*Original Article*
http://mjiri.iums.ac.ir
**Medical Journal of the Islamic Republic of Iran (MJIRI)**

*Med J Islam Repub Iran.* 2022 (21 Feb);36.12. https://doi.org/10.47176/mjiri.36.12

# The Effect of Calorie Restriction and Intermittent Fasting on Impaired Cognitive Function in High-Fat Diet-Induced Obesity Started Post-Weaning in Male Wistar Rat

Nazanin Asghari Hanjani[1], Negar Zamaninour[2], Somayeh Athari Nik Azm[3], AghaFatemeh hosseini[4], Farinaz Nasirinezhad[5], Mohammad Reza Vafa[1]*

Received: 9 Feb 2020 Published: 21 Feb 2022

## Abstract

**Background:** Calorie Restriction (CR) is known as one of the most effective life-extending interventions. Therefore researchers are looking for other interventions or drugs to mimic the mentioned effects. Time-restricted feeding (TRF) has recently gained more attention recently as one of the CR mimetics. Here we evaluate and compare the effects of CR or TRF on cognitive function in young animals fed a high-fat diet (HFD).

**Methods:** This is an experimental study that three-week-old male Wistar rats (n:52) were subjected to a control diet (n:11) or HFD (n:42). Then the HFD group was divided into 1) 30% calorie restriction (CR), 2) Night Intermittent Fasting (NIF), 3) Day Intermittent Fasting (DIF), and 4) Ad-Libitum (AL) with the standard diet for ten weeks (each of 9). An independent T-test or Mann–Whitney test was used for the first phase and in the second phase of the study, one-way analysis of variance (ANOVA), followed by Tukey post-hoc tests, or Kruskal–Wallis and post-hoc Bonferroni test were used. P-values of <0.05 were considered significant

**Results:** Deteriorated mental function was significantly lower in HFD than CON (p= 0.041). CR was still more efficient than NIF in cognitive function in obese subjects. Post-hoc test indicated that from day 2-4, escape latency was significantly shorter in NIF and CR, which was not seen in other groups (p=0.045).

**Conclusion:** While TRF has garnered much attention recently, here we show that CR is still more efficient in learning and memory tasks. Longer fasting times and different fasting periods are recommended to study.

**Keywords:** Cognitive Function, Calorie Restriction, Intermittent Fasting, Circadian

*Conflicts of Interest:* None declared
*Funding:* Iran University of Medical Sciences (Grant Number: 94-03-27-26581).



*Cite this article as*: Hanjani NA, Zamaninour N, Azam SA, Hoseini AF, Nasirinezhad F, Vafa MR. The Effect of Calorie Restriction and Intermittent Fasting on Impaired Cognitive Function in High-Fat Diet-Induced Obesity Started Post-Weaning in Male Wistar Rat. *Med J Islam Repub Iran.* 2022 (21 Feb);36:12. https://doi.org/10.47176/mjiri.36.12

## Introduction

For many reasons, weight control is an important issue for young ages. Firstly, thousands of beliefs and habits are formed during childhood that can be hard to change. Secondly, being overweight or obese in childhood and

*Corresponding author:* Dr Mohammad Reza Vafa, vafa.m@iums.ac.ir

1. Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
2. Department of Minimally Invasive, Surgery Research Center, Iran University of Medical Sciences, Tehran, Iran
3. Cellular and Molecular Nutrition Department, School of Nutritional and Dietetics, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Statistics and Mathematics, School of Health Management and Information Science, Iran University of Medical Sciences, Tehran, Iran
5. Physiology Research Center, Department of Physiology, Iran University of Medical Sciences, Teharn.Iran

***↑What is "already known" in this topic:***
According to literature, obesity and specifically childhood obesity, are increasing worldwide which is accompanied by many chronic diseases. Moreover, there is a correlation between obesity and cognitive function as well. At the opposite extreme, calorie restriction is known as one of the most beneficial interventions to extend lifespan and reduce chronic diseases, and a large number of research have been dedicated to find calorie restriction mimetics to achieve the same outcomes.

***→What this article adds:***
Early life high-fat diet can impair cognitive function in adulthood which can be inverted by calorie restriction. In Comparison, time-restricted feeding according to nocturnal rodent circadian rhythm (not in the form of alternate fasting) was less efficient in this regard.

adolescence may lead to lifelong obesity and increase the risk of the early onset of chronic diseases. Thirdly, lower educational achievements and psychological disorders are related to childhood and adolescent obesity (1, 2). According to the World Health Organization (3), the number of overweight children under the age of five was estimated at more than 41 million in 2016, and its trend is no longer similar to that of adults (4). While it is clear that obesity can lead to many chronic diseases, mounting evidence indicates that there is a correlation between obesity and cognitive function as well. However, fewer studies have focused on childhood obesity and its effects on adulthood learning abilities. Dietary intake is an important factor in hippocampus maturation and will affect learning skills later in life (5, 6).

The major explanation for the current global obesity pandemic is the overconsumption of high-calorie foods. Epidemiological studies in the USA, Canada, and China have indicated that obesity incidence increases by the increment of the average amount of fat in the diet. (7). Generally, food intake can be manipulated to be too low (to cause malnutrition) or too high (to lead to obesity). Calorie restriction (CR) lies between these two extremes and reduces calories without compromising appropriate nutrients intake (8). Since 1935, different studies have shown that CR is the only non-genetic intervention that can extend lifespan and healthspan in many model organisms (9, 10).

A large number of research efforts have been dedicated to finding interventions that mimic the effects of CR. One of these interventions which have been highlighted recently is intermittent fasting (2), which may be more feasible in practice. The protocols of IF in different studies offer an alternate day fasting or time-restricted feeding. Most animal and human studies have worked with alternate day fasting, which is mostly considered as a main IF protocol (11, 12). Applying the IF protocol while considering the timing of food intake may be a good mimetic of calorie restriction. The other concern in such studies is to provide the calorie-restricted groups' food at the beginning of the night phase (which is identical to the light phase in humans) to avoid circadian regulation disturbance, which is usually neglected in such studies.

The comparison of the different effects of these two dietary interventions against nutritional challenges that predispose obesity (and their consequences on general health and cognitive function in vivo) has yet to be investigated. Accordingly, this study aims to investigate the effect of a high-fat diet (started postweaning) on oxidative stress, inflammatory markers and cognitive function in young rats and subsequently to investigate the effects of CR and IF in dark and light cycles on the above-mentioned parameters. The underlying question was to determine which dietary interventions are most effective against obesity-associated cognitive function, especially at an early age.

## Methods

### *Animals and Diets*

This is an experimental study that the protocol was approved by the Iran University of Medical Sciences Ethics Committee (ir.iums.rec. 0327-26581). Postweaning male Wistar rats (three weeks old) were obtained from Pasteur Institute of Iran and were individually housed in cages at a controlled temperature (22 ± 2 C) and humidity (50%) in a designated room under a 12-h light-dark cycle. They were allowed free access to food and water for one week. After one week of accommodation, the animals were divided into two groups, including a high-fat diet (n=41) offering 60% of calories from milk butter and a control diet (n=11) receiving standard chow (18.8 MJ/kg with 23.4% as protein, 4.5% as fat, and 72.1% as carbohydrate, Ralston-Purina and other necessary nutrients for the growth of rats) for 17 weeks. A subsample from HFD was housed in metabolic cages to measure the rat food intake for the second phase of the study and to evaluate calorie restrictions. The experimental diets were freshly prepared every 3 days and were kept at 0-4 °C to avoid any rancidity. After significantly increasing the HFD weight (12.5% higher body weight compared with the control group), the first phase of the study was completed. The behavioral tasks were performed by the animals, which still were on their respective diets at the time of testing. To minimize the stress induced by the behavioral test on biochemical analyses, one week after the behavioral test and 12h fasting, the rats were anesthetized by ketamine and xylazine, and blood samples were collected from the aortic vein. Blood samples were centrifuged (1500 g, 15 min at 4°C), the serum was collected and stored at -80°C. hsCRP and 8-Isoprostane were assessed by ELISA method using (Zell-Bio, Germany). In the second phase of the study, the HFD group was divided into four groups (n=9): 1) 30% Calorie restriction (CR), 2) Night Intermittent Fasting (IFN), 3) Day Intermittent Fasting (IFD), and 4) Ad-Libitum (AL), in such a way that the average weight in all groups was the same. The second phase of the study continued with the four derivative groups from a high-fat diet and control group for ten weeks (13, 14). Under the time-restricted feeding, rats were allowed to access the food between ZT 13 (one hour after light off) and ZT1 (1 hr after light on) (Fig. 1).

At the end of this stage, cognitive function was re-examined and blood samples were taken. Similar to the first phase, a veterinarian regularly monitored the health

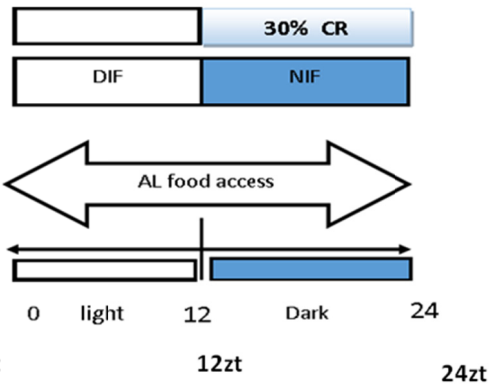


*Fig. 1.* Schematic figure of dietary patterns in the second phase of the study

status of the rats throughout the experiment.

### Morris Water Maze

Morris water maze (MWM) is a broad test used to assess spatial learning and memory. It consists of a round black pool (94-cm diameter and 31-cm depth) filled with 25 ± 0.5°C water. The escape platform was submerged 2cm beneath the water surface and placed in the center of one of the pool quadrants. External cues, including colorful posters and light, were placed around the pool and remained in the same position until the end of the study. The test was conducted on four consecutive training days, which evaluated learning a performance based on escape latency and travel distance, with a probe trial on day five, which assessed spatial accuracy without the platform. During the training periods, rats were gently released into water from different quadrants. Each rat was allowed to find the hidden platform within 1 minute; if it did not find the platform, it was gently placed on the platform and allowed to rest there for 15 s. A probe trial was administered 24 h after the training days, with the platform removed. Rats were released into the water for 1 minute and their spatial learning memory was calculated based on the time which they spent in the target quadrant. After finishing the test, each day, rats were manually dried with a towel and a warm breeze. All data were recorded by means of Nodules software (EthoVision XT 6.1, Netherland). The groups were controlled for the time of the day in which the tests were performed concerning their feeding protocols.

### Cue Test

Thirty minutes after the probe test, a visual cue test was conducted to ensure that poor performance was not due to visual defects. For this purpose, the platform was located one centimeter above the water surface, marked with a white tap to be visible, and rats without visual problems could find them through local visual cues, not with spatial memory (data not shown).

### Statistical Analysis

Investigators were blinded to the group assignment while performing behavioral and biochemical analyses and became unblinded with the statistical analyses. The data are reported as mean±SD. The statistical analyses were performed using SPSS software (2013 - IBM SPSS Statistics for Windows, Version 22.0. Armonk NY: IBM Corp). Levene's test was used to assume the equal variance of each group and the normality of the data distribution was checked through Kolmogorov–Smirnov test. In the first phase of the study, data was analyzed through an independent T-test or its non-parametric alternative, the Mann–Whitney test. In the second phase of the study, one-way analysis of variance (ANOVA), followed by Tukey post-hoc tests, were used to compare the results when the distribution of data was normal. Otherwise, the Kruskal–Wallis and post-hoc Bonferroni test were used. P-values of <0.05 were considered significant.

## Results

### Oxidative stress and inflammation

There were no significant changes in serum concentration of hs-CRP and isoprostane in the first and second phases. Since there was no notable increment in the first phase, changes in the restricted group were not expected (Table 1).

### The Effect of Juvenile High Fat Diet on Spatial Learning and Memory

As indicated in Figure 2, escape latency reduced during the four days of training in the first phase, while the reduction was significantly lower in the HFD group on days three and four (p: 0.027,0.009). The changes in distance traveled were similar, while there was not a significant change in swimming speed, indicating the negative role of post-weaning HFD on adulthood learning, which did not affect motor function. Moreover, data obtained from the probe trial revealed a memory function impairment. In other words, the percentage of time in the target quadrant was significantly lower in HFD than CON (p= 0.041).

It was then evaluated whether 10 weeks of CR or time-restricted feeding regimens could affect impaired learning and memory function. Generally, escape latency and distance traveled significantly decreased in all groups, compared to the first phase (p<0.001) (Fig. 3). A Bonferroni post-hoc test indicated that from day 2-4, escape latency was significantly shorter in NIF and CR, which was not seen in other groups (p=0.045). During 4 days of acquisition, DIF took longer to find the hidden platform while CR and NIF spent a shorter time, which was not significant (p>0.05). These data indicated a protective role of NIF in learning ability, equivalent to CR. The distance traveled was longer in DIF (98.48±40.15) and shorter in NIF (150.40±81.80), which was not statistically significant. Similar to the first phase of the study, no difference in swimming speed was found between groups. The time

*Table 1.* Isoprostane and hsCRP in the first and second phase

| First phase | Isoprostane (ng/L) | hsCRP (ng/L) |
|---|---|---|
| HFD | 22.88±4.86 | 293.6±4.53 |
| CON1 | 28.98±10.75 | 261.3±20.43 |
| p-value | 0.28 | 0.15 |
| Second phase | | |
| CR | 27.37±5.78 | 380.57±122.66 |
| DIF | 29.56±11.25 | 329.38±57.74 |
| NIF | 28.04±8.9 | 316.15±77.14 |
| AL | 25.37±3.30 | 285.82±72.32 |
| CON2 | 31.75±6.38 | 344.05±99.09 |
| P-value | 0.35 | 0.44 |

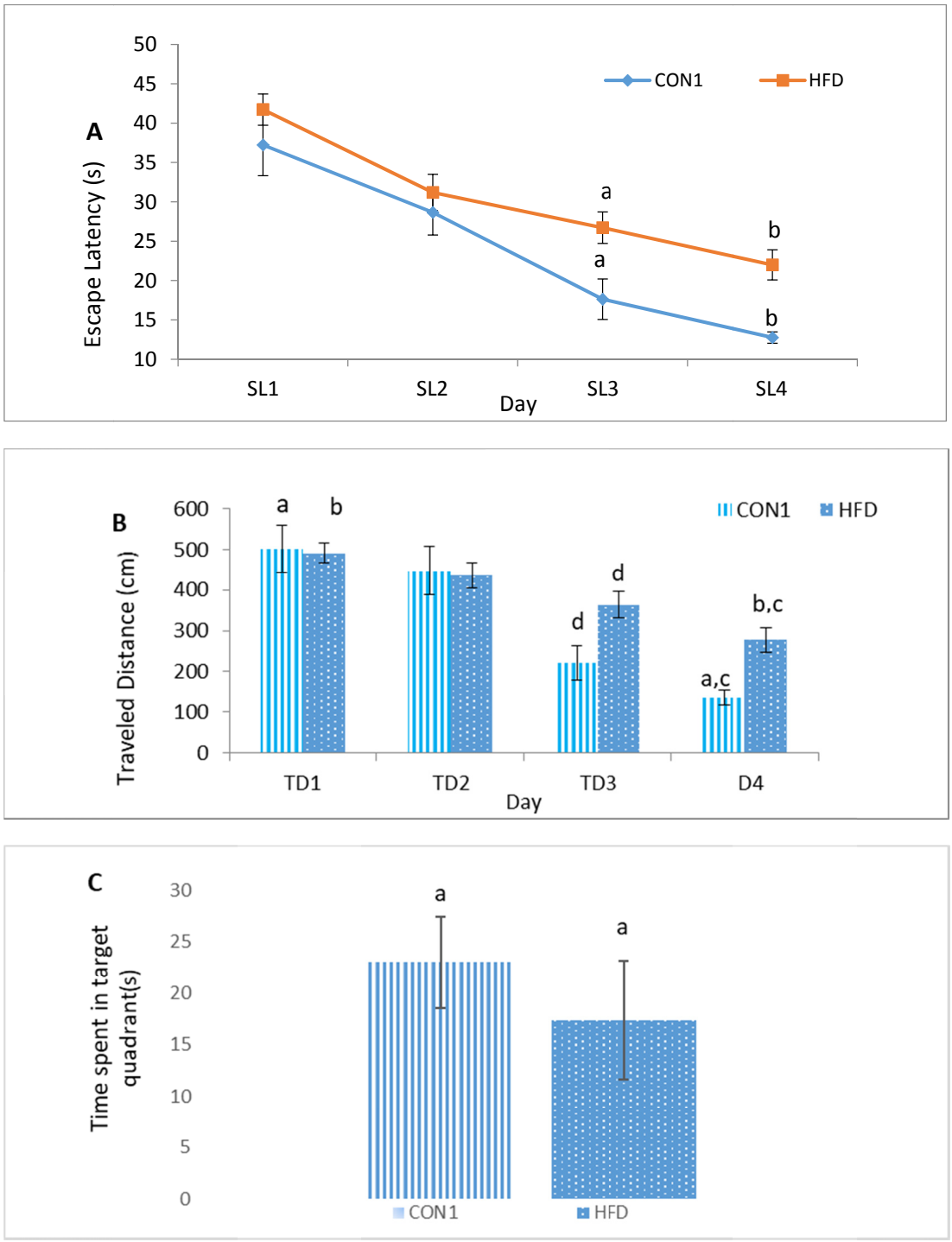


Fig. 2. A: Escape latency during the first phase, B: Traveled distance during the first phase,C: Percentage of time in the target quadrant during the first phase. Data presented as mean± SE. Independent T-test or its non-parametric alternative, the Mann–Whitney test were used to assess data. HFD: high fat diet, CON1: first phase control. P<0.05 was considered as statistically significant. The same letters indicate a significant change between groups.

spent in the target quadrant increased in the second phase of the study in all HFD derivatives groups and was not statically significant (p= 0.552).

The cue test revealed that the rats did not have visual defects.

## Discussion

Lifestyle interventions and dietary patterns play a vital role in mental function and health span enhancement. The primary goal of this study was to elucidate the effects of a juvenile HFD on adulthood learning and subsequently to

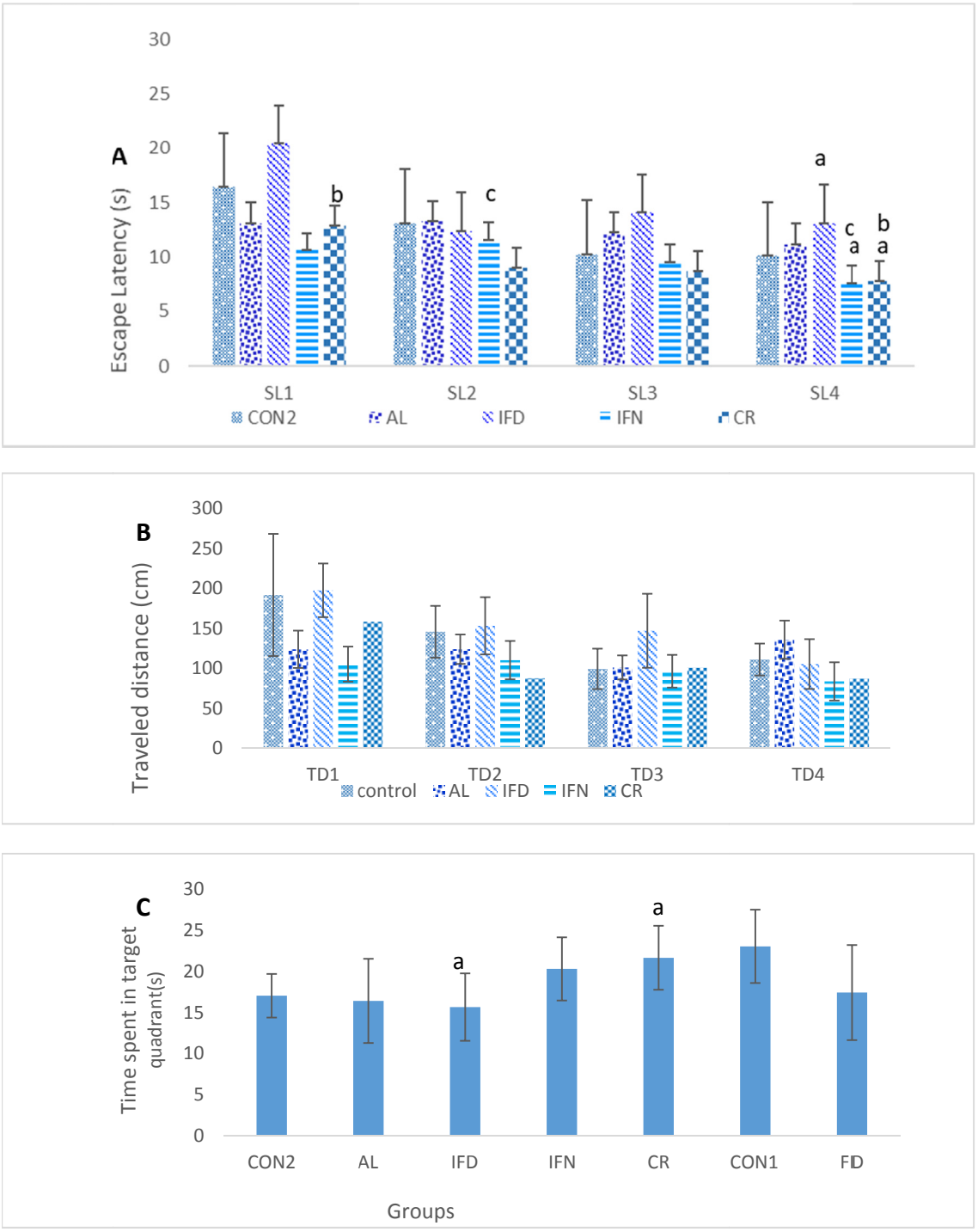


*Fig. 3.* A: Escape latency during the second phase, B: Traveled distance during the second phase, C: Time spent in target quadrant. Data presented as mean± SE. statistical differences between groups were assessed by, one-way (ANOVA), followed by Tukey post-hoc tests or Kruskal–Wallis and post-hoc Bonferroni. AL: adlibitum, IFD: intermittent fasting during the day, IFN: intermittent fasting during night, CON2: second phase control . P<0.05. The same letters indicate a significant change between groups. P-values of <0.05 were considered significant.

compare the effects of CR, NIF, and DIF with AL feeding on learning and oxidative stress.

The findings show that while juvenile HFD is responsible for adulthood cognitive impairment (as assessed in MWM), firstly, CR and then NIF significantly improved learning and memory in comparison to other dietary interventions. Since 1949, ad libitum feeding of a high-fat diet has been used for inducing obesity in animals, which was later called dietary obesity. The combination of fat and sugar (cafeteria diet) or adding a particular fat (30-78% of total energy) are the common protocols that are used to induce obesity (7). Previously, Paul J. et al. reported that a

Western diet (41% fat) and a high-fat diet (60%) in rats causes cognitive impairment due to brain inflammation and brain-dried neurotropic factor reduction (15). Data suggested that a high-fat diet can influence neuronal plasticity and CNS function independently from cerebrovascular function. It has been shown that 22 weeks of HFD reduced the SIRTUIN1 content in the hippocampus, which prevents neuronal regeneration (16).

Interestingly, it was reported that neuroinflammation caused by HFD could happen independently from peripheral inflammation (6); the 8-isoprostane and hsCRP markers of serum concentration did not change following 17 weeks of HFD, while cognitive impairment was reported as assessed by MWM. Engel et al. reported that dietary fat manipulation might not change the hsCRP level in healthy subjects and it is suggested that this factor is not sensitive to diet fat content (17). Two to threefold increment in inflammatory cytokines in the bloodstream is a low-grade, systemic inflammation indicator (18, 19) and while we didn't observe a significant alteration of inflammatory markers in the first phase the desired reduction at the end of the study is not expected. Collectively, the results of this study reinforce the idea of juvenile cognitive function vulnerability to HFD. Subsequently, in the second phase of the study, CR and NIF had positive impacts on learning, while CR could also improve the time spent in the target quadrant in comparison to DIF on the probe test, which indicates memory function's improvement. This suggests a positive effect of NIF on learning while not leading to significant memory improvement in comparison to the DIF. Several pathways have been hypothesized as playing a role in CR's mediated effect on cognitive function, including increasing hippocampus SIRT1 content, mitochondrial biogenesis and neuroprotective factors that decrease neuronal apoptosis (20-22).

The other hypothesis around CR's effects focuses on its hermetic function, which refers to mild stressor effects that are followed by increased expression of resistance molecules and decreased inflammation (23), which is what causes learning improvement in the NIF group. In other words, nutrient metabolism in all organisms has evolved to be cyclical, which means that the metabolism rate is controlled by the circadian rhythm, which is mainly regulated by the Suprachiasmatic nucleus in the hypothalamus. The Suprachiasmatic nucleus transmits signals to neuronal structures in all organs and controls their physiological responses. Hence, perturbation of the circadian oscillator can lead to obesity, metabolic disorders, and cognitive dysfunction.

It is hypothesized that intermittent fasting increases protein chaperones and neurotrophic factors by inducing a mild cellular stress response (24, 25). On the contrary, recently, it has been reported that mistimed feeding results in changes of clock molecules in the hippocampus (corticolimbic) while SCN remains unchanged. This misalignment results in hippocampal-dependent learning impairment (26). The most provocative finding is that despite the positive effect of NIF on learning, CR is still a more efficient intervention on learning and memory, while inappropriate food intake time inversely affects learning and memory.

## Conclusion

In summary, an early life high-fat diet can predispose an organism to adulthood cognitive dysfunction, which may be inverted by CR or NIF. While TRF has garnered much attention recently, the present study points out that CR is still more efficient in learning and memory tasks. It is possible that longer fasting times can have a positive and significant effect on either learning or memory, and study designs with different fasting periods are recommended. Future experiments need to be conducted in order to elucidate SIRTUIN and BDNF levels in the hippocampus alongside the memory task's results for a better conclusion.

### *Conflict of Interests*

The authors declare that they have no competing interests.

## References


1. Collaboration NRF. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128• 9 million children, adolescents, and adults. Lancet. 2017.
2. Caird J, Kavanagh J, O'Mara-Eves A, Oliver K, Oliver S, Stansfiel C, et al. Does being overweight impede academic attainment? A systematic review. Health Educ J. 2014;73(5):497-521.
3. WHO 2011, NA/WHO- FS_ObesityAndOverweight.pdf 2011.
4. WHO. Childhood overweight and obesity. 2016; Available from: http://www.who.int/dietphysicalactivity/childhood/en/.
5. Reinert KRS, Po'e EK, Barkin SL. The relationship between executive function and obesity in children and adolescents: a systematic literature review. J Obes. 2013;2013.
6. Boitard C, Cavaroc A, Sauvant J, Aubert A, Castanon N, Layé S, et al. Impairment of hippocampal-dependent memory induced by juvenile high-fat diet intake is associated with enhanced hippocampal inflammation in rats. Brain Behav Immun. 2014;40:9-17.
7. Hariri N, Thibault L. High-fat diet-induced obesity in animal models. Nutr Res Rev. 2010;23(2):270-299.
8. Jain S, Singh SN. Calorie Restriction â An Approach towards Obesity Management. J Nutr Disord Ther. 2015.
9. McCay C, Crowell MF, Maynard L. The effect of retarded growth upon the length of life span and upon the ultimate body size. J Nutr. 1935;10(1):63-79.
10. Most J, Tosti V, Redman LM, Fontana L. Calorie restriction in humans: an update. Ageing Res Rev. 2017;39:36-45.
11. Varady KA, Roohk DJ, Loe YC, McEvoy-Hein BK, Hellerstein MK. Effects of modified alternate-day fasting regimens on adipocyte size, triglyceride metabolism, and plasma adiponectin levels in mice. J Lipid Res. 2007;48(10):2212-2219.
12. Park S, Min Yoo K, Suk Hyun J, Kang S. Intermittent fasting reduces body fat but exacerbates hepatic insulin resistance in young rats regardless of high protein and fat diets. J Nutr Biochem. 2017;40:14-22.
13. Woodie L, Blythe S. The differential effects of high-fat and high-fructose diets on physiology and behavior in male rats. Nutr Neurosci. 2018;21(5):328-336.
14. Park S, Zhang T, Wu X, Yi Qiu J. Ketone production by ketogenic diet and by intermittent fasting has different effects on the gut microbiota and disease progression in an Alzheimer's disease rat model. J Clin Biochem Nutr. 2020:19-87.
15. Pistell PJ, Morrison CD, Gupta S, Knight AG, Keller JN, Ingram DK, et al. Cognitive Impairment Following High Fat Diet Consumption is Associated with Brain Inflammation. J Lipid Res. 2010;219(1-2):25.
16. Heyward FD, Walton RG, Carle MS, Coleman MA, Garvey WT, Sweatt JD. Adult mice maintained on a high-fat diet exhibit object location memory deficits and reduced hippocampal SIRT1 gene expression. Neurobiol Learn Mem. 2012;98(1):25-32.

17. Engel S, Tholstrup T. Butter increased total and LDL cholesterol compared with olive oil but resulted in higher HDL cholesterol compared with a habitual diet. Am J Clin Nutr. 2015;102(2):309-315.
18. Ridker PM. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory hypothesis toward consensus. J Am Coll Cardiol. 2007;49(21):2129-2138.
19. Calle MC, Andersen CJ. Assessment of dietary patterns represents a potential, yet variable, measure of inflammatory status: a review and update. Dis Markers. 2019;2019.
20 Veronese N, Facchini S, Stubbs B, Luchini C, Solmi M, Manzato E, et al. Weight loss is associated with improvements in cognitive function among overweight and obese people: A systematic review and meta-analysis. Neurosci Biobehav Rev. 2017;72:87-94.
21. Ma L, Wang R, Dong W, Li Y, Xu B, Zhang J. Long-term caloric restriction in mice may prevent age-related learning impairment via suppression of apoptosis. Behav Brain Res. 2016;315:45-50.
22. Parikh I, Guo J, Chuang KH, Zhong Y, Rempe RG, Hoffman JD, et al. Caloric restriction preserves memory and reduces anxiety of aging mice with early enhancement of neurovascular functions. Aging (Albany NY). 2016;8(11):2814.
23. Yang L, Licastro D, Cava E, Veronese N, Spelta F, Rizza W, et al. Long-term calorie restriction enhances cellular quality-control processes in human skeletal muscle. Cell Rep. 2016;14(3):422-428.
24. Ma L, Zhao Z, Wang R, Zhang X, Zhang J, Dong W, et al. Caloric restriction can improve learning ability in C57/BL mice via regulation of the insulin-PI3K/Akt signaling pathway. Neurol Sci. 2014;35:1381-1386.
25. Giller K, Huebbe P, Hennig S, Dose J, Pallauf K, Doering F, et al. Beneficial effects of a 6-month dietary restriction are time-dependently abolished within 2 weeks or 6 months of refeeding—genome-wide transcriptome analysis in mouse liver. Free Radic Biol Med. 2013;61:170-178.
26. Ruby NF, Fisher N, Patton DF, Paul MJ, Fernandez F, Heller HC. Scheduled feeding restores memory and modulates c-Fos expression in the suprachiasmatic nucleus and septohippocampal complex. Sci Rep. 2017;7(1):6755.