

## ORIGINAL ARTICLE

EFFECT OF KETOGENIC DIET ON LEPTIN LEVEL  
IN A DEPRESSED RAT MODEL

Amber Farid, Muhammad Omar Malik, Syed Murtaza Shah Bukhari, Saadia Anwar\*,  
Muhammad Irfan Shereen\*\*, Abdul Haseeb\*\*, Awais ul Islam\*\*\*, Saif ul Islam<sup>†</sup>,  
Muhammad Mustafa\*\*, Muhammad Mobashar<sup>††</sup>,

Department of Physiology, Institute of Basic Medical Sciences, Khyber Medical University, \*Jinnah Medical College, \*\*Muhammad College of  
Medicine, \*\*\*Maqsood Medical Centre, <sup>†</sup>MBBS Student, Jinnah Medical College, <sup>††</sup>Department of Animal Nutrition, Faculty of Animal  
Husbandry and Veterinary Sciences, Peshawar, Pakistan

**Background:** Depression is multidimensional disorder involving neuroendocrine disturbances. Several hormones are affected in depression, including gut related hormones such as leptin. Decreased leptin levels have been associated with worsening depressive symptoms and altered satiety. This study aimed to evaluate effect of ketogenic diet on leptin levels in rat model and its impact on depressive behaviour.

**Methods:** This study included male Sprague Dawley rats (n=39) randomly divided into three equal groups (n=13/group) and housed at Khyber Medical University. Depression was induced using chronic restraint stress model by restraining rats for two hours daily for 21 days. Depression was confirmed using Forced Swim Test and Sucrose Preference Test. Group A served as depressed control group and was sacrificed after confirmation of depression. Group B received normal diet for four weeks, while Group C was administered ketogenic diet for four weeks. Ketosis was confirmed before sacrifice. Blood samples were collected through intra-cardiac puncture, centrifuged, and stored at -80 °C. Serum leptin levels were measured using ELISA. Behavioural tests were repeated to assess effect of dietary intervention. **Results:** Mean leptin levels were 0.92±0.47 in depressed control group and increased to 1.32±0.33 in ketogenic diet-treated depressed group ( $p=0.019$ ). Forced Swim Test mobility significantly increased ( $p=0.001$ ), while Sucrose Preference Test scores significantly improved ( $p=0.026$ ) in ketogenic diet-treated depressed group. **Conclusion:** A four week ketogenic diet increased leptin levels and improved depressive behaviour in depressed rats.

**Keywords:** Depression, Forced swim test, Intra-cardiac puncture, Ketogenic diet, Ketosis, Leptin, Preference test, Sucrose re-strainer

Pak J Physiol 2026;22(1):37–41, DOI: <https://doi.org/10.69656/pjp.v22i1.1924>

## INTRODUCTION

Depression is a sophisticated disorder that disturbs both physical and mental aspects of individual's health. As individual suffering from depression manifest an extensive variations in their clinical symptoms, the exact underlying mechanism of its development remain incomprehensible.<sup>1</sup> However, many risk factors associated with cause of depression are identified in literature including female gender<sup>2</sup>, poor socioeconomic level<sup>3</sup>, depression in past, dysthymia, any physical or cognitive disability and vascular factors, also diseases related to Ageing<sup>4</sup> can cause depressive symptoms, vascular inflammatory diseases, hormonal, and immune changes depress the integrity of fronto-striatal pathways and increase susceptibility to depression and the most important genes.<sup>5</sup> Along with these, studies done recently has also shown strong association of microglial dysfunction with depression.<sup>6</sup> Due to multiple risk factors prevalence of this disorder is increasing day by day and 34% of adolescents globally, aged 10–19 years, are at risk of developing clinical depression, which is exceeding the reported estimates of individuals aged 18–25 years Recent statistics of depression.<sup>7</sup> Hormonal changes and depression are inter related, sometimes

change in hormonal balance can lead to depression while otherwise depression can change hormonal balance in body, likewise change in appetite cycle is marked as a sign of depression due to imbalance of appetite hormones it was suggested in a study that higher depression score will lead to lower appetite score and vice versa.<sup>8</sup> Now it is also a well-established fact that leptin plays a major role in satiety and effect of depression on satiety can be via changes in leptin levels in body. In a recent study it was clearly demonstrated that depression leads to lower level of leptin which in-turn stimulate intense hunger and appetite and leads to increased food consumption and then weight gain.<sup>9</sup> Now to treat depression and altered leptin level many options are available one of them are Ketogenic diet which is a low carbohydrate and high fat diet (70% fats, 27% proteins and only 3% carbs). Many studies in literature have shown that leptin levels gets better with Ketogenic diet and as a result satiety gets better and also depression.<sup>10</sup>

## METHODOLOGY

This experimental study was carried on male Sprague-Dawley rats in KMU animal house after taking permission from ethical committee of the university

under letter no KMU/IBM/IREB/16<sup>th</sup>. Our study included 39 (sample size was calculated according to resource equation method) healthy rats without any overt pathology weighing 200 to 250 grams. Rats were kept in cages having bedding of raw wood chips and were fed with water and normal food. Also rats facility was maintained on a 12:12 hour light n dark cycle under controlled temperature. Acclimatization period was kept seven days for them.

Afterwards to induce depression in rats they were kept in restrainers for 2 hours/day for 21 days according to chronic restrain stress model and depression was assessed and confirmed through FST (It is also known as behavioural despair test, is used to test for depression-like behaviour in rats) and SPT (It's a reward based test, used as an indicator of depression. This task assesses the animal's interest in a sweet-tasting sucrose solution relative to unsweetened water), all rats came out to be depression positive. At this point rats were randomly divided in three groups and first group (group A) was sacrificed through intra-cardiac puncture in post absorptive stage for baseline values of leptin. Collected blood was centrifuged immediately in laboratory and serum was stored at -80 °C in Eppendorf tubes. In the remaining 2 groups, Group B was fed with normal diet for 4 weeks while group C was on Ketogenic diet, then once ketosis was confirmed in group C rats through ketometer (Keto-Mojo GK+Blood Glucose and Ketone Monitoring System, Keto-Mojo, USA) both groups were sacrificed blood was collected and centrifuged at 3,000 rpm for 20 minutes using a laboratory centrifuge (Eppendorf 5702R, Hamburg, Germany) and at the same time the collected plasma was stored in freezer at -80 °C in Eppendorf tubes. Serum leptin levels were measured using a commercially available Rat Leptin ELISA Kit (Elabscience Biotechnology Inc., Wuhan, China; Catalog No: E-EL-R0582) following the manufacturer's protocol. Absorbance was measured using a microplate reader (BioTek ELx800, BioTek Instruments Inc., USA) at 450 nm, and concentrations were calculated from a standard calibration curve.

Data were analysed using SPSS-26. Quantitative variables were expressed as Mean±SD. Independent sample *t*-test was applied to compare differences between groups, and *p*<0.05 was considered statistically significant.

## RESULTS

During the development of depression, the weight of the rats decreased in all the groups. Keto diet rat's weight was decreased more as compared to the control diet group. There was a significant increase in leptin level (*p*=0.019) in depressed rats after being treated with Ketogenic diet (Table-1). Mobility of rats in FST was more in keto group (103.31±10.45 seconds) *p*=0.001 as

compared to control group (69.62±29.51 seconds). Moreover, sucrose water intake was more in keto group (48.46±10.64 mg/mL) as compared to control rats (40.92±2.25 mg/mL) and test came out to be significant (*p*=0.026). For comparison of the groups, independent sample *t*-test was applied.

**Table-1: Comparison of control and Keto group before and after intervention**

Test Parameters	Control	Keto	<i>p</i>
Leptin (ng/L)	0.92±0.47	1.32±0.33	0.019
FST-mobility (Seconds)	69.62±29.51	103.31±10.45	0.001
FST-immobility (Seconds)	50.39±9.51	16.69±10.45	0.001
SPT(g/mL)	40.92±2.25	48.46±10.64	0.026
Ketones (mmol/L)	0.92±0.28	1.47±0.56	0.006
BSR (mg/dL)	177.92±146.34	147.23±86.83	0.522

## DISCUSSION

Our study was to check if the Ketogenic diet intervention for four weeks has beneficial effect on depression scores and metabolic hormones. We found that four weeks of dietary intervention significantly decreased depression scores as measured in our study by FST and SPT. This was accompanied by more decrease in weight in the keto group. Leptin hormone was increased after Ketogenic intervention.

Leptin levels were significantly increased in depression. In contrast to our study it was reported that in depressive patients leptin levels are low as compared to controls and that is linked with primary reports of leptin's antidepressants role in animal models of depression.<sup>11-14</sup> Also on other hand there were results in literature showing parallel results with ours i.e., In any case, there was a strong linear association found between leptin levels and severity of depression. Higher leptin levels were found in patients with moderate to severe depression while lower levels in patients with mild depression.<sup>15</sup> These differences in results may be based on difference in gender, age group, difference in diets in populations under study.

The pathophysiology of depression is complex and involves intricate interaction between biological, psychological and environmental factors. Although exact mechanism of depression is still not known some of the contributing factors could be neurotransmitter dysfunction particularly serotonin, nor epinephrine and dopamine, other could be neuroendocrine dysregulation specially when HPA axis is dysregulated, thirdly could be neuroinflammation like activation of pro inflammatory cytokines can lead to depression.<sup>16</sup> Psychological and social stressors like childhood trauma, isolation and stress can be a leading cause of depression. Lastly genetic and epigenetics could be one of major causes and now they are the domains of most active research nowadays in relation to depression.<sup>17</sup>

The effects of Ketogenic diet on leptin in depress rat model have sparked considerable interest

within the realm of neuroscience and metabolic research. Leptin often referred as satiety hormone plays a crucial role in regulating appetite, metabolism and energy expenditure has also emerged as a potential mediator of mood disorders including depression.

Leptin is primarily produced by adipose tissues and acts on the hypothalamus in the brain to suppress appetite and increase energy expenditure. When leptin levels are sufficient, it signals to the brain that the body has adequate energy stores, thus reducing hunger and promoting weight maintenance. However leptin resistance can occur, wherein the brain becomes less responsive to leptin's signals, leading to increased appetite and weight gain.<sup>18</sup>

Studies on rodents subjected to Ketogenic diet have shown mixed results regarding leptin levels and depressive symptoms.<sup>19</sup> Some research indicates that Ketogenic diet may lead to reduced levels of leptin, suggesting a potential mechanism for appetite suppression and weight loss. However the implications of reduction in this leptin on depressive symptoms remain unclear.<sup>20</sup> Similarly in other study low level of leptin have been associated with depressive symptoms, mentioning that individuals with depression tend to have lower circulating levels of leptin.<sup>21</sup>

On the other hand, there is evidence suggesting that keto diet could have antidepressant effects in animal models mediated through alterations in neurotransmitter level, mitochondrial function, rather than solely through changes in leptin level.<sup>22</sup> The discrepancy in findings may be attributed to difference in experimental protocol, difference in gender, age group, difference in diets composition, duration of exposure and animal models used.

The Ketogenic diet, characterized by its high fat, moderate protein and low carbohydrate has gained popularity for its potential to promote weight loss and improve metabolic health. One intriguing aspect of Ketogenic diet is its influence on hormone levels including leptin. Research on impact of Ketogenic diet on leptin level is still evolving but several studies has provided valuable insights into this relationship. Initially some studies suggested that Ketogenic diet may lead to a decrease in leptin levels potentially due to decrease carbs intake and subsequent change in insulin levels.<sup>23</sup> Since insulin can stimulate leptin secretion, lower insulin level in keto diet might result in decreased leptin production. Additionally changes in adipose tissue mass and distribution, particularly reduction in visceral fats, may impact leptin secretion.

However, recent studies have reported a counter-intuitive rise in leptin levels in individuals adhering to Ketogenic diet. Several factors may contribute to the increased levels of leptin firstly could be unique metabolic effects, characterized by ketosis and increased fat oxidation could play a major role.

Ketone bodies, produced during ketosis, have been shown to influence leptin secretion and sensitivity, potentially leading to elevated leptin level. Moreover changes in dietary composition, particularly the high fat content of the Ketogenic diet, could impact adipose tissue metabolism and leptin production.<sup>24</sup>

The animal model used in this research was based on chronic restraint model which allows investigating physiological, behavioural and neurobiological changes induced by chronic stress exposure. This model involves subjecting animals, typically rodents such as rats or mice, to repeated periods of physical immobilization or confinement. Animals are placed in restraining device for several hours daily over a period ranging from days to weeks. The stressors are applied consistently, mimicking persistent stress experienced in real life situations.

Physiologically, chronic restraint stress elicits a cascade of responses in animals. These include alteration in HPA axis or even dysregulation of cortisol in case of prolonged stress. Similarly behaviourally, chronic restraint stress induces a range of adoptive and maladaptive responses. While some animals may exhibit anxiety like behaviour, like increased vigilance, others may display depressive like behaviour like reduced motivation and anhedonia. Cognitive impairments, like deficits in learning and memory, have also been observed in animals exposed to chronic stress.

The chronic restraint stress model has provided valuable insights into the pathophysiology of stress related disorders, including depression, anxiety disorder, and post-traumatic stress disorder (PTSD). By elucidating the neurobiological mechanisms underlying stress induced changes, researchers can identify potential targets for pharmacological interventions and behavioural therapies aimed at mitigating the adverse effects of chronic stress.<sup>25</sup>

The neurobiological mechanisms underlying the effects of chronic restraint stress are multifaceted and involve complex interactions between brain regions and neurotransmitter systems. Key brain areas implicated in stress response include the prefrontal cortex, amygdala and hippocampus, which regulate emotional processing, fear responses, and cognitive function, respectively.<sup>26</sup> Stress can not only induce structural and function changes in these brain areas but also cause dysregulation of neurotransmitter system such as serotonin, dopamine etc and may cause mood disorders including anxiety and depression.

Animal model play crucial role in depression research, offering insights into the biological and behavioural underpinning of disorder. One such model is the force swim test, traditionally used to assess antidepressant efficacy in rodents. However recent studies have shown its potential as diagnostic tool for depression in animal models.<sup>27</sup> This test involves

placing a rodent in a cylindrical container filled with water from which escape is impossible. Initially animal exhibits vigorous attempts to escape but eventually adopts a state of immobility, floating passively. This immobility is interpreted as measure of behavioural despair. Studies have shown that animal susceptible to depression display prolonged immobility times compared to resilient counterparts. Furthermore these depressive like behaviours often correlate with other hallmark symptoms of depression, such as anhedonia and social withdrawal.<sup>28</sup>

While FST offers a valuable tool for depression research, several challenges must be addressed to ensure its validity and reliability as a diagnostic model. Variability in experimental conditions such as water temperature, container size, lighting, can influence test outcomes and complicate result interpretation.

Another model which play role in understanding the neurobiological mechanisms underlying depression is sucrose preference test. This model is based on the principle that rodents, like humans, exhibit a preference for sweet solution such as sucrose when given a choice between sucrose solution and water. Anhedonia, a core symptom of depression characterized by diminished ability to experience pleasure, is reflected in reduced preference for sucrose consumption. The test typically involves two phases: habituation to sucrose solution followed by a period of preference testing.

Rodents subjected to chronic stress often exhibit decreased sucrose preference compared to control counterparts. This reduction in sucrose preference is interpreted as anhedonia, indicative of depression like state in animals.<sup>29</sup>

Our study revealed relation of Ketogenic diet with depression and leptin. However further research is needed to elucidate the precise mechanisms underlying the effects of Ketogenic diet on leptin level and depressive symptoms in animal model, as understanding the interplay between keto diet, depression and leptin in animal models could provide valuable insights into potential therapeutic strategies for mood disorder in humans. We can apply this model on humans and check the results. Duration of study can be increased in order to stop keto diet in keto group and fed them again with normal food and then check levels of leptin that what keto has done in long term. This project can be used on very young and aged rats to check effects of keto diet on depression and leptin level in extreme ages.

## CONCLUSION

Ketogenic diet for four weeks increased leptin significantly in rats. Keto diet significantly improved depression scores as assessed by force swim test and sucrose preference test.

## REFERENCES

1. Fox ME, Lobo MK. The molecular and cellular mechanisms of depression: a focus on reward circuitry. *Mol Psychiatry* 2019;24(12):1798–815.
2. Moreno X, Gajardo J, Monsalves MJ. Gender differences in positive screen for depression and diagnosis among older adults in Chile. *BMC Geriatr* 2022;22(1):54.
3. Zhang Y, Zhang J, Li H, Jiang Y. The relationship between socioeconomic status and depression: a systematic review and meta-analysis. *Adv Psychol Sci* 2022;30(12):2650.
4. Bedaso A, Mekonnen N, Duko B. Estimate of the prevalence of depression among older people in Africa: a systematic review and meta-analysis. *Aging Ment Health* 2022;26(6):1095–105.
5. Paoli C, Misztak P, Mazzini G, Musazzi L. DNA methylation in depression and depressive-like phenotype: biomarker or target of pharmacological intervention? *Curr Neuropharmacol* 2022;20(12):2267–91.
6. Deng SL, Chen JG, Wang F. Microglia: a central player in depression. *Curr Med Sci* 2020;40(3):391–400.
7. Shorey S, Ng ED, Wong CHJ. Global prevalence of depression and elevated depressive symptoms among adolescents: A systematic review and meta-analysis. *Br J Clin Psychol* 2022;61(2):287–305.
8. Kawaharada R, Sugimoto T, Uchida K, Murata S, Tsuboi Y, Isa T, *et al.* Indirect effects of social activity on appetite via depressive symptoms in community-dwelling older adults: a cross-sectional study. *Appetite* 2022;168:105705.
9. Tasci G, Kaya S, Kalayci M, Atmaca M. Increased ghrelin and decreased leptin levels in patients with antisocial personality disorder. *J Affect Disord* 2022;317:22–8.
10. Morshedzadeh N, Ahmadi AR, Tahmasebi R, Tavasolian R, Heshmati J, Rahimlou M. Impact of low-carbohydrate diet on serum levels of leptin and adiponectin levels: a systematic review and meta-analysis in adult. *J Diabetes Metab Disord* 2022;21(1):979–90.
11. Eikelis N, Esler M, Barton D, Dawood T, Wiesner G, Lambert G. Reduced brain leptin in patients with major depressive disorder and in suicide victims. *Mol Psychiatry* 2006;11(9):800–1.
12. Jow GM, Yang TT, Chen CL. Leptin and cholesterol levels are low in major depressive disorder, but high in schizophrenia. *J Affect Disord* 2006;90(1):21–7.
13. Kraus T, Haack M, Schuld A, Hinze-Selch D, Pollmächer T. Low leptin levels but Normal body mass indices in patients with depression or schizophrenia. *Neuroendocrinology* 2001;73(4):243–7.
14. Yang K, Xie G, Zhang Z, Wang C, Li W, Zhou W, *et al.* Levels of serum interleukin (IL)-6, IL-1 $\beta$ , tumour necrosis factor- $\alpha$  and leptin and their correlation in depression. *Aust NZ J Psychiatry* 2007;41(3):266–73.
15. Morris AA, Ahmed Y, Stoyanova N, Hooper WC, De Stærke C, Gibbons G, *et al.* The association between depression and leptin is mediated by adiposity. *Psychosom Med* 2012;74(5):483–8.
16. Tian H, Hu Z, Xu J, Wang C. The molecular pathophysiology of depression and the new therapeutics. *Med Comm* 2022;3(3):e156.
17. Kendall K, Van Assche E, Andlauer TFM, Choi K, Luykx JJ, Schulte EC, *et al.* The genetic basis of major depression. *Psychol Med* 2021;51(13):2217–30.
18. Picó C, Palou M, Pomar CA, Rodríguez AM, Palou A. Leptin as a key regulator of the adipose organ. *Rev Endocr Metab Disord* 2022;23(1):13–30.
19. Mela V, Samur NS, Vijaya AK, Gálvez VJ, García-Martín ML, Bandera B, *et al.* Ketogenic diet is less effective in ameliorating depression and anxiety in obesity than Mediterranean diet: A pilot study for exploring the GUT-brain axis. *Brain, Behavior, and Immunity* 2025:106167.
20. Mentzelou M, Dakanalis A, Vasios GK, Gialeli M, Papadopoulou SK, Giaginis C. The relationship of ketogenic diet with neurodegenerative and psychiatric diseases: a scoping review from basic research to clinical practice. *Nutrients* 2023;15(10):2270.
21. Zou X, Zhong L, Zhu C, Zhao H, Zhao F, Cui R, *et al.* Role of

- leptin in mood disorder and neurodegenerative disease. *Frontiers in Neuroscience* 2019;13:378.
22. Ge T, Fan J, Yang W, Cui R, Li B. Leptin in depression: a potential therapeutic target. *Cell Death Dis* 2018;9(11):1096.
  23. Chyra M, Swietochowska E, Gorska-Flak K, Dudzinska M, Oswiecimska J. The effect of the ketogenic diet on leptin, chemerin and resistin levels in children with epilepsy. *Neuro Endocrinol Lett* 2021;42(7):489–99.
  24. Park S, Kim DS, Kang S, Daily JW 3<sup>rd</sup>. A ketogenic diet impairs energy and glucose homeostasis by the attenuation of hypothalamic leptin signaling and hepatic insulin signaling in a rat model of non-obese type 2 diabetes. *Exp Biol Med* 2011;236(2):194–204.
  25. Mao Y, Xu Y, Yuan X. Validity of chronic restraint stress for modeling anhedonic-like behavior in rodents: a systematic review and meta-analysis. *J Int Med Res* 2022;50(2):03000605221075816.
  26. Ha GE, Cheong E. Chronic restraint stress decreases the excitability of hypothalamic POMC neuron and increases food intake. *Exp Neurobiol* 2021;30(6):375–86.
  27. Yankelevitch-Yahav R, Franko M, Huly A, Doron R. The forced swim test as a model of depressive-like behavior. *J Vis Exp* 2015;(97):52587.
  28. Brandwein C, Leenaars CH, Becker L, Pfeiffer N, Iorgu AM, Hahn M, *et al*. A systematic mapping review of the evolution of the rat Forced Swim Test: Protocols and outcome parameters. *Pharmacol Res* 2023;196:106917.
  29. Primo MJ, Fonseca-Rodrigues D, Almeida A, Teixeira PM, Pinto-Ribeiro F. Sucrose preference test: A systematic review of protocols for the assessment of anhedonia in rodents. *Eur Neuropsychopharmacol* 2023;77:80–92.

### Address for Correspondence:

**Dr Muhammad Omar Malik**, Department of Physiology, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan. **Cell:** +92-333-5196243

**Email:** omarmalik786@gmail.com

syedmurtazashah3@gmail.com

**Received:** 22 Jan 2026

**Reviewed:** 21 Mar 2026

**Accepted:** 31 Mar 2026

### Contribution of Authors:

Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

**AF:** Methodology, Data collection and analysis, Drafting and Revision

**SMSB:** Data entry and analysis, Tabulation, Writing and Revision of manuscript

**MIS:** Data analysis, Writing and Revision of manuscript

**AI:** Tabulation of Results, Writing and Revision of manuscript

**MM:** Data analysis and Tabulation, Writing and Revision of manuscript

**MOK:** Concept, Design, Writing manuscript, Revision, Final approval

**SA:** Data Analysis, Tabulation, Writing and Revision of manuscript

**AH:** Data analysis, Writing and Revision of manuscript

**SI:** Data analysis and Tabulation, Writing and Revision of manuscript

**MM:** Data analysis and Tabulation, Drafting and Revision

**Conflict of Interest:** The authors declare that they have no conflicts of interest or competing interests

**Funding:** This work was partially supported by a research grant awarded through the Office of Research and Innovation, Khyber Medical University, Peshawar, Pakistan