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Original Article

Intermittent fasting could ameliorate cognitive function against distress by regulation of inflammatory response pathway

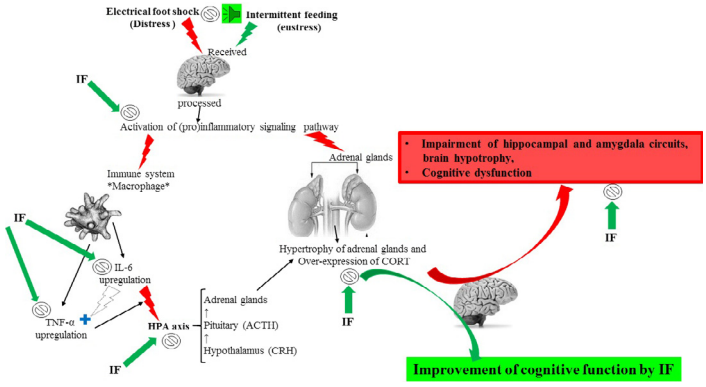
Marjan Shojaie [a], Farzane Ghanbari [b], Nasrin Shojaie [c,*]

[a] Department of Physiology, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
[b] Department of Biology, Islamic Azad University, Tehran, Iran
[c] Faculty of Biological Science, Tarbiat Modares University (TMU), Tehran, Iran

HIGHLIGHTS

- IF could significantly modify pathological effects of distress on brain and adrenal glands.
- In distressful condition, IF leads to reduce the plasma level of the stress hormone (CORT).
- IF has an inhibitory effect on neuro-inflammation caused by distress.
- IF could strongly improve learning and memory function in distressful condition.

GRAPHICAL ABSTRACT



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ABSTRACT

Undesirable and desirable effects of stressors on the body are assigned to distress and eustress, respectively. Immune system and brain are the most susceptible parts to stressful conditions, whereas long-lasting alterations in putative immune proteins involved in tension such as corticosterone (CORT), interleukin 6 (IL-6), and tumor necrosis factor-alpha (TNF-α) can impact learning and memory. Intermittent fasting (IF) is a repeated regular cycle of dietary restriction with well-known beneficial properties on the body. The aim of this study was to identify the eustress effects of IF on cognitive function by assessing the critical inflammatory factors in chronic distress. Forty male mice were divided into four groups (n = 10/group). Distress and control normally received food and water, whereas IF and IF with distress groups were daily deprived of food and water for two hours. In the second week, the electrical foot shock was induced to distress and IF with distress groups. Finally, the cognitive functions of all mice were evaluated by Barnes maze, their blood samples were taken to determine the plasma level of CORT, IL-6 and TNF-α, and the removed brain and adrenal glands were weighed in the third week. A significant gain in plasma level of CORT, IL-6 and TNF-α with a considerable brain hypotrophy and adrenal hypertrophy was found in distress group, whereas IF caused a remarkable reduction of the plasma inflammatory factors, especially in IF with distress mice ($P \leq 0.05$). In conclusion, IF could improve cognitive function and preserve the brain against distress by regulation of inflammatory response pathway.

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* Corresponding author.
E-mail address: Nasrinbiochem@yahoo.com (N. Shojaie).

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Introduction

Stress was nominated as an illness by Hans Selye, however, it was only accepted as a unique concept to find the influence of environmental factors (stressors) on living beings in the 1990s [1]. Generally, chronic stress leads to an adaptation between organism and its environment [2,3], however distress disturbs homeostasis by its adverse effects on the heart [3], digestive system, and brain in the long term [4,5]. Brain messages are changeable and remade by stress to control anxiety, temper, and make right decision [4]. Moreover, stress is an intensive regulator of memory and learning functions [6,7] via the hypothalamic–pitui tary–adrenal (HPA) axis [8]. The main part of the brain relevant to learning and memory is the hippocampus (HC) region, which is very sensitive to corticosterone and cytokines [9]. Therefore, brain has a central role in response to all favorable or unfavorable environmental changes for the transmission of the essential signals to all organs via immune system, endocrine, autocrine, and neural mechanisms [5]. Corticosterone is a well-known stress hormone [10]. In fact, it was overexpressed in distress conditions with hypertrophy of adrenal glands and subsequently leads to impairments in hippocampal and amygdala circuits, which play key roles in cognitive function [10].

IL-6 produced by the committed immune cells, is a critical factor in inflammation and subsequently neurodegeneration and brain dysfunction mediated by distress [10]. Besides, IL-6 and TNF-α, other important cytokine in inflammation, is a potent activator of the hypothalamic-pituitary adrenal axis, key element in communication with the neuroendocrine system, and glucocorticoid (e.g. corticosterone) production. Interestingly, this cytokine with its dual conflicting properties, pro- and anti-inflammatory, can alter cognitive function in stressful condition [11,12]. In contrast to distress, the positive responses in the body arise from eustress such as intermittent fasting (IF) [2,9,13]. IF is actually a food deprivation period, which is repeated between a fasting and non-fasting duration with no malnutrition effects [14,15]. The amelioration of neurodegenerative disorders, such as Alzheimer's disease [16,17], regulation of inflammatory responses [13] even in animals with systemic bacterial infection [18], reduction of risk factors for cardiovascular disease [3] and cancer [19] are some positive effects attributed to IF [13,15]. In addition, food deprivation may promote lifespan and brain functions even in the mice with high fat diet fasting [15,20]. There is no reference relevant to IF ability to manage distress. Therefore, with respect to the importance of prevention of learning and memory loss caused by distress, the aim of the current study was to evaluate the eustress effects of IF on cognitive function by assessment of the vigorous inflammatory factors; corticosterone, IL-6 and TNF-α during chronic distress (electrical foot shock).

Experimental

Animals

Forty male BALB/c mice (Pasteur Institute of Iran) with mean weight 25 g were used in this study. They were specifically assigned to four groups (n = 10/group); control, distress and IF as well as IF with distress [16,20]. *Animal experiments were performed based on Ethical Research Committee guidelines of Hormozgan University of Medical Sciences that comply with NIH guidelines (Registration no. 0436).*

Stress induction

Control and distress mice daily received enough food and water for 18 days, while the other groups were under food and water deprivation for two hours (time: 12–2 pm) daily in the second week. Besides, at this week, while all mice were under sociopsychological stress in the communication box for one hour (time: 9–10 am), electrical foot shock (40 mV, 10 *Hz*) was induced for 100 s only for distress and IF with distress groups in the box [21].

Evaluation of cognitive function by Barnes maze

Barnes maze is a round platform (diameter, 92 cm) with 20 equal holes (diameter, 7.5 cm) on the circumference and the escape box ((30 × 30 × 20 cm) with some food placed under one of the holes. To determine the cognitive functions in the third week (15th to 18th day), the tool was located 120 cm above the floor. All mice were placed at the center of Barnes maze and examined their learning and memory functions one by one. The elapsed time for finding escape box in the maze was registered by a digital camera connected to a computer system with open control program (as a video-tracking software) [18]. Escape latencies of 15th–18th day were considered as criteria for learning and memory assessment.

Blood and tissue collection

After the last cognitive function assay (18th day), blood samples (Max. 1 mL) were taken from retroorbital sinus of all mice and diluted by 0.5 mL sodium citrate 1%. Finally, the mice were sacrificed and their adrenal glands and brains were removed and weighed.

Determination of TNF-α, IL-6, and corticosterone

TNF-α and IL-6 ELISA KITs were purchased from Diaclone Company (Besancon Cedex, France) and corticosterone ELISA was provided by DRG Company (Marburg, Germany). The plasma levels of these critical factors were measured exactly via the protocols stated by the manufacturer.

Statistical analysis

The data analysis was performed by SPSS version 17.0 (IBM Corporation, USA) with significant $P < 0.05$. The results were presented as mean ± SD and statistical significance was assessed by ANOVA with Tukey's method (HSD). Based on the calculated *P*, all alterations of the determined parameters were compared between distress and other three groups.

Results

IF could significantly modify pathological effects of distress on adrenal glands and brain

The adrenal glands and brain of the mice were removed, dried, and weighed at the end of the study. Data analysis of weighing showed that there was a significant weight gain in adrenal glands between distress and the other groups, however IF could suppress hypertrophy of adrenal glands in IF with distress mice (Fig. 1A). Besides, a significant weight loss was found in the brain between distress and the other groups, IF could remarkably moderate the brain hypotrophy in the mice with distress (Fig. 1B).

IF led to reduce the plasma level of the stress hormone (CORT) in distressful condition

Distress significantly increased plasma corticosterone level without any notable alteration in the other groups. However, IF could firmly inhibit this elevation in the mice with distress (Fig. 2).

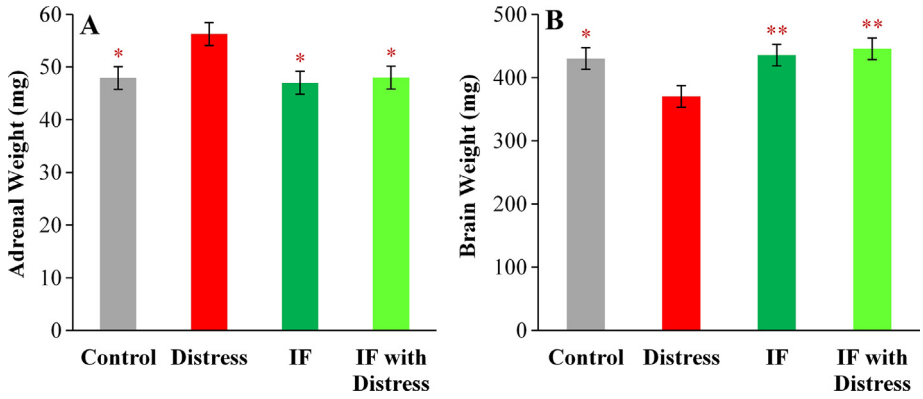

Fig. 1. The alterations of the adrenal glands and the brain weight in different groups. A significant adrenal glands hypertrophy (A) and a notable brain hypotrophy (B) were determined in distress group, however, these elements had no significant alterations in IF with distress mice.*: $0.05 < P \leq 0.01$, **: $0.01 < P \leq 0.001$, ***: $P < 0.001$ versus distress group.

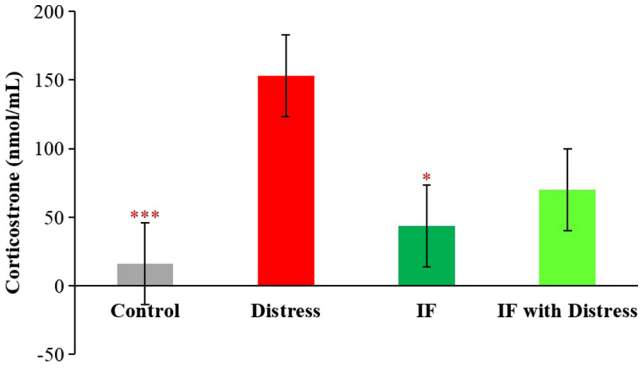

Fig. 2. The alterations of plasma corticosterone (CORT) level in different groups. A remarkable enhancement of plasma CORT level was revealed in distress group especially in comparison with control. However, IF could inhibit this elevation by 50% in the mice with distress.*: $0.05 < P \leq 0.01$, **: $0.01 < P \leq 0.001$, ***: $P < 0.001$ versus distress group.

IF had inhibitory effect on neuro-inflammation caused by distress

As indicated in Fig. 3A, in comparison with control, IL6 was significantly upregulated by distress, however; it was considerably downregulated in IF group. IF also could strongly suppress this elevation in the mice with distress. Moreover, it was found that TNF-α was significantly upregulated only in distress group, however, IF caused a notable reduction particularly in the mice with distress (Fig. 3B).

IF could strongly improve learning and memory in distressful condition

The statistical analysis of the registered escape latencies revealed that there was a significant reduction in the learning and memory functions by IF. In addition, no remarkable effect of distress on cognitive function was determined in distress and control mice, because both of them had a long delay to find escape box. However, IF led to a considerable improvement of cognitive functions particularly in the mice with distress (Fig. 4).

Discussion

Stress is a priority for all health systems in the world and a wide range of psychotherapy techniques [22] and drugs [4,23] were utilized to manage it. However, in agreement with the definition of stress, these strategies, themselves, could be considered as a stressor [4,5]. Tension is generally referred to as the impact of environmental factors and even genetic variations on the mind [4]. It leads to various individual biochemical reactions associated with behavioral changes in the body [4]. Based on stress influence on the body, it is classified into eustress and distress. However, simultaneously

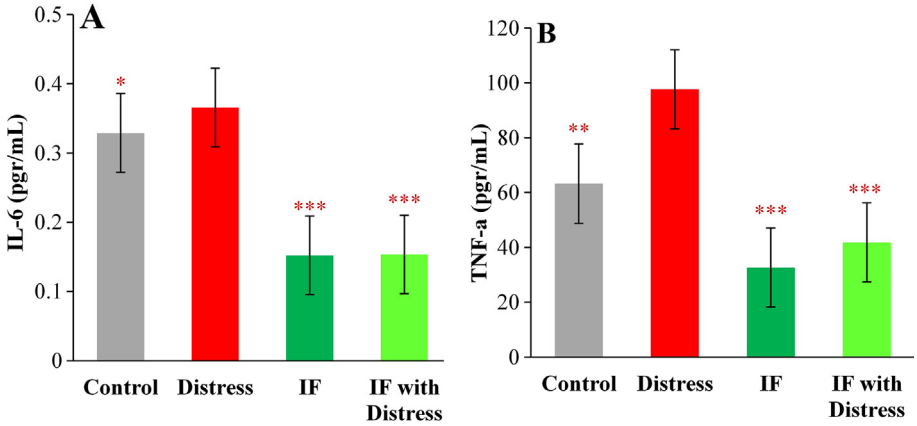

Fig. 3. The alterations of plasma interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α) levels in different groups. A remarkable production of IL-6 and TNF-α were determined in distress group, however, IL-6 and TNF-α were significantly downregulated by 58% and 57%, in IF with distress mice, respectively.*: $0.05 < P \leq 0.01$, **: $0.01 < P \leq 0.001$, ***: $P < 0.001$ versus distress group.

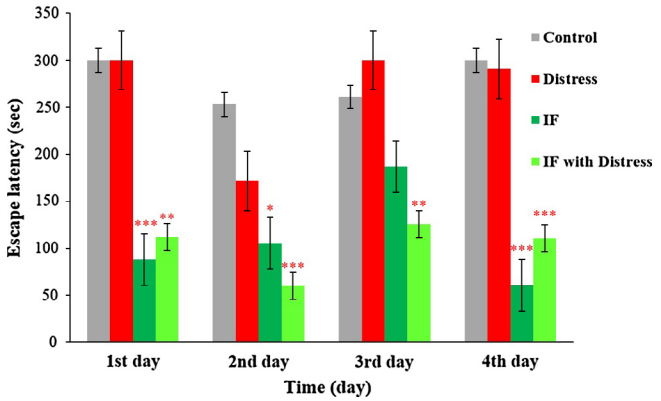

Fig. 4. The alterations of learning and memory functions. Escape latencies registered from the 15th to the 18th day represented learning and memory functions. There was no notable difference between control and distress groups in cognitive function, however the escape latencies significantly reduced in IF and particularly IF with distress mice.*: $0.05 < P \leq 0.01$, **: $0.01 < P \leq 0.001$, ***: $P < 0.001$ versus distress group.

they can mutually neutralize each other's effects on their joint target organs [2,24].

Fig. 5 displayed major feasible events during stressful conditions. The message of stress is processed by brain and triggers pro- or anti-inflammatory response pathways. IL-6 with dual contradictory features, pro- and anti-inflammation, in inflammatory response pathway can lead to neurodegeneration and neuroplasticity [25] and both distress and eustress usually cause IL-6 secretion. However, its tissue origin is a critical factor to determine its pro- or anti-inflammatory response. Generally, in the presence of distress, including obesity, IL-6 is upregulated in the committed immune cells, such as macrophages, and subsequently results in pro-inflammatory response and neurodegeneration [26]. Whereas, in the presence of eustress, such as intermittent fasting, IL-6 is downregulated in these cells to ameliorate inflammation [27]. However, exercise is a typical form of eustress that leads to IL-6 upregulation in muscles. This isoform of IL-6 has neuroprotection property with anti-inflammatory and immune regulatory effects [11,12]. Besides, stress-exposure time is another critical parameter to evaluate stress effects on the health as short-time exposure to exercise results in CORT overexpression, however, chronic exercise rapidly downregulates it [28]. In agreement with these studies, it was revealed that pro-inflammatory signaling pathway was hyperactivated under distress conditions to produce TNF-α [29], IL-6 [25], and CORT. Furthermore, IL-6 produced by the committed immune cells stimulates macrophages to over-express TNF-α [11,30]. These cytokines are the main elements in brain impairments (brain hypotrophy) and HPA axis stimulation to elevate plasma CORT level and adrenal glands hypertrophy. Besides, chronic exposure to CORT leads to neuro-inflammatory and neurotoxic responses as well as cognitive dysfunction (Fig. 5) [31].

Intermittent fasting (every other day plan) is a known positive stressor revealed to have eustress effects in both animal and human studies [18]. It was observed that both acute and chronic IF could improve cognitive function (Data not shown). In accordance to several studies, these findings also showed that IF could stimulate brain to downregulate inflammatory response pathway by different molecular mechanisms [17,31]. Particularly, this message was transmitted to the committed immune cells in distress conditions to decrease the upregulated IL-6 and TNF-α and subsequently, it led to a significant reduction in over-expressed CORT. With respect to the main role of CORT in cognitive dysfunction [10], it was indicated that IF could strongly suppress CORT upregulation and its adverse effects on the brain and also it causes a notable significant improvement in learning and memory functions (Fig. 5). Furthermore, IF group had no significant enhancement of plasma CORT level, therefore in agreement with a

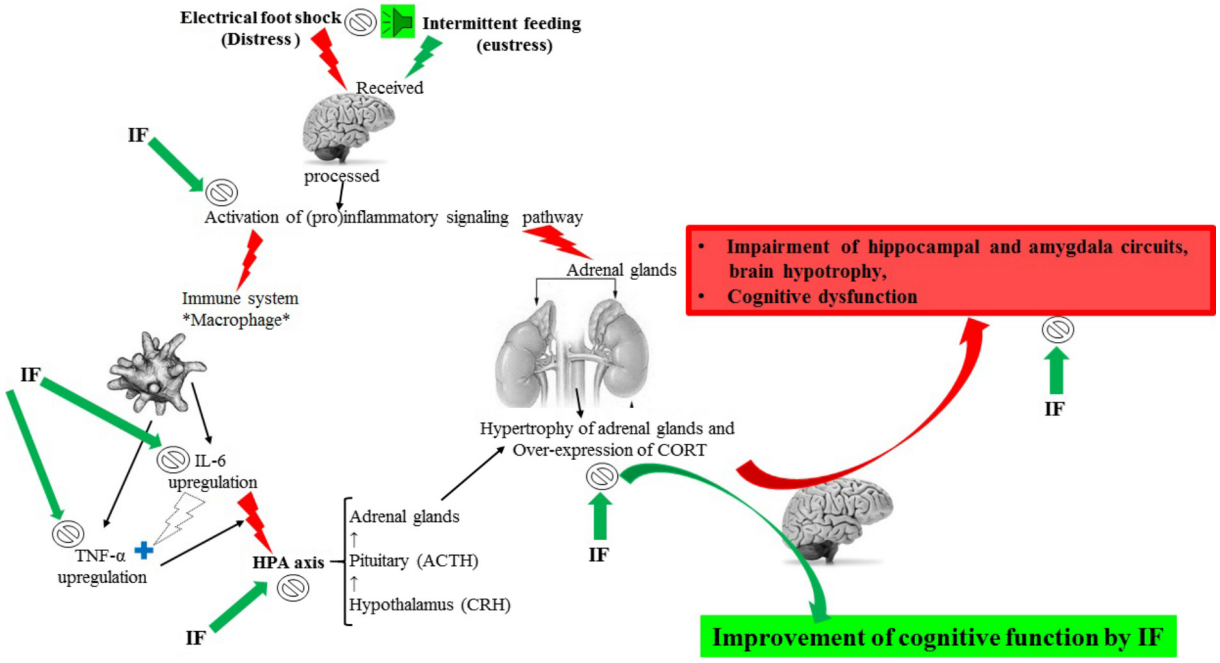

Fig. 5. The susceptible organs and pathways to stress. The red symbols and black arrows imply in distress (electrical foot shock) footprints while the green ones represent IF (Intermittent fasting) traces. Both distress and eustress messages have the joint targets in the body. They are also received and processed by brain. The processed distress signals activate (pro) inflammatory responses and stimulate adrenal glands to over-express corticosterone (CORT) and the committed immune cells to upregulate interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α). IL-6 can also trigger (✚) TNF-α-induced macrophages. Hypothalamic–pituitary–adrenal (HPA) axis is the main targets of these secreted cytokines and subsequent CORT overexpression, adrenal glands hypertrophy and loss of spatial learning and memory. As it has been shown in the figure, the activated sites by distress can potentially suppress via IF (⃠) and finally lead to amelioration of cognitive function.

number of researches, neuroendocrine cells are independently sensitive to IF signal to attenuate CORT production [9] and it seems that CORT is a distress hormone. Together, the findings revealed that IF is a preferable modifier for inflammatory response pathway and the function of hippocampus as the processor of cognitive function [32]. These organs are also the targets of chronic stressors [6] therefore, with respect to these joint points between IF and distress, brain may have complicated distinctive mechanism(s) to recognize stress-induced different messages and regulate a variety of aspects relevant to the health.

Conclusions

The current findings demonstrated that intermittent fasting would be a useful tool in distressful condition to improve learning and memory by downregulation of the putative molecular factors involved in neuro-inflammation, although chronic stressors are generally well-known for adverse effects on the body particularly cognitive decline [4,5].

Conflict of Interest

The authors have declared no conflict of interest.

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