International Journal of Research Studies in Biosciences (IJRSB)
Volume 3, Issue 11, November 2015, PP 38-45
ISSN 2349-0357 (Print) & ISSN 2349-0365 (Online)
www.arcjournals.org

Effect of Intermittent Fasting on Brain Neurotransmitters, Neutrophils Phagocytic Activity, and Histopathological Finding in Some Organs in Rats

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Abstract: *Many previous studies had differed in answering question if the fasting has benefits or drawbacks. This work carried out on animal model to evaluate the impact of intermittent fasting on body and organs weight, hematological parameters, neutrophils phagocytic activity, brain neurotransmitters, some hepatic, renal biochemical parameters, electrolyte balance, as well as histopathological effect of fasting on liver, kidney, and spleen. Twenty male Wister rats were randomly divided into two groups of ten animals each. Group 1 served as control and group 2 was fasted for 12 h/ daily for 30 day. The results showed that fasting caused a significant decrease in the weight of body, liver and stomach while caused a significant increase in PCV, neutrophil phagocytic activity, phagocytic index and brain neurotransmitters (serotonin and norepinefrine). Additionally fasting caused a significant decrease in AST, ALT, Urea and Creatinine. Fasting has no effect on hemoglobin, WBC count, total protein, albumin, globulin, dopamine level and electrolyte balance. No pathological alteration could be detected in examined tissues; only proliferation of the lymphoid cells in the mantie zone in the splenic lymphoid follicle was detected in fasting group. On conclusion, intermittent fasting has beneficial influences on the immunity, neurotransmitter and other biochemical parameters.*

Keywords: *Brain neurotransmitters, histopathological finding, intermittent fasting, phagocytic activity, rats*

1. INTRODUCTION

Food restriction has marked effects on the body weight development, accompanied by reduction in the muscle mass and fat stores, this could be explained by the limited food supply which was not enough to meet the caloric requirement [1]. Ramadan fasting leads to weight loss and fat-free mass reductions [2].On the other hand, Nagra et al. [3] observed that the overall body weight did not show any significant variation during or at the end of fasting period.

Several studies have reported on the effect of fasting on the values of certain haematological factors [4, 5]. Red blood cells (RBCs) count, haemoglobin and hematocrit remained unchanged in fasting [4, 6], while other studies showed a slight degree of hemoconcentration [7]. Conversely, Dewanti et al. [8] showed a significant decrease in haemoglobin and hematocrit values. Maughan et al. [9] reported that the total leukocytic count of sporting players during Ramadan fasting was unchanged. On the other hand Unalacak et al. [10] recorded that white blood cells (WBCs) count was significantly lower after Ramadan fasting. Phagocytosis is the main part of innate immune system. Since decrease the phagocytotic cell activity is generally leading to immunosuppression and patients may expose to infections. The innate immune system, including phagocytotic cells, is the first line of defense against microbial disease, especially intracellular pathogens [11] .Some studies reported that fasted rats have higher neutrophils counts than control rats [12] and the percentage of neutrophils participating in phagocytosis increased with fasting [13]

The forebrain included important regions such as the thalamus, hypothalamus and hippocampus; neurotransmitters (serotonin, norepinefrine and dopamine) in these regions play a key role in the regulation of some brain functions such as emotion and behavior [14]. The catecholamines norepinephrine (NE) and epinephrine (EPI) have been implicated as important efferent immune modulators following exposure to stressors, often acting in concert with activation of the hypothalamic-pituitary axis. Catecholamines modulate a range of immune cell activities, including cell proliferation, cytokine and antibody production, lytic activity, and migration [15].

On human study, serum total proteins and albumin levels declined while the levels of serum globulin increased significantly with the advancement of fasting days [3]. Kamal et al. [16] reported, that Ramadan fasting induce significant decrease in the total protein level by the 14th Ramadan fasting days and statistically significant increase between 14th Ramadan and 26th Ramadan. On the other hand, fating increase the total proteins [17] and albumin [7]. Liver function tests such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase ALP were unchanged by fasting [18] in contrary to Unalacak et al. [10] which reported that, post-Ramadan ALT levels were significantly lower compared to pre-Ramadan values.

Creatinine clearance and the plasma/serum concentrations of creatinine, urea and uric acid are all potential measures of renal function [19]. Effect of fasting on serum creatinine and urea in healthy individuals were slight and were statistically not significant [20]. Nagra et al. [3] reported that the levels of blood urea nitrogen and serum creatinine remained within the normal physiological limits with the advancement of fasting days.

Serum sodium concentration is potential indicator of hydration status [21]. Trabelsi et al. [22] noted that serum electrolytes concentrations (sodium, potassium and chloride) did not change during fasting if aerobic training was undertaken after breaking the fast, whereas sodium and chloride concentrations increased if aerobic training was performed before breaking the fast.

By screening the previously published literatures about fasting, a very little data were written about histopathological changes in different body organs. Effect of long-term fasting on organ mass and intestinal morphometric parameters was investigated by Funes et al. [23], but for the author knowledge there is no histopathological data discussing the effect of intermittent fasting on body organs and tissues. Therefore we want to investigate that intermittent fasting could induce pathological alteration in tissues or not.

The effect of fasting on different body systems has not fully understood and there are many differences in the published data. Therefore, we aimed to investigate the effect of intermittent fasting on body and some organs weight, some hematological, immunological, biochemical parameters, and brain neurotransmitters in addition to investigate the histopatholoical changes in liver, kidney and spleen in healthy male rats as animal model.

2. MATERIALS AND METHODS

2.1. Animal model

Twenty male Wister rats (100-120 g) were used in this experiment. Animals were obtained from Al-Zyade experimental animal production center, Giza, Egypt. Animals were quarantined and allowed to acclimate for a week prior to the experiment. The animals were handled under standard laboratory conditions of a 12-h light/dark cycle in a temperature and humidity-controlled room. Water and feed were supplied ad libitum. All animal-handling procedures were carried out following the regulations of Institutional Animal Ethics Committee and with their prior approval for using the animals.

2.2. Experimental protocol

Rats were randomly divided into two groups of ten animals each (n=10). Group 1 served as control and received water and feed ad libitum. Group 2 was fasted for 12 hours/ daily for 30 consecutive days. Fasting was performed by moving rat to clean cages depraved from diet and water.

2.3. Blood sampling and brain tissue preparation

At the end of the experiment, rats examined externally, weighted, and blood sample collected from retro-orbital puncture under diethyl ether anesthesia. Blood samples were drawn into dry tubes (for obtaining serum) and heparinized tubes (for obtaining whole blood). Serum were separated by

centrifugation and stored at -20°C for subsequent analysis. The brain was rapidly excised according to the method of Heffner et al. [24], and transferred to a dry ice-cold glass plate and the forebrain was rapidly excised, plotted dry on a filter paper to remove excess fluid. Forebrain samples were stored at -80 C till taken for an analysis of monoamine content (neurotransmitters).

2.4. Gross weight

Body weight and weight of liver, stomach, spleen, heart, kidney and testis were determined.

2.5. Hematological investigations

Hematological parameters were determined by standard methods. Hemoglobin concentration was determined by the cyanomethemoglobin method [25]. Packed cell volume (PCV) was determined by microhematocrit method as described by Feldman et al. [26] using microhematocrit centrifuge. WBC were counted under the low power of microscope by using double improved Neubauer hemocytometer [27].

2.6. Determination of the neutrophils phagocytic activity

Phagocytic power of neutrophils using Candida Albicans was performed according to the method described by Ortega et al. [28].

2.7. Estimation of brain neurotransmitters

Each brain tissue sample was homogenized in 10 volumes of cold acidified n-butanol using a glass homogenizer; the sample weighting less than 300 mg was homogenized in 3 ml of acidified n-butanol [29]. The estimation of dopamine, norepinephrine and serotonin (5-HT) levels in the forebrain of rats were carried out according to the fluorometric method described by Ciarlone [30].

2.8. Biochemical investigation

Total protein and albumin were estimated by the method of Lowry et al. [31]. Globulin was determined by subtracting albumin values from total protein values and A/G ratio was calculated by dividing albumin values by globulin values. The level of serum AST, ALT was estimated by the method of Young [32]. Urea was estimated by the method of Patton and Croush [33] and creatinine was estimated by the method of Young [32]. Sodium (Na^+) and potassium (K^+) ions were determined by flame photometry.

2.9. Histopathological examination

After evisceration, the liver, kidney and spleen of each rat was removed, washed in saline and fixed in 10% neutral buffered formalin (NBF) for histopathological investigation. After 72 h of fixation, samples from the above-mentioned organs were dehydrated, embedded in paraffin wax and sectioned (3µm) for haematoxylin and eosin (HE) staining.

2.10. Statistical analysis

All data were expressed as mean ± standard error (SE). Paired t-test was used to compare control and fasting variables. Differences were considered significant when p values were less than 0.05. All analysis was performed using the statistical package (SPSS) version 16.0 (Chicago, IL, USA).

3. RESULTS

3.1. Gross weight

There is a significant decrease ($P < 0.05$) in body weight and weight of liver and stomach in fasting group in compare with control group while there is no significant change in weight of spleen, heart, kidney and testis between control and fasting group (Table 1).

Table1. *Effect of fasting on Body weight and weight of liver, stomach, spleen, heart, kidney and testis*

Parameters	Control	Fasting group
Body weight	92.67±4.15 [a]	74.07±4.56 [b]
Weight of liver	5.28±0.3 [a]	3.39±0.16 [b]
Weight of stomach	2.08±0.45 [a]	0.87±0.06 [b]
Weight of spleen	0.3±0.04	0.41±0.04
Weight of heart	0.45±0.03	0.46±0.03
Weight of kidney	0.99±0.05	0.94±0.07
Weight of testis	1.98±0.25	2.08±0.27

Different Letters in the same raw show significant difference at p <0.05

Hematological parameters

The results of hematological parameters have been summarized in table 2. Hemoglobin and WBCS showed no significant changes While PCV was significantly increased (P < 0.05) in fasting group.

Table2. *Effect of fasting on blood hemoglobin (Hb), packed cell volume (PCV) and white blood cells*

Parameters	Control	Fasting group
HB (g/dl)	12.85±0.55	13.39±0.48
PCV%	34.10±0.60[b]	40.70±1.04[a]
WBCs $1\times10^3/mm^3$	8.424±0.67	9.055±0.30

Different Letters in the same raw show significant difference at p <0.05

3.2. Neutrophil phagocytic activity

There is a significant increase (P < 0.05) in the phagocytic activity and phagocytic index in fasting group (Table 3).

Table3. *Effect of fasting on phagocytic activity and phagocytic index*

Parameters	Control	Fasting group
Phagocytic activity %	68.20±0.77[b]	73.50±0.86[a]
Phagocytic index	2.36±0.04[b]	2.71±0.06[a]

Different Letters in the same raw show significant difference at p <0.05

3.3. Brain monoamine content (neurotransmitters)

The effects of fasting on serotonin, dopamine and norepinephrine levels are shown in tables 4. The data indicated that levels of serotonin and norepinephrine in the forebrain of adult male rats were significantly increased (p<0.05) in fasting rats while there is no significant change in the dopamine level.

Table4. *Effect of fasting on monoamines content (Seratonin, Dopamine and Norepinephrine) in the forebrain of adult male albino rats*

Parameters	Control	Fasting group
Serotonin (µg/g)	0.35±0.05[b]	0.43±0.02[a]
Dopamine (µg/g)	0.81±0.05	0.92±0.07
Norepinephrine (µg/g)	0.63±0.01[b]	0.89±0.07[a]

Different Letters in the same raw show significant difference at p <0.05

3.4. Biochemical parameters

The results of biochemical parameters have been summarized in tables 5, 6 and 7. There is no significant change in the levels of total protein, albumin, globulin, albumin/ globulin ratio, sodium and potassium between control and fasting group while there is a significant decrease (P < 0.05) in ALT, AST, Urea and Creatinine in fasting group.

Table5. *Effect of fasting on Total protein, Albumin, Globulin, Albumin globulin ratio, AST and ALT*

Parameters	Control	Fasting group
Total protein (g/dl)	4.82±0.19	5.31±0.13
Albumin (g/dl)	3.18±0.09	3.32±0.06
Globulin (g/dl)	1.63±0.17	1.99±0.12
Albumin/ Globulin ratio	1.95±0.22	1.67±0.11
AST (IU/L)	48.20±0.83[a]	41.80±0.57[b]
ALT (IU/L)	71.80±0.99[a]	67.60±0.78[b]

Different Letters in the same raw show significant difference at p <0.05

Table6. *Effect of fasting on Urea and Creatinine*

Parameters	Control	Fasting group
Urea (mg/dL)	59.88±0.90[a]	54.41±0.87[b]
Creatinine (mg/dL)	2.27±0.1 4[a]	1.80±0.07[b]

Different Letters in the same raw show significant difference at p <0.05

Table7. *Effect of fasting on Sodium and Potassium*

Parameters	Control	Fasting group
Sodium (mmol/L)	142.8±1.25	141.5±2.86
Potassium (mmol/L)	5.16±0.23	5.04±0.11

Different Letters in the same raw show significant difference at p <0.05

3.5. Histopathological finding

3.5.1. Gross Lesions

Rats in the fasting group basically had no gross lesions but smaller liver and stomach than that of the control group were observed. No gross lesions were detected in control rat group.

3.5.2. Histopathology

Histopathological investigation of liver, kidney and spleen of both control and fasting groups (Fig. 1a, 1b, 2a, 2b, 3a, and 3b) reveals that, there is no detectable histopathological alteration in any of these organs. The only observed change were in the liver and spleen of the fasting group (Fig. 1b & 3b) in which, the cytoplasmic vacuolation in hepatocytes were decreased comparing to the control group (Fig. 1a), in addition to increase the size of the mantie zone (MaZ) of the lymphoid follicle due to proliferation of its lymphoid cells (Fig. 3b) comparing to the control group (Fig. 3a).

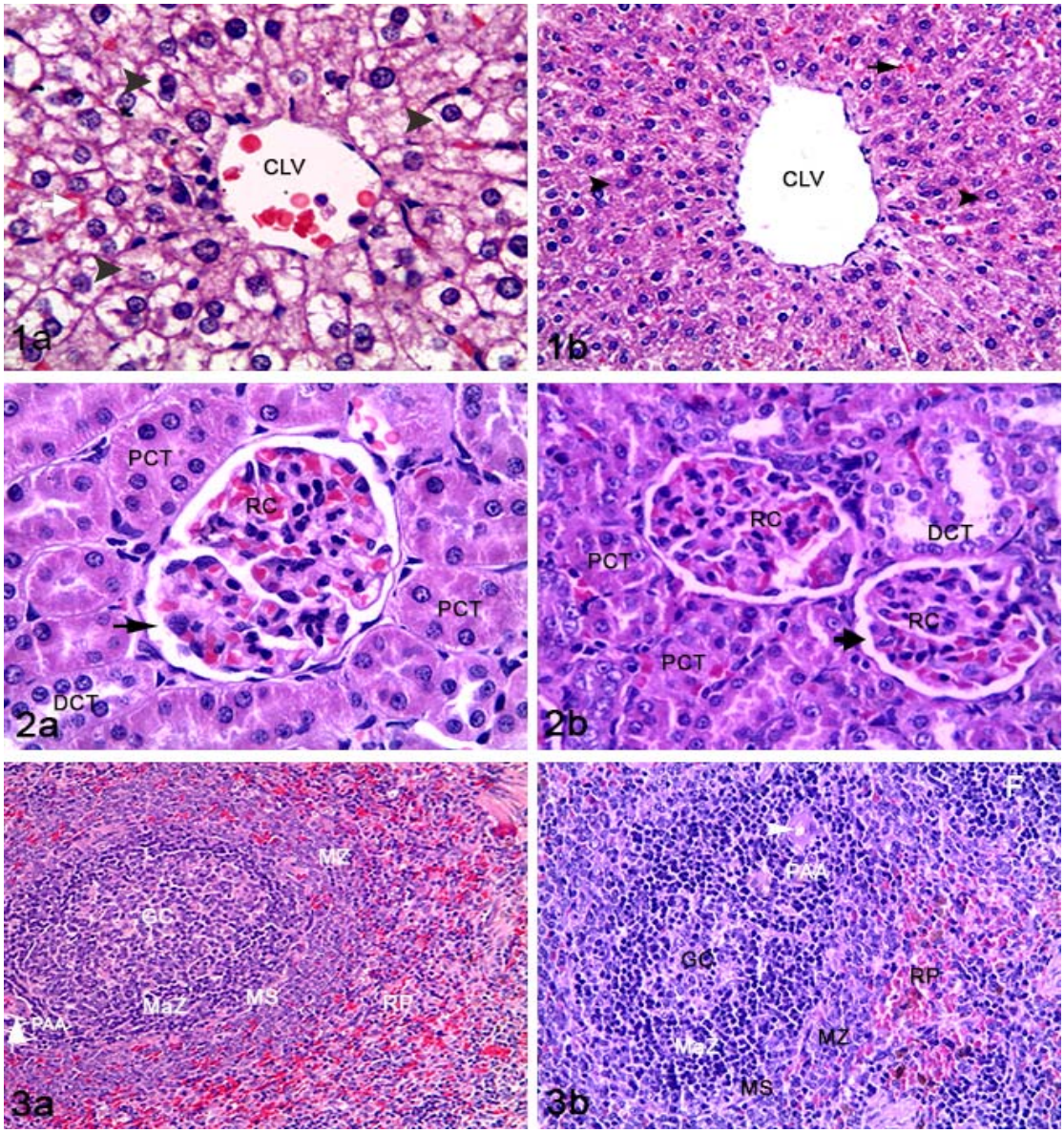

Fig1a. *Liver; rat, control group: normal histological architecture. Centrilolular venule (CLV), hepatocytes (black arrowheads), hepatic sinusoid (white arrow). HE. X40.*

Fig1b. *Liver; rat, fasting group: decrease the cytoplasmic vaculation in hepatocytes (black arrowheads). Centrilolular venule (CLV), hepatic sinusoid (black arrow). HE. X20.*

Fig2a. *Kidney; rat, control group: normal histological architecture. Renal corpuscle (RC), proximal convoluted tubules (PCT), distal convoluted tubules (DCT), and Bowman's space (arrow). HE. X40.*

Fig2b. *Kidney; rat, fasting group: normal histological architecture. Renal corpuscle (RC), proximal convoluted tubules (PCT), distal convoluted tubules (DCT), Bowman's space (arrow) . HE. X40.*

Fig3a. *Spleen; rat, control group: normal histological architecture. Germinal center of the lymphoid follicle (GC), mantie zone (MaZ), marginal sinus (MS), marginal zone (MZ), periarterial area of the follicle (PAA), central arteriole (arrowhead), red pulp (RP). HE. X20*

Fig3b. *Spleen; rat, fasting group: proliferation of the lymphoid cells in the mantie zone (MaZ) of the lymphoid follicle. Germinal center (GC), marginal sinus (MS), marginal zone (MZ), periarterial area of the follicle (PAA), central arteriole (arrowhead), red pulp (RP). HE. X20*

4. DISCUSSION

Our results indicated that intermittent fasting causes a significant decrease ($P<0.05$) in body weight, liver weight and stomach weight Which may be attributing to the reduction of meal frequency during fasting, which often results in reduction of energy intake and loss of body mass and body fat. Other contributing factors are extracellular fluid volume reduction secondary to lower sodium and fluids intake, and the moderate degree of dehydration [34]. However, there are many studies that have shown no significant loss of body mass [35, 36]

In this study, it is clear that fasting causes a significant increase ($P<0.05$) in PCV%, while no significant difference in hemoglobin level and WBCS count could be detected. Packed cell volume is the ratio of RBC volume to the total blood volume, an increase in PCV may be attributing to relative hemoconcentration which occurred as a result of fasting in agreement with the results of Born et al. [37] Who observed that fasting for 12–14 h caused a significant increase in haematocrit percentage due to water deprivation. On the other hand Ramadan [5] recorded that blood hemoglobin concentration, red and white blood cell counts were unchanged during both Ramadan fasting and non-Ramadan testing periods.

Phagocytic activity and phagocytic index were significantly increased ($P<0.05$) in fasted rats in this study. Fasting may induce stimulation of the immune system which may leads to increase in the phagocytic power of neutrophils. This theory is supported by the results of norepinefrine and serotonin described blow.

The interesting findings of the present study were the significant increase in the level of monoamines (norepinefrine and serotonin) in the forebrain of adult male rats after fasting. Experimental studies have demonstrated increase brain availability of serotonin and tryptophan during fasting [38]. These results were agree with Pérez-Cruet et al. [39] who reported that synthesis rate of brain serotonin was about 30% lower in rats fed than in rats fasted. Food intake produced changes in plasma tryptophan opposite to those produced in brain. Feeding also decreased brain serotonin turnover in rat. As well as Fond et al. [40] observed that fasting was frequently accompanied by an increased level of vigilance and a mood improvement, a subjective feeling of well-being and sometimes of euphoria. Garabal et al. [41] showed that serotonin increase the in vitro activity of cell phagocytosis. Fasting is a strong physiological stimulus equivalent to a biological stress that activated the hypothalamic pituitary-adrenal (HPA) axis. This activation leads to massive catecholamines release [40]. The studies of Hritcu et al. [15] reported that Catecholamines modulate a range of immune cell activities, including cell proliferation, cytokine and antibody production, lyric activity, and migration. These studies make a focus on the stimulatory effect of fasting on phagocytic activity of neutrophils as a part of immune cell activities and natural immunity.

AST and ALT activities measurements are considered to be two of the most important tests to detect liver injury, ALT is more specific to the liver than is AST. Our data indicated that fasting causes a significant decrease ($P<0.05$) of ALT and AST, while no significant difference in total protein albumin, globulin was found between fasting and control groups. The decrease in serum level of ALT and AST in fasted rats may be attributed to decrease in releasing of tissue specific enzymes and other intracellular proteins which secondary to oxidative stress during metabolism .These results in agreement with studies of Unalacak et al. [10] which reported that, post-Ramadan ALT levels were significantly lower compared to pre-Ramadan values.

Moreover, there was a significant improvement in the kidney function test in fasted rats, which could be explained by Bernieh et al. [34] who observed some improvement in the glomerular filtration rate (GFR) during and post fasting. There were no disturbances in serum electrolytes (sodium and potassium) during fasting in this study which agree with the result of Trabelsi et al. [22] who noted that serum electrolytes concentrations (sodium, potassium and chloride) did not change during fasting.

Generally no detectable histopathological alteration can be observed in the liver, kidney and spleen after fasting. The cytoplasmic vacuoles in hepatocytes in fasted rats were decreased comparing to the

control group and this point need further investigation to determine which of the cell components are responsible for that. In addition, proliferation of lymphoid cells in the mantie zone (MaZ) of the splenic lymphoid follicle was detected in fasted rats. Lymphoid cells proliferation can be explained by the action of norepinephrine which increased by fasting in this study , norepinephrine as a member of Catecholamines group is reported to modulate a range of immune cell activities, including cell proliferation [15].

In conclusion, our results showed that intermittent fasting may induce decreasing in the weight of body and some organs. Fasting increase brain neurotransmitters (serotonin and norepinefrine) as well as leading to enhancing neutrophil phagocytic activity, phagocytic index and immune cells proliferation. Additionally, fasting may have positive effects on liver and kidney function tests. Fasting has no effect on hemoglobin, WBC count, total protein, albumin, globulin, dopamine level and electrolyte balance but causing a significant increase in PCV. No detectable histopathological lesions could be induced by fasting in liver, kidney and splenic tissues. Finally, intermittent fasting may have some beneficial influences on healthy body.

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