

ORIGINAL ARTICLE

EFFECT OF 20-HOUR FASTING AND LOW FAT DIET ON GHRELIN HORMONE, GLUCOSE LEVEL AND LIVER FUNCTION IN ALBINO RATS MALE

DOI: 10.36740/WLek202204109

Ahmed Zwain, Husham Qassim Mohammed

UNIVERSITY OF KUFA, KUFA, IRAQ

ABSTRACT**The aim:** It aims to study the effect of fasting and low fat diet on ghrelin hormone, glucose level, the liver enzymes AST and ALT.**Materials and methods:** The experimental study was conducted using 24 healthy young male albino rat weighing 95 ± 5 gram and age 2 month, one-way (ANOVA) were employed to determine a significance of differences.**Results:** A significant increase $p \leq 0.05$ in glucose level of non-fasting control group compare with non-fasting low fat diet group, significant increase $p \leq 0.05$ in glucose level of control group fasting for 20h compared with low fat diet fasting for 20h group, significant decrease $p \leq 0.05$ when compares non-fasting low fat diet compares to 20h fasting low fat diet and significant decrease $p \leq 0.05$ when compares non-fasting control compares to 20h fasting control, while the effect of fasting and low fat diet on ghrelin hormone. A significant decrease $p \leq 0.05$ in ghrelin hormone level of non-fasting control group compare with non-fasting low fat diet group, significant increase $p \leq 0.05$ in ghrelin hormone of control group fasting for 20h compared with low fat diet fasting for 20h group, non-fasting control compares to 20h fasting control show a significant ($p \leq 0.05$) increase, Fasting with low fat diet cause a significant decrease $p \leq 0.05$ in ALT level, also in AST level there was a significant decrease $p \leq 0.05$ after 20h fasting.**Conclusions:** The fasting and low fat diet have effected on ghrelin hormone, glucose level and fasting with low fat diet cause decrease in ALT level, also in AST level decrease after 20h fasting in male albino rats.**KEY WORDS:** Fasting fat diet, low fat diet, Ghrelin hormone, Glucose level, liver function

Wiad Lek. 2022;75(4 p1):798-802

INTRODUCTION

Fasting is not a new concept; it has been practiced for hundreds of years and is recommended by a variety of religions for purification and cleansing purposes. Is fasting, nevertheless, beneficial to our physiology? Is there a chance that specific conditions will be harmed? Science nowadays is no longer based on empirical observations of fascinating things. Modern scientific activity demands that each given question be evaluated under well controlled and highly reproducible settings. Animal models, particularly rats, are crucial in this process, and a growing body of research suggests that fasting can improve human physiology and improve symptoms and quality of life in a variety of conditions. Obesity is one of the most evident, but there are also neurodegenerative illnesses, cancer, diabetes, cardiovascular disease, and sleep problems on the list [1]. Intermittent fasting (IF) is a kind of eating that alternates between fasting and eating intervals. It doesn't tell you which meals to consume, but rather when you should eat them. In this way, it's more correctly defined as an eating habit than a diet in the traditional sense [2]. Nutrients are substances that an organism needs in order to survive, develop, and reproduce. Carbohydrates, dietary fiber, fats, proteins, minerals, vitamins, and water are the seven primary types

of important nutrients for animals (including humans). Nutrients can be grouped as either macronutrients (carbohydrates, dietary fiber, fats, proteins, and water require in gram quantities) or micronutrients (vitamins and minerals needed in milligram or microgram quantities) [3]. Ghrelin is a hormone generated mostly by the stomach and released in lesser amounts by the small intestine, pancreas, and brain. Ghrelin serves a variety of purposes. Because it stimulates appetite, increases food intake, and promotes fat accumulation, it is known as the "hunger hormone." When given to people, ghrelin increases food intake by up to 30%; it circulates in the circulation and effects on the hypothalamus, a brain region important for hunger regulation [4]. Ghrelin also stimulates the pituitary gland to release growth hormone, which, unlike ghrelin, breaks down adipose tissue and encourages muscular development [5]. Alanine transaminase, or ALT, is an enzyme found mostly in the liver. ALT is released into the circulation when liver cells are destroyed. The quantity of ALT in the blood is measured by an ALT test. High levels of ALT in the blood can suggest a liver problem even if you don't have any symptoms of liver illness, such as jaundice (yellowing of the skin and eyes). Early diagnosis of liver illness may be aided by an ALT blood test. SGPT (serum glutamic-pyruvic transaminase) was

Table I. Component of low fat diet

Low fat diet			
Nutritional ingredient	(100 kg)	metabolizable energy	crud protein
yellow corn	51.5	1725	4.3
Soybean meal	35	780	16.8
bran	10	160	1.6
Premix	2.5	147	0.83
corn oil	-----		
limestone	0.3		
Di-Calcium Phosphate	0.7		
Total	100		
chemical analysis of low fat diet			
metabolizable energy		2812	
crud protein		23.5	
total fat		2.2	
The ratio of fat energy to total food energy		7%	

Table II. Component of control diet

Control diet			
Nutritional ingredient	(100 kg)	metabolizable energy	crud protein
yellow corn	47	1575	4
Soybean meal	38	847	18.24
Premix	2.5	147	0.83
corn oil	11.5	1035	-----
limestone	0.3		
Di-Calcium Phosphate	0.7		
Total	100		
chemical analysis of control diet			
metabolizable energy		3604	
crud protein		23.07	
total fat		13.7	
The ratio of fat energy to total food energy		34.2%	

the previous name for ALT. The SGPT blood test was previously known as the ALT blood test [6]. The enzyme AST (aspartate aminotransferase) is mostly located in the liver, although it can also be found in the muscles. AST is released into the circulation when your liver is damaged. The quantity of AST in your blood is measured by an AST blood test. The test can assist your doctor in determining whether you have liver injury or illness. Hepatitis, cirrhosis, mononucleosis, and other liver disorders can all be indicated by high AST levels in the blood. Heart issues or pancreatitis can also be indicated by high AST values [7].

THE AIM

It aims to study the effect of fasting and low fat diet on ghrelin hormone, glucose level, the liver enzymes AST and ALT.

MATERIALS AND METHODS

METHODOLOGY

The experimental study was conducted during the period from April 2021 to August 2021, and the experiment was conducted using using 24 healthy young male albino rat (*Rattus norvegicus*) weighing (95±5) gram and age 2 month. The animals were integrated with wooden shelves, under natural light 12 hours and 12 hours in the dark. The animals were placed in cages at laboratory temperature (23-25°C). Food and water were introduced daily, and kept for a week before the acclimatization experiment began. Adult Male

Rat divided into two groups as following:

1- Non-fasting group: Low fat diet group 6 animal and Control group 6 animal.

2- Fasting for 20 hours group: Low fat diet group 6 animal and Control group 6 animal.

Table III. Effect of fasting period and LFD on glucose and ghrelin hormone levels of albino male rats (Mean \pm SE)

Parameter Group (n=6)	Glucose (mg/dl)	Ghrelin (ng/mL)
CON1	156 \pm 1.140 A b	0.8384 \pm 0.026 B a
LFD1	215.8 \pm 2.596 A a	0.5520 \pm 0.010 B b
CON2	126.4 \pm 3.043 B b	1.3042 \pm 0.033 A b
LFD2	156.6 \pm 3.695 B a	1.5872 \pm 0.030 A a
LSD	9.169	0.0878

Table IV. Effect of fasting period and LFD on AST level and ALT level of albino male rats (Mean \pm SE)

Parameters Groups (n=6)	AST level (IU/L)	ALT level (IU/L)
CON1	309 \pm 1.048 A a	142 \pm 2.712 Aa
LFD1	303.2 \pm 0.916 A b	140.2 \pm 2.576 A a
CON2	302.2 \pm 1.319 B a	146 \pm 2.738 A a
LFD2	300.2 \pm 1.854 Aa	117 \pm 2.701 B b
LSD	3.49	14.3

Measurements include estimation of Serum Glucose, Serum Ghrelin hormone, liver enzyme Serum Alanine aminotransferase (ALT) and the activity of the liver enzyme aspartate aminotransferase (AST) in the blood is measured, the component of low fat diet, is shown in table (I), while the component of control diet is shown in table (II) [8].

RESULTS

The difference capital letters refer to significant change ($p \leq 0.05$) when comparing fasting period of study group. The difference in small letter refer to significant change ($p \leq 0.05$) when comparing the nutrition type of the study group.

CON1 = non-fasting control diet group, CON2=20 hours fasting control diet group.

LFD1= non-fasting low fat diet, LFD2=20h fasting low fat diet.

The result of study shows that Effect of fasting and low fat diet on glucose level of control non-fasting group compare to non-fasting low fat diet show a significant $p \leq 0.05$ increase, control group fasting for 20h compared with low fat diet fasting for 20h group displayed a significant increase $p \leq 0.05$. The result of non-fasting low fat diet compares to 20h fasting low fat diet show a significant $p \leq 0.05$ decrease, non-fasting control compares to 20h fasting control show a significant $p \leq 0.05$ decrease in glucose level. The result of study shows that Effect of fasting and low fat diet on ghrelin hormone level of control non-fasting group compare to non-fasting low fat diet illustrated a significant decrease $p \leq 0.05$, control group fasting for 20h compared with low fat diet fasting for 20h group displayed a significant increase $p \leq 0.05$. The result of non-fasting low fat diet compares to 20h fasting low fat diet displayed a significant increase $p \leq 0.05$, non-fasting

control compares to 20h fasting control show a significant $p \leq 0.05$ increase in ghrelin hormone level.

The difference capital letters refer to significant change $p \leq 0.05$ when comparing fasting period of study group. The difference in small letter refer to significant change $p \leq 0.05$ when comparing the nutrition type of the study group.

CON1 = non-fasting control diet group, CON2=20 hours fasting control diet group.

LFD1= non-fasting low fat diet, LFD2=20h fasting low fat diet.

The results shows that Effect of fasting and low fat diet on AST level of control non-fasting group compared with low fat diet non-fasting group illustrated a significant decrease $p \leq 0.05$. The result of control group fasting for 20h compared with low fat diet fasting for 20h group displayed a non-significant change $p \leq 0.05$. The result of non-fasting low fat diet compares to 20h fasting low fat diet show a non-significant change $p \leq 0.05$. The result of non-fasting control compares to 20h fasting control show a significant $p \leq 0.05$ decrease in AST level. The results shows that Effect of fasting and low fat diet on ALT control non-fasting group compared with low fat diet non-fasting group illustrated a non-significant change $p \leq 0.05$. The result of control group fasting for 20h compared with low fat diet fasting for 20h group displayed a significant decrease $p \leq 0.05$. The result of non-fasting low fat diet compares to 20h fasting low fat diet show a significant decrease $p \leq 0.05$. The result of non-fasting control compares to 20h fasting control show a non-significant $p \leq 0.05$ change on ALT level.

DISCUSSION

Fasting cause increase metabolic rate that lead to increase glycolysis to produce energy. Since this diet contains a

natural percentage of fat, the body does not activate the hormone glucagon, and at the same time the body will depend on the percentage of glucose come from this diet to generate energy. The results demonstrate that fasting with LFD induces a considerable drop in glucose levels [9]. Since the percentage of fat in this diet is very low, it caused a decrease in the level of glucose in the blood, and the glucose was clearly reduced after a 20-hour fasting period, and this led to an increase in the breakdown of glucose in the blood [10], and the other cause associated to ghrelin hormone level, fasting raises ghrelin levels, which lowers blood glucose levels because ghrelin raises the amount of insulin-secreting beta cells and serum insulin levels, which promote glucose metabolism [11]. Fasting increased the level of the ghrelin hormone in the fasting group. Different circumstances, including as fasting and pathological states, alter ghrelin production and secretion. Ghrelin levels in the blood rise during fasting and fall during eating. The rise in ghrelin levels during fasting is noradrenergic-mediated, while the subsequent drop is mediated by an increase in glucose and insulin [12]. After giving a low-fat diet to rats and measuring liver enzymes, we found decrease in ALT in group fasting for 20-hour. these results agree with human study and with animal model study [13-14]. There was a small decrease in the liver enzyme AST in the control diet and low fat diet in the case of fasting 20 hours with the control diet, as fasting improves liver function and reduces the liver enzyme this agree with [15]. Measurements of AST and ALT activity are two of the most essential assays for detecting liver damage; ALT is more specific to the liver than AST. The drop in ALT serum levels in fasting rats might be due to a decrease in tissue-specific enzymes and other intracellular proteins being released as a result of oxidative stress during metabolism [16-17].

CONCLUSIONS

Overall, this study strengthens the idea that fasting causes an increased level of ghrelin hormone that leads to a decreased level of glucose, Fasting with low fat diet cause decrease in ALT level, also in AST level decrease after 20h fasting

RECOMMENDATION

- The more penefet fasting priod is 20 houer.
- A low-fat diet is considered beneficial for health, because it lowers the level of sugar in the blood and increases the level of the hormone ghrelin.
- Use the same proportions of the diet, but with different ingredients such as casein and starch
- Ethical Clearance : Taken from University of Kufa ethical committee

REFERENCES

1. Mattson M.P., Moeh K., Ghena N. et al. Intermittent metabolic switching, neuroplasticity and brain health. *Nature Reviews Neuroscience*. 2018; 19(2): 63.
2. Longo V.D., Mattson M.P. Fasting: molecular mechanisms and clinical applications. *Cell metabolism*. 2014; 19(2): 181–192. doi:10.1016/j.cmet.2013.12.008.
3. Cena H., Calder P.C. Defining a Healthy Diet: Evidence for The Role of Contemporary Dietary Patterns in Health and Disease. *Nutrients*. 2020; 12(2): 334. doi:10.3390/nu12020334.
4. Pradhan G., Samson S.L., Sun Y. Ghrelin: much more than a hunger hormone. *Current opinion in clinical nutrition and metabolic care*. 2013; 16(6): 619–624. doi:10.1097/MCO.0b013e328365b9be.
5. Khatib N., Gaidhane S., Gaidhane A.M. et al. Ghrelin: ghrelin as a regulatory Peptide in growth hormone secretion. *Journal of clinical and diagnostic research:JCDR*. 2014; 8(8): 13–17.
6. Hinkle J., Cheever K., Suddarth's. *Handbook of Laboratory and Diagnostic Tests*. 2nd Ed, Kindle. Philadelphia: Wolters Kluwer Health, Lippincott Williams & Wilkins; c. Alanine Aminotransferase (ALT). 2014. 31p.
7. Ribeiro A.J.S., Yang X., Patel V. et al. Liver Microphysiological Systems for Predicting and Evaluating Drug Effects. *ClinPharmacolTher*. 2019; 106(1): 139-147.
8. Al-Kassar A.M. Nutritional requirements of experimental animals. College of veterinary medicine, University of Kufa. 2020, 189p.
9. de Toledo F.V., Grundler F., Cesare R. et al. Unravelling the health effects of fasting: a long road from obesity treatment to healthy life span increase and improved cognition. *Annals of medicine*. 2020; 52(5): 147–161.
10. Albosta M., Bakke J. Intermittent fasting: is there a role in the treatment of diabetes? A review of the literature and guide for primary care physicians. *Clinical Diabetes and Endocrinology*. 2021; 7(3).
11. Elabaddlah H., Hameed R., D'Souza C. et al. Exogenous Ghrelin Increases Plasma Insulin Level in Diabetic Rats. *Biomolecules*. 2020; 19; 10(4): 633. doi: 10.3390/biom10040633.
12. Akalu Y., Molla M.D., Dessie G., Ayelign B. Physiological Effect of Ghrelin on Body Systems", *International Journal of Endocrinology*. 2020, 26p.
13. Jang E.C., Jun D.W., Lee S.M. et al. Activity, and Histopathological Finding in Some Organs in Rats. *International Journal of. Comparison of efficacy of low-carbohydrate and low-fat diet education programs in non-alcoholic fatty liver disease: A randomized controlled study. Hepatol Res*. 2018; 48(3): 22-29.
14. Krugner-Higby L., Caldwell S., Coyle K. et al. The effects of diet composition on body fat and hepatic steatosis in an animal (*Peromyscus californicus*) model of the metabolic syndrome. *Comp Med*. 2011; 61(1):31-38.
15. Cong Y., Zihan L., Yulin X. et al. Effect of Intermittent Fasting on Non-Alcoholic Fatty Liver Disease: Systematic Review and Meta-Analysis. *Frontiers in Nutrition*. 2021; 8: 405.
16. Shawky S.M., Zaid A.M., Orabi S.H. et al. Effect of Intermittent Fasting on Brain Neurotransmitters, Neutrophils Phagocytic Activity, and Histopathological Finding in Some Organs in Rats. *International Journal of Research Studies in Biosciences*. 2015; 3: 38-45.
17. Nasiri J., Kheiri S., Khoshdel A., Boroujeni A.J. Effect of Ramadan Fast on Liver Function Tests. *Iran J Med Sci*. 2016; 41(5): 459-460.

ORCID and contributionship:

Ahmed Zwain: 0000-0002-8572-503X ^{A-F}

Husham Qassim Mohammed: 0000-0002-7183-8148 ^{A-F}

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Husham Qassim Mohammed

University of Kufa

29CG+62H, Kufa, Iraq

e-mail: hushamq.mohammed@uokufa.edu.iq

Received: 14.11.2021

Accepted: 08.03.2021

A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis,
D – Writing the article, **E** – Critical review, **F** – Final approval of the article