C) Nrf2 area percentage among four studied groups in dentate gyrus

The body weight was statistically significantly different between the studied groups with the highest value in HFD group (p<0.001). Post hoc testing with Tukey correction revealed that HFD group had a significantly higher mean body weight than other groups (p <0.001), while the IF group had a significantly lower mean body weight, compared to other groups (p<0.001), **(Table.2 and Fig.9).**

Table 2. Rats' weight among the studied groups

Parameter	Control group	HFD group	IF group	HFD +IF group	P value*
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Body weight (gm)	150.00 ± 2.507	350.00 ± 2.752	90.00 ± 2.00	200.00 ± 2.00	**<0.001**
Pairwise comparison (P value)**	**P1<0.001**	**P2<0.001**	**P3<0.001**	**P4 <0.001**	
	P5<0.001		**P6 <0.001**		

* One-way ANOVA; ** Post hoc testing with Tukey correction for pairwise comparison; P1: Control group vs. HFD group; P2: Control group vs. IF Group; P3: Control group vs. HFD+IF group; P4: HFD group vs. IF group; P5: HFD Group vs. HFD+IF group; P6: IF group vs. HFD+IF group. HFD: High fat diet, IF: Intermittent fasting; Bold: Significant.

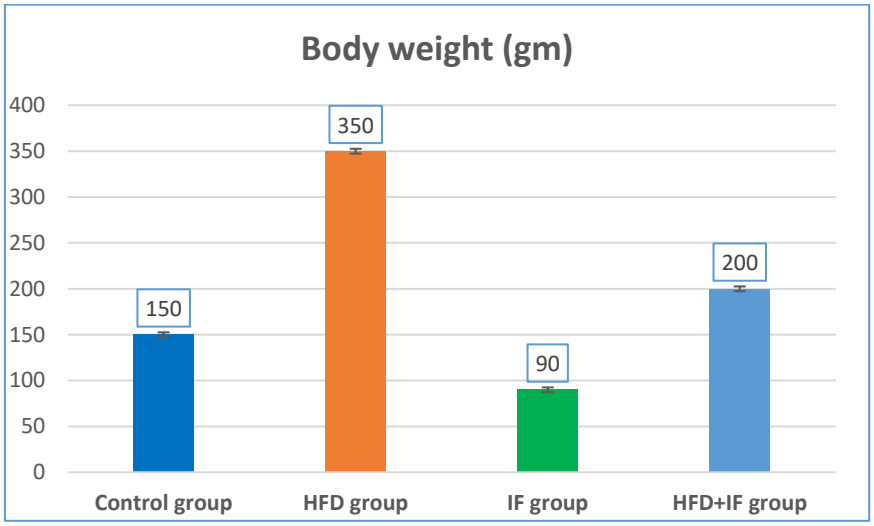

Fig. 9. Mean of body weight among study groups

Discussion

Research in animal studies has revealed that eating high-calorie diets can impact learning and memory in the DG **(López-Taboada et al., 2020).**

In the present study, in the HFD group, the DG region exhibited disruption to the granular layer of both outer and inner blades, an increase in size of the subgranular zone, and vacuolization of granule cells accompanied by nuclear pyknosis. Our findings are consistent with those of Hazzaa et al. **(Hazzaa et al., 2020),** who observed numerous degenerated cells in brain tissue samples from the HFD group, characterised by marked apoptosis and a reduction in the thickness of all hippocampus layers. Niculescu et al. **(Niculescu andLupu, 2009)** found that a HFD of the mother affected the formation of new neurons in the hippocampus and cortex of E17 mouse fetal brains, as well as the differentiation of neurons. In fetuses born to mothers on a high-fat diet, the number of neural progenitors in the neuroepithelium of the hippocampus and cortex was found to be higher compared to controls, whereas their count in the DG was lower. Ledreux et al. **(Ledreux et al.,**

2016) discovered a high concentration of densely packed pyramidal neurons in the DG across all participant groups.

In IF group, the results showed aggregation of rounded granule cells with deeply stained nuclei in DG. The Molecular and Polymorphic layers had many blood capillaries and glial cells. Hazzaa et al. **(Hazzaa et al., 2020)** revealed that the thickness of all hippocampal layers was substantially increased, whereas the number of apoptotic and degenerated cells was decreased in comparison to the HFD group. Our results showed that the HFD+ IF group exhibited restoration of the normal structure of granular neurons in the DG. In a prior investigation, the group that adhered to intermittent fasting and concurrently consumed a HFD exhibited a substantially greater increase in the thickness of all layers of the hippocampus **(Hazzaa et al., 2020)**.

In current study, semithin section of DG in HFD group revealed neuronal degeneration in the form of disintegrated vacuolated cytoplasm, dark shrunken nuclei with chromatin clumping. There was numerous vacuolization in Neuropil. On the other hand, when the rats were fed the HFD regimen then exposed to IF they showed improvement of neuronal degeneration. Huali et al. **(Wu et al., 2018)** demonstrated that the intercellular gaps in the pyramidal cell layers were expanded and irregularly structured are in agreement with the reported results. Research revealed that mice on a HFD lost 14.5% more neurones in the DG than mice fed a normal diet. This finding confirms that the high-fat diet was responsible for hippocampal neuronal loss. Furthermore, these results are consistent with a previous study by Hazza et al. **(Hazzaa et al., 2020)** which also showed a decrease in the number of hippocampal cells undergoing apoptosis and degeneration in the HFD group compared to others.

The research findings suggested that IF reduced oxidative stress, resulting in improved brain structure and function. IF improves the brain's redox state and raises glutathione level, according to a study by Rebrin et al. **(Rebrin et al., 2007)** which discovered that a 40% decrease in caloric intake reduces oxidative stress in various brain regions of aged mice. Additionally, studies have shown that post-operative IF increases the concentration of GSH in brain tissues while decreasing the concentration of MDA. The detrimental effects of HFD on body weight and brain oxidative stress may be mitigated by the concurrent neuroprotective activity of IF. Histological and immunohistochemical staining results showed that the hippocampus's pyramidal and granular cells had increased in thickness and vitality. In the HFD+IF groups, there were notable decreases in GFAP levels.

Regarding the EM examination, the neurons from HFD group exhibited cytoplasmic vacuoles, destructed mitochondria, disrupted nuclear and cell membranes, and numerous vacuoles in the neuropil. The IF group demonstrated normal neurons with euchromatic nuclei, prominent nucleoli, regular nuclear membrane, rough endoplasmic reticulum cisternae, and mitochondria, with abundant mitochondria in nerve cell axons. The HFD+IF group exhibited nerve cell bodies with euchromatic nuclei, parallel cisternae of rough endoplasmic reticulum, and normal mitochondria, with few vacuolization in the neuropil. These results were in concordance with Alkan et al. **(Alkan et al., 2021)** reported neurodegeneration of the obese rats fed on HFD including, swelling of mitochondria, dilated cisternae of rough endoplasmic reticulum, increased lysosomes and cytoplasmic vacuoles.

Our findings showed a substantial rise in the count of GFAP positive astrocytes in rats belonging to the HFD

group relative to those in the control and IF groups. The findings are in line with those of Hazzaa et al. **(Hazzaa et al., 2020),** who discovered that the hippocampal pyramidal cell damage was linked to reduced viability, diminished thickness, increased apoptosis, and a significant increase in GFAP immunostaining in the HFD group. Conversely, Teixeira et al. **(Teixeira et al., 2017)** observed that the number of GFAP-positive cells did not vary between the groups.

The current research revealed that administering intermittent fasting to the HFD group resulted in a significant reduction in the number of GFAP positive cells. Morgan et al. **(Morgan et al., 1997)** revealed that limiting food intake reduced GFAP gene transcription in older rats. Furthermore, restricting food intake resulted in decreased microglial activation over time, implying that GFAP expression is susceptible to oxidative stress.

The current research found a low immunohistochemical response for Nrf2 in the DG area from the HFD group compared to the control and IF groups. Notably, this response was higher in rats from the HFD+IF group. Morrison et al. **(Morrison et al., 2010)** reported that 20-month-old male mice on a HFD had lower Nrf2 levels. The disruption of Nrf2 signalling may be a contributing component to the cognitive dysfunction and reduced activity linked to HFD; following 4 months of HFD, the mice showed increased oxidative stress in the hippocampus and cognitive deterioration in comparison to controls. Batandier et al. **(Batandier et al., 2020)** discovered that a meal heavy in fat and sugar caused decreased Nrf2 gene expression and increased oxidative stress in the frontal cortex and brain mitochondria.

The current study reported that DG from control and IF groups had strong positive TH expression, the HFD group showed negative to weak positive TH expression, and the HFD+IF group showed mild positive TH expression. In concordance with these results; Kao et al. **(Kao et al., 2019)** found that the percentage of TH-positive cells in the SN of HFD mice was significantly less than those in control group.

Current study revealed that the body weight was statistically significantly different between the studied groups with the highest value in HFD group ($p<0.001$), as this group had a significantly higher mean body weight than other groups ($p <0.001$), while the IF group had a significantly lower mean body weight, compared to other groups.This results are agree with **(Hebatoallah et al, 2024)** which revealed that HFD resulted in increased body weight, lipids, visceral adiposity and induced hyperglycemia and insulin resistance compared to controls. Findings revealed significant higher AT IL-6 levels and lower IL-10 levels with significant upregulation of CD11c and CD206 mRNA expressions in all age groups. The histological findings showed increased inflammation and presence of crown like structures in adult and old HFD groups. Moreover, the HFD-induced obesity in groups resulted in significant reduction in p-AMPK levels and SIRT1 expression in AT as compared to controls. AMPK and SIRT1 was positively correlated with IL-10 and CD206 and negatively correlated with TG, HOMA-IR, IL-6 and CD11c in obese groups of different ages.

(Dina S. et al, 2023) study showed that fasting (24 h of fasting nonconsecutive day/week) combination with basal diet caused a significant decrease ($P<0.05$) in weight gain, feed intake, peritoneal fat pad, serum (glucose, insulin, leptin, ALT, AST, uric acid, creatinine, TC, TG, LDL-c, VLDL-c) and significant increase ($P<0.05$) in HDL-c level compared to the control group (–ve). Group of rats were fed 50%fat, 20%protein, 30%

carbohydrates had best result in weight loss compared other tested groups.

Conclusion

HFD had significant neuronal degeneration, disrupted DG architecture, and alterations in astrocyte activity and molecular markers. Conversely, IF showed protective and restorative effects on DG integrity and function, as evidenced by the improved morphology of neurons, decreased astrocyte reactivity, and increased expression of neuroprotective markers such as Nrf2 and TH. Importantly, introducing IF after HFD exposure (HFD+IF group) markedly mitigated the HFD-induced DG damage, restoring neuronal architecture, reducing astrocyte activation, and improving molecular profiles. IF may serve as a potential intervention to counteract the adverse neural effects of HFD by enhancing neuroplasticity, reducing inflammation, and improving antioxidant responses in the hippocampus.

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Conflict of Interest: Nil

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