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Impact of Gender on Short-Term- Alternate-Day Fasting´s Neuronal Autophagy Induction in Albino Rats

Angie Mohammad Ameen[a,e], Rasha Atta[a,e], Noha M. Abd El-Fadeal[b,c,d,e], Mona F. Mansour[a,e]

[a] Department of Human Physiology, Faculty of Medicine, Suez Canal University, 41522, Ismailia, Egypt.
[b] Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Suez Canal University, 41522, Ismailia, Egypt.
[c] Department of Biochemistry, Ibn Sina National College for Medical Studies,22421, Jeddah, Saudi Arabia.
[d] Oncology Diagnostic Unit, Faculty of Medicine, Suez Canal University, 41522, Ismailia, Egypt.
[e] Center of Excellence in Molecular and Cellular Medicine (CEMCM), Faculty of Medicine, Suez Canal University, 41522, Ismailia, Egypt.

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- Alternate day Fasting
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- Gender

Abstract

Fasting enhances metabolic balance and cognitive function, by triggering autophagy. Autophagy may have a varied impact on disease mechanisms and consequences depending on gender differences. With the hypothesis that these processes differ between both genders, this study investigates sex variations in autophagy and cognitive responses generated by short-term alternative day fasting. **Aim:** The purpose of this study was to examine the impacts of short-term alternate-day fasting on neuronal autophagic induction, with a specific focus on identifying gender differences. **Methods:** Rats were assigned into four groups: Normal diet male group, Normal diet female group, Alternate fasting male group, and Alternate fasting female group. After 1 week, rats were examined for neurocognitive function, metabolic and autophagic induction. **Result:** Short-term alternate day fasting improve cognitive functions in both sexes with female rats being superior in stress response, and spatial memory. Reduced energy intake in alternative day fasting decreased insulin, leptin production equally in both sexes and elevated levels of AMPK. Elevated cortisol levels were significantly higher in alternate fasting female. Decreased mTOR levels in both genders. Upregulation of PPAR gamma, adiponectin, and autophagy markers like LC3 and Beclin-1. **Conclusion:** Both sexes exhibited higher autophagic responses with female exhibit superior results in several aspects. Higher cortisol levels and more exploratory behavior were seen in females, suggesting that their coping mechanisms for stress had changed. Cognitive performance increased for both sexes, while females' BDNF elevation was higher. In terms of metabolism, fasting enhanced glucose regulation and decreased leptin levels in both sexes.

Corresponding author: Rasha Atta , Lecturer of Physiology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.
E-mail: rasha atta@med.suez.edu.eg. ORCID: **https://orcid.org/0000-0003-1547-1588**

Introduction

The brain's complex activity consumes ~25% of the body's energy expenditure at rest. The majority of this energy demand is utilized for membrane potential preservation and neuronal signaling; the remaining portion is employed for fundamental cellular functions such as axonal transport along with turnover of macromolecules and organelles. During food deprivation, the brain's energy sources are strongly controlled, and a metabolic shift to ketones occurs (1,2).

Recent data suggests that metabolic changes brought on by fasting may enhance brain functions in terms of cognitive functions, synaptic plasticity, and protection against diseases (3). Alternate day fasting (ADF), is a form of intermittent fasting, which is characterized by recurring intervals when access to food is limited or nonexistent and times when access is unrestricted. In rodent models, ADF showed beneficial outcomes in insulin and glucose control in addition to cognitive functions (4).

Molecular studies have shown that low energy or a lack of vital nutrients, like glucose and amino acids, can lead to a depletion of ATP and a rise in the AMP/ATP ratio, which then triggers autophagy. Autophagy is an essential homeostatic mechanism involved in the defense against cancer, infections, and neurological illnesses (5).There is growing evidence that autophagy is a gender-dependent mechanism (6). To date, the main regulatory mechanisms of sex differences in autophagy are poorly identified.

There are gender differences in several different types of diseases, and these differences affect many different elements of the disease, including its mechanism, occurrence, development, therapy, and prognosis. Numerous factors can contribute to illness variations, including genetic and environmental factors. Among these, autophagy which is impacted by a few sex-related diseases. In certain circumstances, sex variations in autophagy control may operate independently of other sex-related factors, but they may increase the risk of certain diseases and the degree of pathology (7).

The autophagy process can occur or be upregulated under normal physiological conditions or in response to stressors such as cytotoxic chemicals, DNA damage, hypoxia, and caloric deprivation (8,9). Autophagy is initiated by specific genes known as ATG genes (10,11). The stimulation or inhibition of different stress-responsive pathways, such as those involving AMP-activated protein kinase (AMPK—energy sensing) and the mammalian target of rapamycin (mTOR—nutrient sensing), affects the regulation of each step of autophagy via unique protein complexes (12,13).

Under the influence of the PI3K III complex, the ULK1 complex initiates the process by signaling the nucleation of autophagosomes and PIP3 (phosphatidylinositol 3 phosphate) production, which attracts additional Atg proteins to construct the phagophore (14). After that, the phagophore is recruited by two ubiquitin-like conjugation systems to produce the mature autophagosome by attracting ATG12–ATG5 and microtubule-associated protein light chain 3 (LC3) proteins (15). Through conjugation with

phosphatidylethanolamine, this mechanism transforms soluble LC3-I into the membrane-associated LC3-II form, which mostly localizes to both the inside and outside membranes of the autophagosome(16). Finally, LC3-II directs cargo to autophagosomes for lysosome destruction, the last stage of autophagy, by interacting with adaptor proteins including p62 (sequestosome-1/SQSTM1) (17,18).

This study hypothesized that sex differences are present in neural autophagy induced by short term ADF and has differential cognitive efficiency in adult male and female rats.

Materials and methods

The Faculty of Medicine's Research Ethics Committee of Suez Canal University in Egypt gave its approval to this experimental investigation (4732#). The experiment was conducted on Twenty-four male and female adult albino rats with average weight 200 mg.

Study groups: Four groups of rats were randomly assigned.

Normal diet male group, n =6: Males with free availability to ad libitum.

Normal diet female group, n=6: Females with free availability to ad libitum.

Alternate fasting male group, n=6: Male rats subjected to 24-hr feeding and then 24-hr fasting for 1 week) with free water access fasting period (19).

Alternate fasting female group, n=6: Female rats subjected to 24-hr feeding and then 24-hr fasting for 1 week) with free water access fasting period (19).

After 1 week, rats were examined for a battery of neurocognitive tests to assess stress response, anxiety and spatial memory.

Forced swimming test (FST):

Stress response was evaluated by FST and formed of one session (5 min. duration). Each rat was placed in a transparent glass container; 60 cm height, 38 cm width dimensions filled with ≈ 23°C fresh water. The diving percentage, struggle percentage and immobility percentage were measured (20).

Open field test (OFT):

Locomotor and anxiety behaviors were evaluated by OFT. Each rat was allowed individually to explore the arena for 5 min. duration. To avoid olfactory cues 70% ethanol was used to clean the device after every experiment. Total distance traveled (TDM), the center time (CT), the peripheral time, frequency of entry to the central area and rearing frequency were measured (21).

Barnes maze test (BMT):

Spatial memory was evaluated by BMT and assessing rat´s ability to learn and memorize escape box location. The test is formed of two stages. The first stage is the training period in which the rat was allowed for 3 min to explore the maze with aversion stimuli till reach the escape box. Each animal had one training trail daily for 3 days. The second stage was the probe test on the 4^{th}day which each rat was allowed to find the target hole after removal of the escape box, and latency time was recorded. Following each test, the maze was wiped with 70% alcohol (22).

Blood and tissue sampling:

At the end of the study, the rats were anesthetized by xylazine (10 mg/kg) and ketamine (90 mg/kg) following a fasting period of 8 h.

Serum samples and brains were stored at -20°C for further analysis.

Chemical analysis

Serum was used to measure leptin, insulin, and cortisol levels using ELISA kits purchased from MyBioSource (San Diego, CA, USA). The specific kit details are as follows: Cortisol (Catalog Number: MBS727040), Leptin (Catalog Number: MBS701500), and Insulin (Catalog Number: MBS281388). The ELISA tests were conducted using an Agilent BioTek 800TS-SI instrument from Winooski, Vermont, USA. Additionally, serum was used to measure fasting glucose levels using a colorimetric technique with the glucose kit from Sigma (Saint Louis, MO, USA), Product Code GAGO-20.

Supernatants from tissue homogenates were used to assess the following markers using the ELISA technique: BDNF, AMPK, mTOR, and the NADP/NADPH ratio. The Rat BDNF ELISA Kit (Catalog No.: MBS355345) and the AMPK Kit (Catalog No.: MBS765897) were obtained from MyBioSource, while the mTOR kit (Catalog No. LS-F175531) was purchased from Lifespan Biosciences (WA, USA). The NADP/NADPH kits were sourced from Abcam (Catalog No.: ab186033, Cambridge, Massachusetts, USA).

Molecular analysis:

A 15 mg portion of homogenized brain tissue was used for total RNA extraction with the miRNeasy Mini Kit from QIAGEN (Catalog No. 217004, Germany). Complementary DNA (cDNA) was synthesized using the QuantiTect Reverse Transcription Kit, also from QIAGEN (Catalog No. 205311, Germany). Gene expression analysis of Beclin1, LC3, Adiponectin, and PPAR-ɣ was performed using a Step One real-time PCR instrument (Thermo Scientific, Catalog No. 4376357, UK). The reaction mixture consisted of 10 µL of HERA SYBR® Green qPCR Master Mix (Willowfort, Birmingham, UK), 15 pmol of each primer pair (listed in Table 1), and 200 ng of cDNA. The PCR conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. mRNA fold changes for the target genes were calculated using the 2-△△Ct method (23), with gene expression normalized to β-actin levels.**(Table 1)**

Table (1): Primer's sequence for target genes.

Primers	Sequence		Annealing Temp.
Beclin1	Forward 5'CAGCCTCTGAAACTGGACACGA 3' Reverse 5'CTCTCCTGAGTTAGCCTCTTCC 3'	NM_019584	57 °C
LC3	Forward 5'GTCCTGGACAAGACCAAGTTCC 3' Reverse 5'CCATTCACCAGGAGGAAGAAGG 3'	NM_026160	60 °C
Adiponectin	Forward 5'AGATGGCACTCCTGGAGAGAAG3' Reverse 5'ACATAAGCGGCTTCTCCAGGCT3'	NM_009605	60 °C
PPAR-ɣ	Forward 5'GTACTGTCGGTTTCAGAAGTGCC 3' Reverse 5'ATCTCCGCCAACAGCTTCTCCT 3'	NM_011146	60 °C
β -actin	Forward 5'TCCTCCTGAGCGCAAGTACTCT3' Reverse 5'GCTCAGTAACAGTCCGCCTAGAA3'	NM_007393.5	60 °C

Results

Impact of Alternate Fasting on neurobehavioral studies:

Forced swimming test:

Diving percentage was significantly highest in alternate fasting female (14.85%±4.61) compared to all other groups. Struggle percentage was significantly lower in alternate fasting female group (41.68%±0.76) compared to all other groups, including both normal diet groups and alternate fasting males. whereas alternate fasting male group showed lower significant struggle percentage (57.23%±5.57) compared to normal female group. Immobility percentage were 43.47%±5.1 and 41.11%±6.1 in alternate fasting female and male respectively that were significantly higher compared to normal diet female. **(Figure1)**

Open field test:

There was no significant difference in the total distance traveled among the different groups. However, alternate fasting female showed significantly more distance travelled in the central area (52.5± 2.5)compared to both sexes on a normal diet and alternate fasting males (17.5 ± 2.5). In addition, alternate fasting females visited the central area more frequently (4.5±0.5) compared to the normal diet males (1±0.0) and females (1±0.0). Alternate fasting male group showed no significant difference in central area travelled or frequency to central area entry with other studied groups. Finally, there was no significant difference in rearing behavior. **(Table2)** Overall, the significant findings highlight changes in central area exploration, particularly under alternate fasting conditions, which could be related to the diet's impact on behavior or anxiety levels.

Barnez maze test:

Both alternate fasting male (36.5± 2.5) and female (17.5±0.5) showed significant decrease in latency escape time compared to normal male (67.25± 4.02). **(Figure2)**

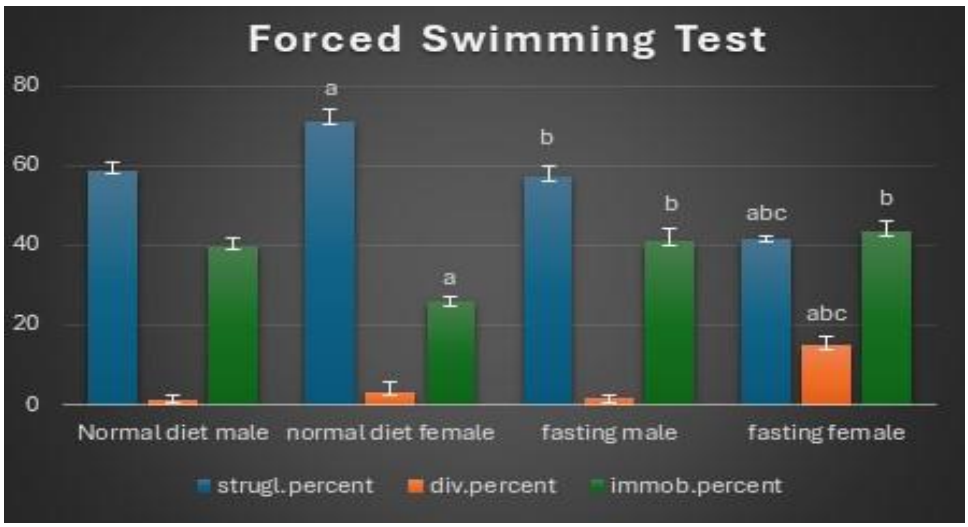

Figure 1: Forced swimming test among study groups. Data are represented by Means ± SEM. All data were analyzed using ANOVA followed by Bonferroni post hoc test. a (significant versus normal diet male group), b (significant versus normal diet female group), and c (significant versus alternate fasting male)

Table (2): Open field test parameters among study groups

Mean ± SEM	Normal diet male	Normal diet female	Alternate Fasting male	Alternate fasting female	Significance
Total distance	174 ± 4 squares	230 ±0.0 squares	250±0.0 squares	262.5 ± 52 squares	0.230
Distance in peripheral area	167 ± 2.5 squares	220 ±0.0 squares	235 ± 5 squares	210 ± 50 squares	0.383
Distance in central area	6.50± 1.5 squares	10 ± 0.0 squares	17.50 ± 2.5 squares	52.50± 2.5[abc] squares	0.000*
Frequency to central area	1±0.0	1±0.0	2±1	4.5±0.51[ab]	0.031*
Rearing	9±1	8±0.0	9±0.0	14±4	0.29

*** Significant P value <0.005 using one way ANOVA.**
a (significant versus normal diet male group), b (significant versus normal diet female group), and c (significant versus alternate fasting male) using Post-hoc Bonferroni test.

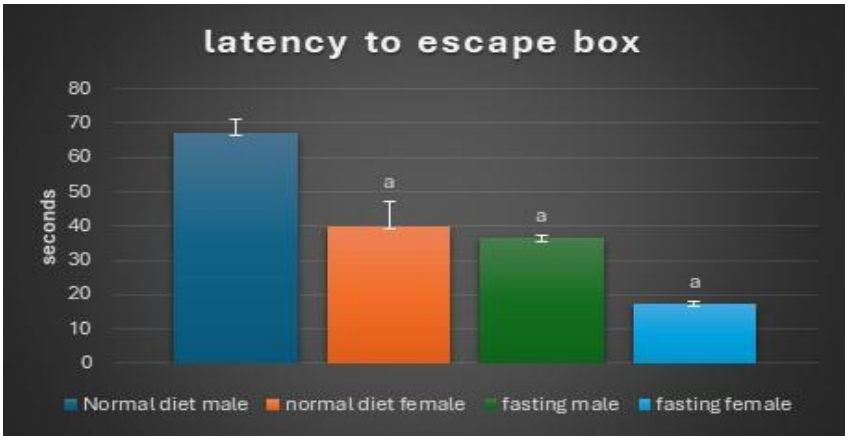

Figure 2: Barnes maze test among study groups. Data are represented by Means ± SEM. All data were analyzed using ANOVA followed by Bonferroni post hoc test. a (significant versus normal male group).

Impact of alternate fasting on blood glucose, insulin and cortisol levels:

Both males and females show a significant reduction in blood glucose levels under alternate fasting (93.6±3.7 and 97.7±0.3respectively, P < 0.05) compared to those on a normal diet (148±4.6 and165.7±5.5). Accordingly, blood insulin level was significantly reduced in both males and females on an alternate fasting diet (3.7±0.9 and 3.9±1.1respectively, P < 0.05) in comparison to their normal diet female group (11.4±2.9). There wasn't significant difference in insulin levels in between fasting males and females.**(Figure 3)**

The cortisol levels were measured after introducing rats in forced swimming test to assess stress response and results showed a quite similar between normal diet males and females (60.6±2.4 and 61.03±3.7 respectively).There was a significant elevation in cortisol in female rats under alternate fasting conditions (186.5±47.3, P < 0.05) in comparison to normal diet male and female groups (60.6±2.4 and 61.03±3.7 respectively), with cortisol levels nearly tripling compared to normal diet females, while male rats under alternate fasting show a non-significant increase (100.8±6.02). **(Figure 3)**

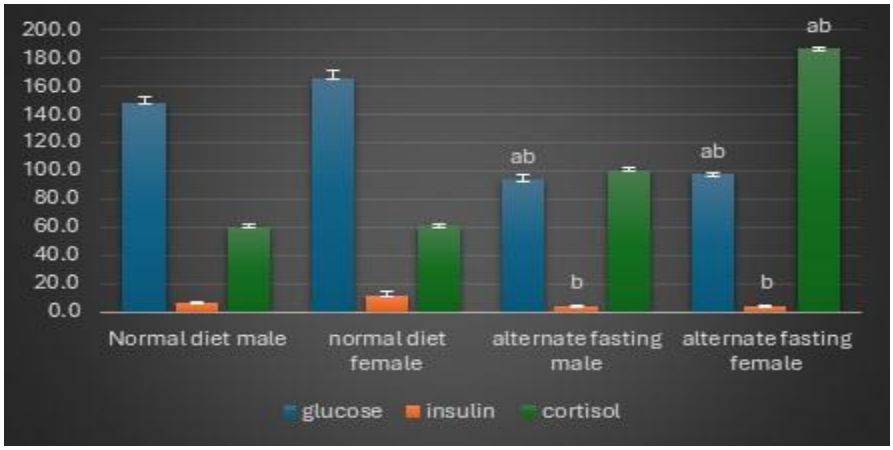

Figure 3: Blood glucose, insulin and cortisol hormones level among study groups. Data are represented by Means ± SEM. All data were analyzed using ANOVA followed by Bonferroni post hoc test. a (significant versus normal diet male group), and b (significant versus normal diet female group). Units of measuring: Glucose [mg/dl], Insulin [µIU/ml]&Cortisol [pg/ml]

Impact of alternate fasting on serum leptin level:

Analysis of serum leptin among studied groups showed that normal diet female group had significantly higher leptin levels (7.31±0.8) compared to normal diet male group (3.37±0.6). Both alternate fasting male group (2.33±0.1) and alternate fasting female group (1.79±0.3) had significantly lower leptin levels compared to normal diet female group. Alternate fasting male group showed no statistically significant difference (2.33±0.1) with normal diet male group (3.37±0.6). **(Figure 4)**

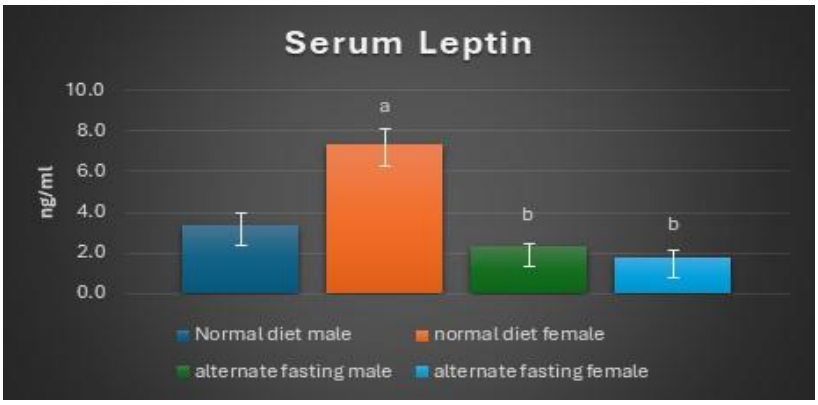

Figure 4: Serum leptin level among study groups. Data are represented by Means ± SEM. All data were analyzed using ANOVA followed by Bonferroni post hoc test. a (significant versus normal diet male group), and b (significant versus normal diet male group).

Impact of Alternate Fasting on brain BDNF Levels:

Alternate fasting male group (126.8±4.8) showed significant higher levels compared to normal diet male and female groups (65.1±3.5 and 84.6±3.6 respectively). The increase in BDNF levels was even more significant in females under alternate fasting (163.7±5.4, $P < 0.05$) in comparison to other studied groups. **(Figure5)**

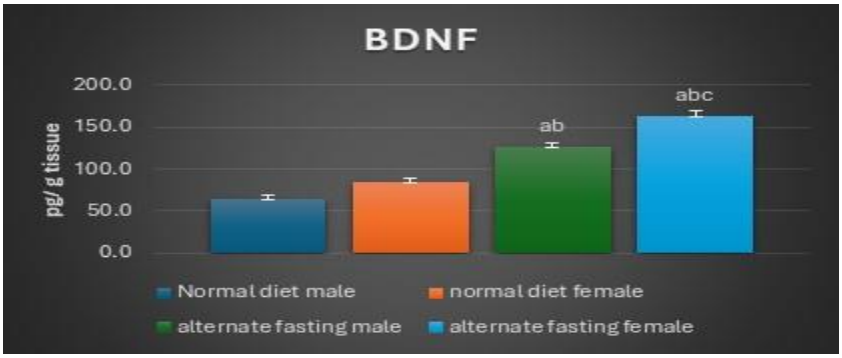

Figure 5: Brain BDNF level among study groups. Data are represented by Means ± SEM. All data were analyzed using ANOVA followed by Bonferroni post hoc test. a (significant versus normal diet male group), b (significant versus normal diet female group), and c (significant versus alternate fasting male)

Impact of Alternate Fasting on AMPK Levels:

Alternate fasting male group showed significantly higher levels (106.6±27) compared to the normal diet male and female groups (27.8±2.0 and 22.6±1.95,P < 0.05) respectively. The increase in AMPK levels was also observed in females under alternate fasting (60.8±1.4), though it wasn't significant with other groups. **(Figure 6)**

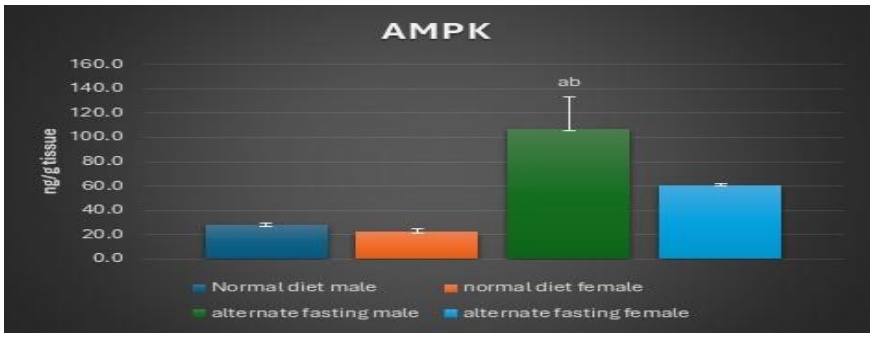

Figure 6: Brain AMPK level among study groups. Data are represented by Means ± SEM. All data were analyzed using ANOVA followed by Bonferroni post hoc test. a (significant versus normal diet male group), b (significant versus normal diet female group).

Impact of Alternate Fasting on mTOR Levels:

Alternate fasting male group showed significantly lower levels (1.75±0.6) compared to the normal diet male and female groups (4.23±0.1and 3.91±0.4,P < 0.05) respectively. A significant decrease in mTOR levels was found in the alternate fasting female group (1.88±0.1) when compared to male and female normal diet groups. **(Figure 7)**

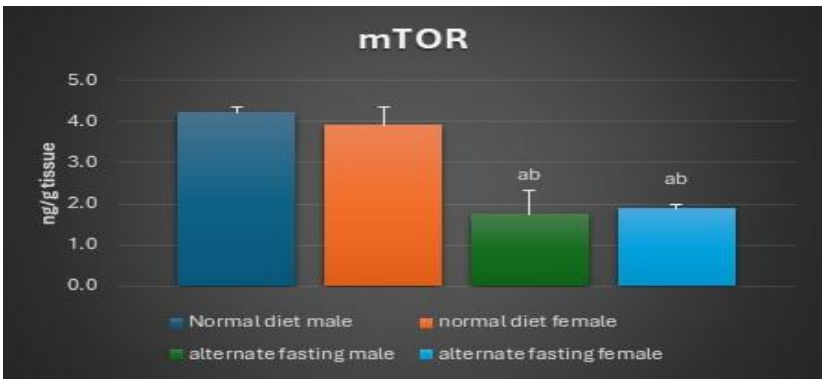

Figure 7: Brain mTOR level among study groups. Data are represented by Means ± SEM. All data were analyzed using ANOVA followed by Bonferroni post hoc test. a (significant versus normal diet male group), and b (significant versus normal diet female group).

Impact of Alternate Fasting on NADP/NADPH Levels:

Alternate fasting male group showed significantly higher levels (13.5±1.1) compared to the normal diet male group (8.8±0.4). A significant increase in NADP/NADPH ratio levels was observed in the alternate fasting female group (21.7±1.1), compared with both normal male and female group, and alternate fasting male groups (8.8±0.4, 9.7±0.5 and 13.5±1.1,P < 0.05). **(Figure8)**

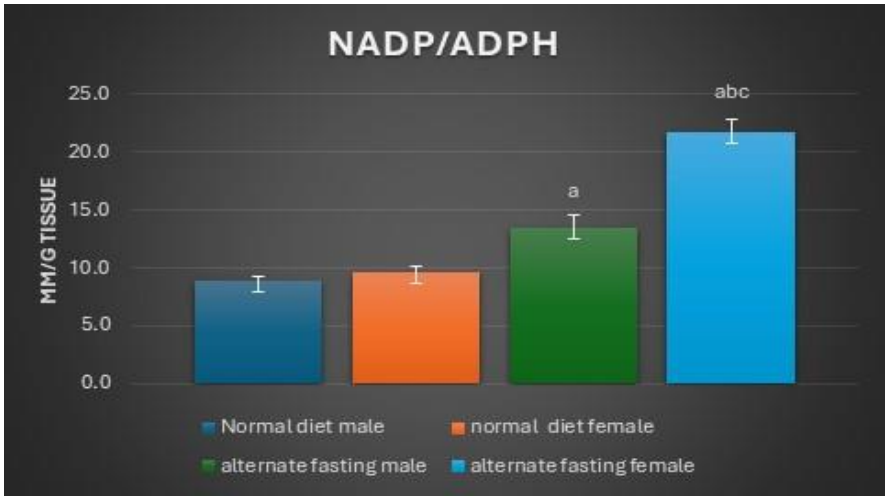

Figure 8: Brain NADP/ADPH ratio among study groups. Data are represented by Means ± SEM. All data were analyzed using ANOVA followed by Bonferroni post hoc test. a (significant versus normal diet male group), and b (significant versus normal diet female group).

Impact of Alternate Fasting on metabolic and autophagic gene expression:

There is a significant increase in PPAR gamma expression under alternate fasting in both males and females (2.4±0.5, 4±0.3, P< 0.005), with females showing a significant higher expression (P< 0.005) in comparison to normal diet groups. Adiponectin gene expression also increased significantly under alternate fasting, particularly in females (3.9±0.3, P< 0.005) in comparison to normal diet groups. LC3 gene expression, a marker of autophagy which is crucial for cellular maintenance and repair, increased significantly in both genders under alternate fasting (2.8±0.3, 3.8±0.6, P< 0.005) in comparison to normal diet groups. Beclin gene expression also increased significantly under alternate day fasting, in both sexes (2±0.3, 2.9±0.3,P< 0.005) in comparison to both sexes under normal diet. **(Table3)**

Table (3): Metabolic and autophagic gene expressions among study groups

Mean±SEM	Normal diet male	Normal diet female	Alternate Fasting male	Alternate fasting female	Significance
PPAR gamma	1±0.0	1±0.0	2.4±0.5	4±0.3abc	0.00*
adiponectin gene	1±0.0	1±0.0	1.6±0.2	3.9±0.3abc	0.00*
LC3 gene	1±0.0	1±0.0	2.8±0.3ab	3.8±0.6ab	0.001*
Beclin gene	1±0.0	1±0.0	2±0.3	2.9±0.3ab	0.002*
*** Significant P value <0.005 using one way ANOVA. a (significant versus normal diet male group), b (significant versus normal diet female group), and c (significant versus alternate fasting male) using Post-hoc Bonferroni test.**					

Discussion

In the present study, we have evaluated whether gender difference had a role in neuronal autophagy induction after short term alternative day fasting in rat model.

Results of the forced swimming test showed interesting behavioral variations between male and female rats that occur during periods of alternate fasting. The most remarkable finding is the significant rise in the dive percentage among the alternative fasting females, indicating that under stress, these animals might behave more exploratorily. This increased diving habit may be a stress-adaptive response, maybe related to the physiological modifications brought on by fasting. Levina et al., 2020 found that diving behavior in forced swimming test and Morris water maze was strain dependent and determined the exploratory activity (24).

The lower struggle percentage observed in alternate fasting females, and to a lesser extent in alternate fasting males, suggests that fasting may reduce the active coping responses typically seen in stressful situations. This could indicate that alternate fasting diminishes the motivation or energy to struggle, or it might reflect a shift in how the animals perceive and react to stress. In the FST, stress-induced behavior shifts to passive coping, which is characterized by immobility. Adaptation to these circumstances is believed to encourage energy saving, which prolongs survival(25,26).

Increased immobility in both alternate fasting females and males, compared to normal diet females, further supports the idea that alternate fasting alters behavioral responses to stress. Immobility is often interpreted as a measure of behavioral despair or resignation, and its increase in the fasting groups could indicate a heightened stress response or a reduced ability to engage in active coping mechanisms. The similarity in immobility between fasting males and females suggests that while there are sex differences in active behaviors like diving and struggling, the overall impact of fasting on passive behaviors like immobility may be similar across sexes. Our results were inconsistent with Cheng *et al.*, 2023 who found that the immobility time was significantly decreased with acute fasting ovariectomized mice (27). The difference may be due to differences in methodology and animal species.

Overall, these results suggest that alternate fasting has a significant impact on stress-related behaviors in rats, with more pronounced effects in females. The findings imply that alternate fasting may modulate how animals cope with stress, potentially through alterations in energy metabolism, hormonal balance, or neural pathways involved in stress responses. Our findings were similar to Alfheeaid et al., 2023 who found that alternative fasting improved cognitive functions in type 2 diabetes mellitus rat model (28).

Alternative day fasting showed differences in cognitive functions in rats based on gender difference. Female rats showed more explorative behavior during open field test based on increased central area entry and exploration.

Barnes maze testing results showed that alternative fasting had a positive impact on short term memory in both sexes which is an indirect measure of cognition. An improvement in cognition was also confirmed in a study by Li et al. (2013), in which Barnes maze test was used to

evaluate short term memory in 7-week-oldCD-1 wild type male mice after they had been exposed to control, high fat diet, or alternate-day fasting for 11 months. Mice in the fasted state performed better than those in the other groups (29).

Short term alternative day fasting was able to lower blood glucose levels in both sexes with approximately the same range. Similar study revealed the same effect on blood glucose levels after the use of several intermittent fasting protocols including alternative day fasting in type 2 diabetes mellitus rat model (28).

Alternative day fasting helps lower insulin resistance by changing metabolism and consuming fewer calories. Moreover, through energy/nutrient limitation (such as calorie restriction), increased AMPK activation could support healthy aging and limit chronic disease development (30). As demonstrated in this study, reduced energy intake in alternative day fasting should lead to long-term decreases in insulin production as well as elevated levels of AMPK, which is believed to contribute to enhanced insulin sensitivity and glucose homeostasis.

Cortisol is a steroid hormone secreted from adrenal cortex in response hypothalamic pituitary adrenal axis, autonomic signals and local clocks in the adrenal cortex (31). In our study female rats in alternative day fasting showed significant elevated cortisol level compared to other groups including alternative day fasting male after exposed to forced swimming test session. In human, several hypotheses linked this gender variation to women exhibit a shorter circadian rhythm period, an earlier phase of the biological clock gene expression rhythm, and are more of a "morning chronotype" than men (32,33).

Leptin hormone plays an essential function in regulating energy balance and food intake. It is secreted by adipocytes and the stomach, with its levels reflecting the body's energy reserves and food intake. In alternative day fasting both male and female groups showed significant decrease in serum leptin indicating hunger and increased food intake to restore energy balance (34).

Consistent with improved cognitive functions in alternative day fasting, BDNF levels were elevate in both genders with more significant results to female rats. This gender difference may be attributed to estrogen induced effect on brain BDNF levels in animal studies (35).

The research by Krisztina Marosi and colleagues highlights the relationship between fasting and BDNF levels. Fasting can enhance expression of BDNF in cognitive related neuronal circuits. Their findings suggest that cellular energy shift to ketones utilization during fasting contributes to the increased activity of these circuits. Additionally, both intermittent fasting and exercise appear to promote neurogenesis by encouraging stem cell neuronal differentiation and supporting the synaptic incorporation of newly formed neuronal cells. This study provides insight into the potential cognitive and neuroprotective benefits of fasting and exercise, linked to increased BDNF levels (36).

Autophagy is initiated through the assembly of various autophagy-related genes (Atg1-Atg12) and other proteins to form a phagophore, which includes essential components for autophagosomes (AP) and lysosomes fusion(37). The initial action involves Beclin1 and class III PI3K, crucial for vesicle isolation. Following this, the elongation stage uses

conjugation systems to convert LC3 to LC3-II, eventually leading to the fusion of APs and lysosomes, forming autolysosomes for degradation and recycling (38). Our findings demonstrate that intermittent fasting significantly upregulates autophagy markers, specifically Beclin-1, LC3, and PPAR gamma, supporting the role of these markers in autophagy initiation and cellular maintenance. This aligns with previous studies highlighting Beclin-1's essential function in autophagosome formation, aiding cellular recycling for repair and maintenance (39).

In addition, our findings indicate that intermittent fasting downregulates mTOR levels, a primary autophagy inhibitor under nutrient-rich conditions. This mTOR suppression during fasting enables the dephosphorylation of ULK1, triggering autophagy (40). Increased LC3 levels under alternate fasting further validate the enhancement of autophagosome maturation, promoting effective cellular cleanup, particularly in females. The regulatory balance between AMPK and mTOR is fundamental in this context, with our data showing significantly elevated AMPK levels, especially in males, and decreased mTOR levels in both genders under fasting conditions. Increased AMPK under fasting promotes autophagy by phosphorylating ULK1 and counteracting mTOR's autophagy-suppressive effects(41). This shift supports cellular adaptation and energy maintenance during nutrient scarcity. The rise in PPAR gamma expression, especially in females, enhances autophagic and metabolic processes by promoting fat metabolism, improving insulin sensitivity, and upregulating autophagy-related genes (42).

Furthermore, elevated NADP/NADPH ratios under fasting across both genders indicate an enhanced antioxidant response, contributing to cellular resilience by minimizing oxidative stress and fostering an autophagy-friendly environment (43). Collectively, upregulation of PPAR gamma, adiponectin, and autophagy markers like LC3 and Beclin-1 under intermittent fasting suggests broad metabolic and protective benefits. These benefits may be particularly valuable in metabolic disorders such as obesity and diabetes by enhancing insulin sensitivity and lipid metabolism and in neurodegenerative conditions by facilitating damaged protein clearance (44,45).

Conclusion

This study examined the role of short-term alternate-day fasting on neuronal autophagy induction, stress responses, metabolic health, and cognitive function in rats, focusing on gender differences. The results showed that fasting upregulated autophagy markers like Beclin-1, LC3, and PPAR gamma, while reducing mTOR and increasing AMPK activity, particularly in females. These changes support improved cellular maintenance and metabolic adaptation.

Behaviorally, fasting altered stress-coping responses, with females showing increased exploratory behavior and higher cortisol levels. Both sexes demonstrated improved cognitive performance, supported by elevated BDNF, particularly in females. Fasting also reduced leptin levels and improved glucose regulation, highlighting metabolic benefits. Overall, the study suggests that alternate-day fasting promotes neuroprotection, enhances autophagy, and improves metabolic health, with potentially stronger effects in females.

Conflict of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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