Kurdish Studies
May 2024
Volume: 12, No: 4, pp. 989-996
ISSN: 2051-4883 (Print) | ISSN 2051-4891 (Online)
www.KurdishStudies.net

DOI: 10.53555/ks.v12i4.3097

Adiponectin Level In Transgenerational Chronic Stress Rat Model: Comparison Between Offspring Of Stressed Versus Non-Stressed Parents

Robina Usman[1], Muhammad Omar Malik[2*], Madiha Khattak[3], Syed Hamid Habib[4], Rifat Ullah Khan[5]

[1]MBBS, MPhil Physiology, MHPE, Professor, Physiology Department, Peshawar Medical College, Riphah International University, Islamabad., https://orcid.org/0009-0005-1949-878X
[2*,4]MBBS, MPhil Physiology, PhD (Glasgow), Associate Professor, Physiology Department, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, [2]https://orcid.org/0000-0001-5168-6258, [4]https://orcid.org/0000-0003-3992-7279
[3]MBBS, MPhil Physiology, Assistant Professor, Physiology Department, Khyber Medical College, Peshawar, https://orcid.org/0000-0001-5617-0904
[5]PhD, Associate Professor, College of Veterinary Sciences, Faculty of Animal Husbandry and Veterinary Sciences, University of Agriculture, Peshawar, https://orcid.org/0000-0002-0924-0479

***Corresponding author:** Dr. Muhammad Omar Malik
MBBS, MPhil Physiology, PhD (Glasgow), Associate Professor, Physiology Department, Institute Of Basic Medical Sciences, Khyber Medical University, Peshawar, Email address: omermalik@kmu.edu.pk

Abstract
This study investigated the changes in adiponectin level in offspring of case and control parents after exposure to chronic stress at early, late and both early and late life stress. 130 healthy Wistar rats, 11 weeks of age were raised. Ten were sacrificed for baseline values. Parent generation was divided into case parents(n=70) and control parents (n=40). Only, case parents were exposed to chronic stressors for 21 days. Ten rats of case parents were sacrificed to find serum adiponectin (ELISA). The remaining were bred in their respective groups with 6 rats per cage. Offspring of both the groups were exposed to chronic stress for three weeks, early life stress (ELS) at 5 weeks of age, late life stress (LLS), at 11 weeks of age, both early and late life stress (ELS+LLS) and results were compared. Open field test was carried out. Adiponectin (ng/ml) level was increased in all groups of case offspring compared to offspring of control parents. It was highest after both early and late life stress in case 31.87 (28.57-35.18) compared to control offspring,19.62 (14.30-24.94)(geometric means and confidence interval) with $p < 0.01$. Increased adiponectin was observed in case compared to control offspring, also revealing anxiolytic behaviour in open field behavioural test.

Key words: Early life stress, late life stress, restraint stress, adiponectin

1. INTRODUCTION

Stress whether psychological or physical can activate the hypothalamic-pituitary-adrenal (HPA) axis, and chronic stress raises baseline cortisol levels that take longer to return to pre-stress levels [1].Adiponectin is highly expressed in lean, healthy individuals having anti-inflammatory, anti-atherogenic and anti-diabetic properties. [2] It's level is reduced in obesity. [3] In circulation, adiponectin is found as trimers, hexamers and multimers having a high-molecular-weight. [4] Isoforms such as trimers and hexamers can pass through the blood brain barrier (BBB) and can be found in the mice's and human's cerebrospinal fluid. [5] AdipoR1 and AdipoR2 are adiponectin receptors present in high concentration in hippocampus and prefrontal cortex, areas linked to depression and anxiety so the level of adiponectin has an effect on anxiety and depression. [6]

An important transcriptional factor acting as controller of adipokine gene expression is nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) having two isoforms: PPAR1 is present in adipose tissue significantly but is present elsewhere too, whereas PPAR2 is only detected in adipose tissue. [7] The development of adipocytes depends on PPARγ. [8] Adipose tissue of mice susceptible to stress but not the resilient ones had lowered PPAR mRNA levels. [9] This might be due to overactive sympathetic nervous system as is seen in chronic social defeat stress. [10] Release of norepinephrine causes inhibition of gene expression of PPARγ2 in adipocytes resulting in reduced adiponectin. [11] Mice given multiple injections of rosiglitazone, a PPAR-selective agonist, an hour before testing their behavior on elevated plus maze, exhibited reduced anxiety as greater number of entries and duration in open arms area was observed. [12]

Furthermore, glucocorticoids inhibit movement of adiponectin through endothelial cells which means that stress can decrease availability of adiponectin to the tissues even if the serum levels remain unaltered. [13] Deficiency of adiponectin makes the anxiety worst, [14] as seen in firefighters with post-traumatic stress disorder [15] and also in anxiety disorders. [16] It is seen that adiponectin upregulates two molecules, ADAM10 and Notch1 and activate Notch signaling in the hippocampus. Adiponectin-Notch pathway is inhibited by chronic stress and causes cognitive dysfunction. [17]

Mesolimbic dopamine system through dopaminergic neurons in the ventral tegmental area (VTA) processes emotions.[18]These neurons express adiponectin receptor 1 (AdipoR1) and action of adiponectin inhibits them lessening anxiety. [19] Loss of AdipoR1 in dopamine neurons resulted in defective signaling. [20] This study was designed to find out the effect of chronic stressors on adiponectin level of offspring of stressed versus non-stressed parents and the effect of altered adiponectin level on behaviour of case and control offspring.

2. MATERIALS AND METHODS

2.1 Study setting: Peshawar Medical College, Animal research laboratory in University of agriculture, Khyber Medical University, Peshawar

2.2 Study duration: two years, November 2019 to December 2021

2.3 Study design: Experimental case control study

2.4 Inclusion criteria: Healthy Wistar rats of appropriate age.

2.5 Exclusion criteria: Unhealthy rats or not of required age

2.6 Animals

Ethical approval was taken by the Ethical Committee of Peshawar Medical College: IRB approval number: Prime/IRB/2019-167 and Khyber Medical University No. Dir/KMU-EB/HM/000713. A total of 130 adult healthy Wistar rats weighing, 280-300 g; age 11 weeks, were raised in animal house of Peshawar Medical College. They were maintained at 25±2°C temperature with free availability of food (standard laboratory diet and water in a humidity of 40-60% under a 12-h light/dark cycle. [21] Ethical guidelines for animal care, UK were followed. For baseline values, ten rats were sacrificed after been given isoflurane anaesthesia by open drop procedure [22] (Supplementary 1). Blood was collected through cardiac puncture[23] (Supplementary 2).Supplementary 1 and 2 are part of article by Usman et al.[24] After the procedure was complete, the rat was immediately euthanized by exsanguination. [25] Blood samples were cold centrifuged (- 4◦C) at 4,000 revolutions per minute for 20 minutes to separate sera. The supernatant was transferred to Eppendorf tubes and preserved at −80◦C until further analysis by ELISA.

2.7 Timing of stress and chronic stressors

Chronic stress for three weeks: Seventy rats from cases parents' group were exposed to chronic stress at 11 weeks of age for three weeks i.e., from 11 to 14 weeks of age. The chronic stressors applied, (one stressor per day for a short time) were alteration of day-night cycle for 12 hours from 7am to 7 pm. [26,27] On day 2, rats were immersed in cold water at 16±2°C for 5 min in a tank with water depth of 15.5 cm. The rats were subjected to cold water immersion stress by placing them individually in a tank of water. [28] On day 3, restraint stress was applied by keeping them in a cylindrical tube having ventilation holes for a duration of 120-180 minutes. [29] The size of the cylindrical tubes was such that the limbs could not be moved. Details of stressors are mentioned in an article, "Developing chronic unpredictable/Alternating stress Model in Wistar Albino Rats" by Khattak et al. [30]

2.8 Experimental design

It was an experimental case control study design. Out of seventy rats of the case parents group subjected to chronic alternating stress, ten were found to be non- stressed after behavioural tests so they were removed. Ten out of remaining sixty rats were sacrificed for hormonal assays and histology. Fifty rats remaining in case parents' group and forty in control parents' group were allowed to mate. Parents of cases and controls were kept in respective cages, housing 6 rats per cage, under standard conditions of water, food and temperature. There were approximately 19 pregnancies out of 25 couples in the case parents while in the control parents, there were 18 pregnancies out of 20 couples. Same stress protocol was followed for the offspring of both groups. After birth, litter was kept with the mother for four weeks as they attain sexual maturity at the age of 6 weeks. [31] A litter had 8-10 rats at an average that were divided into two groups of approximately four each. After 4 weeks, the pups were separated from the mother and the male pups were kept separate from the female pups. These pups were distributed to one of the six groups in case offspring if they were from the case parents**.** The pups of the parent control group were also separated from their mothers after 4 weeks and the male pups were kept separate from the female pups. They were assigned randomly to one of the six groups of the control offspring. Offspring of cases were assigned to 6 different groups (F1A, F1A1, F1A2, F1A3, control of F1A1 and control of F1A2) and similarly, offspring of controls were selected to fill F1B, F1B1, F1B2 and F1B3, control of F1B1 and control of F1B2) groups. Offspring of parents exposed to stress were labelled as case offspring and those of parents unexposed to stress were named as control offspring. For details of methodology see figure1 and 2 in article by Usman et al, "Development of protocol for transgenerational stress in Wistar rats." [24]

2.9 Defining timing of exposure to chronic stress

2.9.1 Early life stress (ELS) for three weeks

As the offspring reached the age of 5 weeks, they were given early life stress for three weeks i.e., from 5th to 8th week of age.

2.9.2 Late life stress (LLS)
At the age of 11 weeks, they were given late life stress for three weeks i.e., from 11 to 14 weeks.

2.9.3 Both early and late life stress (both ELS and LLS)
For both early and late life stress, offspring were exposed to early life stress from 5th to 8th week of age then stressors were applied from 11th to 14th week of age.[24]

2.10 Open field test [32]
After applying stressors for 21 days, open field test was performed on 22nd day. It's conduction and analysis were carried out by the same person at 9 a.m. Testing session lasted for 5 minutes and was recorded by a digital camera, Sony super steady shot D5C-H50. It tested locomotion, rears, duration of rearing, number and duration of central entries, percentage of time spent in central square of open field and periphery as well. Rats with greater anxiety have lesser locomotion, more thigmotaxis and lesser ambulation in centre. [33] [34] A lower rearing frequency is suggestive of decreased exploration and more anxiety. [35] Between the trials, floor was swept with 30% ethanol and dried. [36]

2.11 Measuring adiponectin level by ELISA
We compared these offspring in terms of adiponectin by using commercially available rat enzyme-linked immunosorbent assay (ELISA) kit. (ELab Science USA, Rat ADP/Acrp 30 (Adiponectin) ELISA kit, Catalogue no. E-EL-R 3012).

2.12 Statistical analysis:
Normality of data was checked using tests of normality, Kolmogorov-Smirnov and Shapiro-Wilk test. Adiponectin was not normally distributed. It was log transformed. Data of adiponectin was presented as geometric means (confidence interval). Mann Whitney U test was applied to compare different groups. SPSS version 25 was used for data analysis and graph pad prism version 9.1.0 was used to make graphs.

3. RESULTS

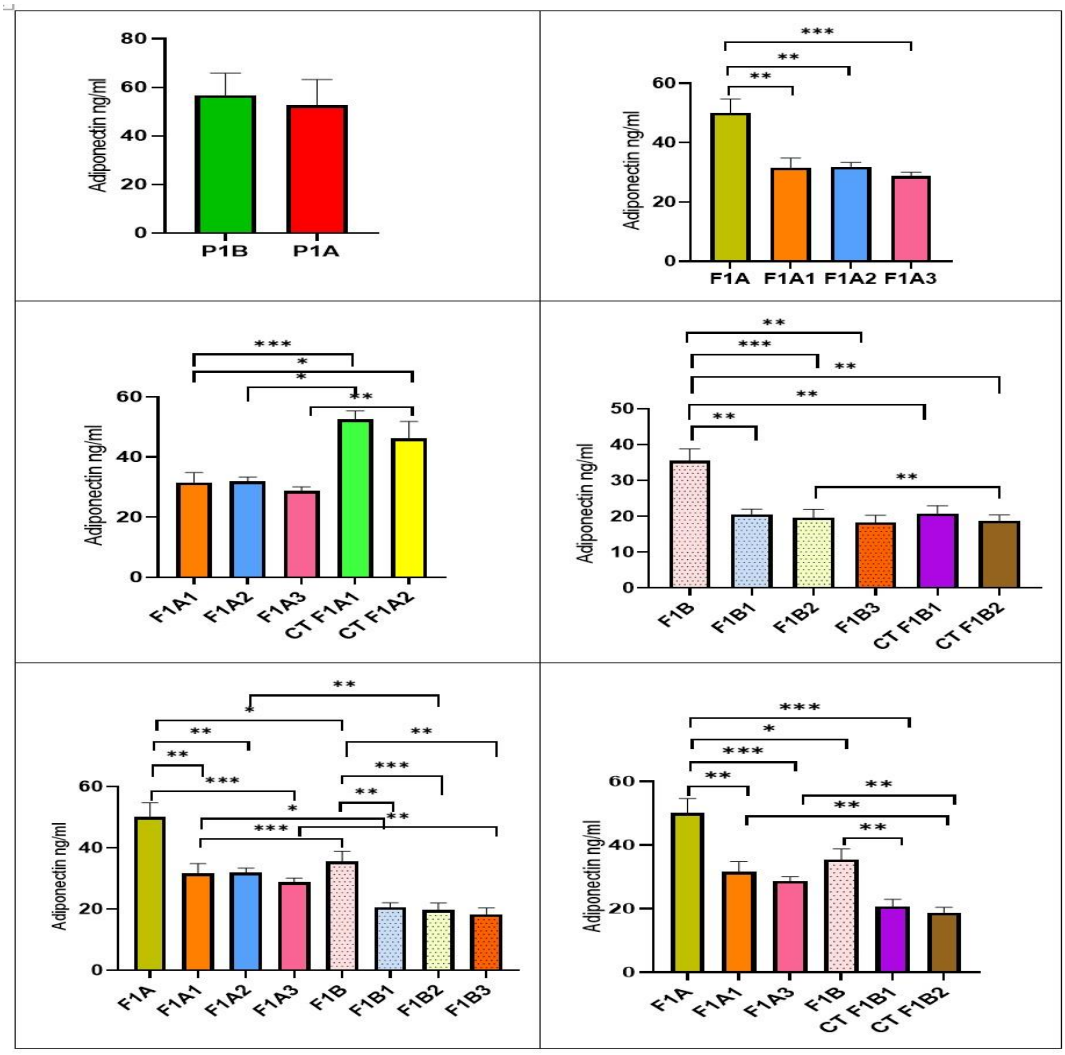
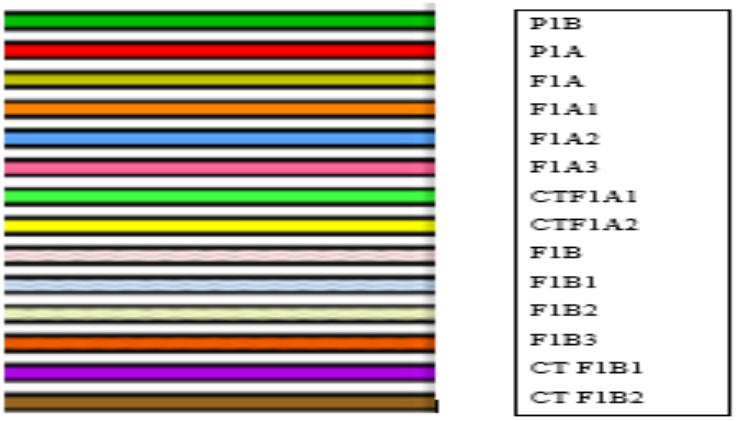


Figure 1: Comparison of adiponectin level (ng/ml) in case and control parents' and their offspring Values are mean ± SEM

Key: p* ≤ 0.05
p**≤ 0.01
p***≤0.001
P1A, Case parents, age=11 weeks
F1A Off-spring of case parents, age=5 weeks, unexposed to stress
F1A1, Off-spring of case parents, age=8 weeks (after early life stress)
F1A2, Off-spring of case parents, age=14 weeks (after early and late life stress)
F1A3, Off-spring of case parents, age= 14 weeks (after late life stress)
Control for F1A1, age=8 weeks, unexposed to stress
Control for F1A2, age=14weeks, unexposed to stress
P1B, Control parents age=11 weeks, unexposed to stress
F1B Off-spring of control parents, age=5 weeks, unexposed to stress
F1B1, Off-spring of control parents, age=8 weeks (after early life stress)
F1B2, Off-spring of control parents age=14 weeks (after early and late life stress)
F1B3, Off-spring of control parents, age=14 weeks (after late life stress)
Control of F1B1, age=8 weeks. unexposed to stress
Control of F1B2, age=14 weeks, unexposed to stress



The above colour coding was used for the various experimental groups while the names designated to different groups are the same in both the figure 1 and figure 2.

Table 1: Adiponectin level (ng/ml) in all experimental groups of Wistar rats

P1A	**F1A**	**F1A1**	**F1A2**	**F1A3**	**CT F1A1**	**CT F1A2**
52.62 (45.01-60.22)	50.09 (39.67-60.50)	31.57 (24.21-38.93)	31.87 (28.57-35.18)	28.73 (25.70-31.76)	52.53 (46.20-58.85)	46.23 (33.55-58.91)
P1B	**F1B**	**F1B1**	**F1B2**	**F1B3**	**CT F1B1**	**CT F1B2**
56.73 (50.15-63.31)	35.46 (27.87-43.04)	20.60 (17.43-23.77)	19.62 (14.30-24.94)	18.27 (13.64-22.90)	20.73 (15.70-25.75)	18.77 (15.08-22.46)

Adiponectin level (ng/ml) is presented as geometric means (confidence interval)

The Adiponectin level was higher in control compared to case parents but not statistically significant. The levels were higher in all groups of case offspring even in the group at 5 weeks of age unexposed to stress and the groups of controls of case offspring compared to their control counterparts. It remained statistically significant between case and control offspring unexposed to stress at 5 weeks of age at a lower level($p<0.05$). It was high in case offspring exposed to early life stress compared to its control offspring counterpart at a lower level of statistical significance. It was high in group of case offspring exposed to both early and late life stress (F1A2) compared to their counter part of control offspring (F1B2) group showing statistical significance of ($p<0.01$). In comparison of control offspring exposed to late life stress (F1B3), their case offspring counterpart (F1A3) showed higher adiponectin level with statistical significance of ($p<0.01$) (Figure 1, Table 1)

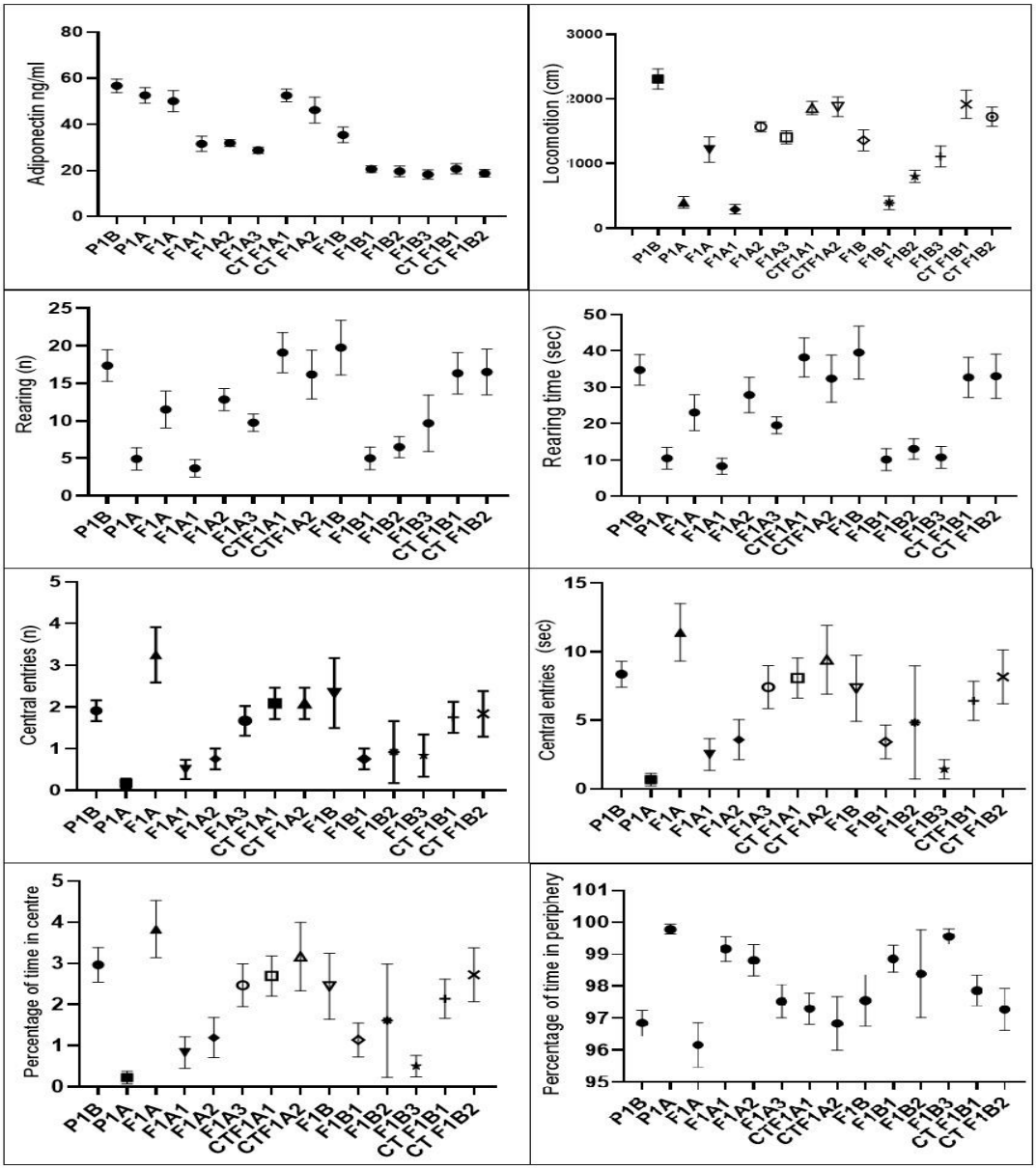


Figure 2: Adiponectin level and open field tests in all groups of experimental animals

Values are mean ± SEM
Key:
p* ≤ 0.05
p**≤ 0.01
p***≤0.001

Case offspring exposed to both early and late life chronic stressors or the ones that got the stress late in life and even their controls had greater locomotion (cm), rearing and rearing time compared to control offspring that got the stress at the same points in life. Central entries were more in all the case offspring groups even in their control groups compared to their control counter parts. Groups with higher adiponectin level showed more locomotion, rearing and rearing time i.e., lesser anxiety, with the exception of case offspring after early life stress.

4. DISCUSSION

The stressful events triggering chronic stress response are unavoidable in daily life and overcoming barriers is integral to success. One cannot live in an environment free of stress and response to stress also varies.[37] Stress response to stressors for a short duration may act as a motivation for success but a chronic stress response may be devastating. [38] Some parents are more exposed to chronic stressors than others in their lives. This study depicts the comparison of stress response of offspring of case versus offspring of control parents whether they have become vulnerable or resilient. We found different responses in groups that were defined by the different time of exposure to stress.

Offspring of case parents exposed to stress showed raised adiponectin levels compared to control offspring in all groups whether they were exposed to chronic stress only in early life, late life or both early and late life. Highest adiponectin(ng/ml)

was observed in offspring group of case parents exposed to both ELS+LLS than offspring of control parents exposed to stress at the same time. (Figure 1). This may be due to resilience shown by offspring of cases to chronic stress compared to control offspring due to which adiponectin levels are better in cases offspring compared to the control ones. Case offspring exposed to both early and late life chronic stressors or the ones that got the stress late in life and even their controls had greater locomotion (cm), rearing and rearing time compared to control offspring that got the stress at the same points in life. Central entries were more in all the case offspring groups even in their control groups compared to their control counter parts. Groups with higher adiponectin level showed more locomotion, rearing and rearing time i.e., lesser anxiety, with the exception of case offspring after early life stress (Figure 2).

Literature suggests that PPARγ is a key transcription factor controlling adiponectin expression. [39] Its activation causes formation of adiponectin and adiponectin- dependent anxiolytic behaviour. In chronic social defeat stress, decreased adipose PPARγ and reduced adiponectin production was observed in susceptible mice but not in the resilient ones. In our study, the control offspring were susceptible to stress. This may be attributed to suppression of adipose PPARγ-adiponectin axis resulting in PPARγ downregulation, leading to decreased adiponectin level. These showed anxiogenic behaviour in open field (lesser locomotion, rearing and central entries). The case offspring showed resilience which might be due to absent PPARγ downregulation and normal adiponectin levels. The decrease in anxiogenic behaviour observed in groups of case offspring having high adiponectin is the same as was observed by Reader et al in their study in which Rosiglitazone, a blood–brain barrier-impermeant PPARγ-selective agonist, was given to wild-type mice, with a concurrent increase in plasma adiponectin levels and they elicited antidepressant- and anxiolytic-like behavioural effects in elevated maze and open field test. [10]

21-day chronic restraint stress resulted in decrease in adiponectin receptors. This was like our study in which control offspring groups had relatively lower adiponectin after 21 days of exposure to chronic stress. [40] In contrast to our study in which the case offspring groups had higher adiponectin level compared to control offspring groups, total adiponectin levels in plasma were decreased after social defeat in cases compared to controls (24 h: control 18.37 ± 0.87, defeat 12.61 ± 0.96, $t_{(19)} = 4.467$, $P < 0.001$; 48 h: control 18.46 ± 1.15, defeat 13.11 ± 0.61, $t_{(20)} = 4.538$; $P < 0.001$). This reduction of adiponectin level had no relation with the change in adipose tissue mass. Reduced adiponectin level showed a positive correlation with social withdrawal. [6] Glucocorticoids lead to inhibition in gene expression and secretion of adiponectin both in vivo and in vitro.[41,42]

Furthermore, lower level of adiponectin in offspring of control parents at different ages may be due to decreased movement of adiponectin across endothelial cells in the presence of glucocorticoids. [13] Chronic stress impairs the adiponectin's downstream signaling pathways. [16] Deficiency of adiponectin further enhanced anxiety and deteriorated memory in transgenic Alzeihmere's animal model [14]
It is reported that action of adiponectin is carried out through Adiponectin-Notch pathway. The two important molecules in notch signaling are ADAM10 and Notch1 that are upregulated by adiponectin and play an important role in hippocampal neurogenesis and cognitive function. Chronic stress inhibits the Adiponectin-Notch pathway and induces impaired hippocampal neurogenesis and cognitive dysfunction. Lesser adiponectin in control offspring after exposure to chronic stress at different times in life may be due to inhibition of the Adiponectin-Notch pathway leading to impaired hippocampal neurogenesis and increased anxiety in open field test. [17] On the other hand, case offspring had higher adiponectin levels compared to their control offspring counter parts.

Another important system through which adiponectin acts is mesolimbic dopamine system in ventral tegmental area (VTA). It has importance in exhibiting emotions along with processing of rewards. [18] Its dysfunction can lead to depression, anxiety and other psychiatric disorders. [43] Dopamine neurons not only exhibit activation in response to rewards but also to stressors. [44] Restraint stress induced an increase in firing of dopaminergic neurons. [45] Metabolic hormones may influence stress processing by altering activity of dopamine neurons. [20]

Adiponectin alters the activity of dopamine neurons in ventral tegmental area and anxiety-related behavior through AdipoR1 present on these neurons as reported by Sun et al in their study. [20] In our study, offspring of control parents showed increased anxiety like behaviour after exposure to both E+L and late life stress. These were the groups with lower adiponectin level compared to their counter parts. In control offspring, there is less adiponectin, less adipo R1 stimulation, more anxiety revealed by less locomotion and rearing due to increased firing of dopamine neurons. In case offspring, there is more adipoR1 stimulation as adiponectin level is more thus decreasing firing of dopamine neurons showing decreased anxiety depicted by greater locomotion and rearing.

Conclusion: Adiponectin (ng/ml) level was increased in case offspring compared to control offspring after exposure to same chronic stressors in early, both early and late and late life. Similarly, lesser anxiety was observed in groups with high adiponectin level.

Future recommendation: Finding the mechanism of adaptation in transgenerational model is recommended for further research.

Authors contribution

1. Dr. Robina Usman
Manuscript write up, idea, critical reading, lab work, statistics, data collection

2. Dr. Muhammad Omar Malik (corresponding author)
Manuscript write up, Idea of study, statistics, critical reading

3. Dr. Madiha Khattak
Idea of study, lab work, statistics, critical reading

4. Dr. Syed Hamid Habib
Idea of study, statistics, critical reading

5. Dr. Rifat Ullah Khan
Critical reading, lab work, data collection

Conflict of interest: The authors declare that there is no conflict of interest.

Acknowledgements: This study has no funding.

REFERENCES

1. Juster R-P, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. Neurosci Biobehav Rev. 2010;35(1):2–16.
2. Albarracín MLG, Torres AYF. Adiponectin and Leptin Adipocytokines in Metabolic Syndrome: What Is Its Importance? Dubai Diabetes Endocrinol J. 2020;1–10.
3. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 1999;257(1):79–83.
4. Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, et al. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin: implications for metabolic regulation and bioactivity. J Biol Chem. 2003;278(11):9073–85.
5. Neumeier M, Weigert J, Buettner R, Wanninger J, Schäffler A, Müller AM, et al. Detection of adiponectin in cerebrospinal fluid in humans. Am J Physiol Metab. 2007;293(4):E965–9.
6. Liu J, Guo M, Zhang D, Cheng SY, Liu M, Ding J, et al. Adiponectin is critical in determining susceptibility to depressive behaviors and has antidepressant-like activity. Proc Natl Acad Sci U S A. 2012;109(30):12248–53.
7. Vidal-Puig A, Jimenez-Liñan M, Lowell BB, Hamann A, Hu E, Spiegelman B, et al. Regulation of PPAR gamma gene expression by nutrition and obesity in rodents. J Clin Invest. 1996;97(11):2553–61.
8. Barak Y, Nelson MC, Ong ES, Jones YZ, Ruiz-Lozano P, Chien KR, et al. PPARγ is required for placental, cardiac, and adipose tissue development. Mol Cell. 1999;4(4):585–95.
9. Guo M, Li C, Lei Y, Xu S, Zhao D, Lu X-Y. Role of the adipose PPARγ-adiponectin axis in susceptibility to stress and depression/anxiety-related behaviors. Mol Psychiatry. 2017;22(7):1056.
10. Reader BF, Jarrett BL, McKim DB, Wohleb ES, Godbout JP, Sheridan JF. Peripheral and central effects of repeated social defeat stress: monocyte trafficking, microglial activation, and anxiety. Neuroscience. 2015;289:429–42.
11. Lindgren EM, Nielsen R, Petrovic N, Jacobsson A, Mandrup S, Cannon B, et al. Noradrenaline represses PPAR (peroxisome-proliferator-activated receptor) γ2 gene expression in brown adipocytes: intracellular signalling and effects on PPARγ2 and PPARγ1 protein levels. Biochem J. 2004;382(2):597–606.
12. Rodgers RJ, Dalvi A. Anxiety, defence and the elevated plus-maze. Neurosci Biobehav Rev. 1997;21(6):801–10.
13. Dang TQ, Yoon N, Chasiotis H, Dunford EC, Feng Q, He P, et al. Transendothelial movement of adiponectin is restricted by glucocorticoids. J Endocrinol. 2017;234(2):101–14.
14. Ng RC-L, Jian M, Ma OK-F, Bunting M, Kwan JS-C, Zhou G-J, et al. Chronic oral administration of adipoRon reverses cognitive impairments and ameliorates neuropathology in an Alzheimer's disease mouse model. Mol Psychiatry. 2021;26(10):5669–89.
15. Na K-S, Kim E-K, Park J-T. Decreased plasma adiponectin among male firefighters with symptoms of post-traumatic stress disorder. J Affect Disord. 2017;221:254–8.
16. Formolo DA, Cheng T, Yu J, Kranz GS, Yau S-Y. Central adiponectin signaling–a metabolic regulator in support of brain plasticity. Brain Plast. 2022;8(1):79–96.
17. You J, Sun L, Wang J, Sun F, Wang W, Wang D, et al. Role of Adiponectin-Notch pathway in cognitive dysfunction associated with depression and in the therapeutic effect of physical exercise. Aging Cell. 2021;20(6):e13387.
18. Nestler EJ, Carlezon Jr WA. The mesolimbic dopamine reward circuit in depression. Biol Psychiatry. 2006;59(12):1151–9.
19. Labouèbe G, Liu S, Dias C, Zou H, Wong JCY, Karunakaran S, et al. Insulin induces long-term depression of ventral tegmental area dopamine neurons via endocannabinoids. Nat Neurosci. 2013;16(3):300–8.
20. Sun F, Lei Y, You J, Li C, Sun L, Garza J, et al. Adiponectin modulates ventral tegmental area dopamine neuron activity and anxiety-related behavior through AdipoR1. Mol Psychiatry [Internet]. 2019;24(1):126–44. Available from: http://dx.doi.org/10.1038/s41380-018-0102-9
21. Radahmadi M, Alaei H, Sharifi MR, Hosseini N. Stress biomarker responses to different protocols of forced exercise in chronically stressed rats. J Bodyw Mov Ther. 2017;21(1):63–8.

22. Risling TE, Caulkett NA, Florence D. Open-drop anesthesia for small laboratory animals. Can Vet J. 2012;53(3):299.
23. Beeton C, Garcia A, Chandy KG. Drawing blood from rats through the saphenous vein and by cardiac puncture. J Vis Exp JoVE. 2007;(7).
24. Usman R, Malik MO, Khattak M, Habib SH, Khan RU. DEVELOPMENT OF PROTOCOL FOR TRANSGENERATIONAL STRESS IN WISTAR RATS. J Popul Ther Clin Pharmacol. 2023;30(19):151–70.
25. Beeton C, Garcia A, Chandy KG. Drawing blood from rats through the saphenous vein and by cardiac puncture. J Vis Exp. 2007;(7):1–2.
26. Fonken LK, Finy MS, Walton JC, Weil ZM, Workman JL, Ross J, et al. Influence of light at night on murine anxiety- and depressive-like responses. Behav Brain Res. 2009;205(2):349–54.
27. Munn E, Bunning M, Prada S, Bohlen M, Crabbe JC, Wahlsten D. Reversed light–dark cycle and cage enrichment effects on ethanol-induced deficits in motor coordination assessed in inbred mouse strains with a compact battery of refined tests. Behav Brain Res. 2011;224(2):259–71.
28. Agrawal A, Jaggi AS, Singh N. Pharmacological investigations on adaptation in rats subjected to cold water immersion stress. Physiol Behav. 2011;103(3–4):321–9.
29. Padovan CM, Guimaraes FS. Restraint-induced hypoactivity in an elevated plus-maze. Brazilian J Med Biol Res. 2000;33(1):79–83.
30. Khattak M, Malik MO, Usman R, Habib SH, Saddique U. DEVELOPING CHRONIC UNPREDICTABLE/ALTERNATING STRESS MODEL IN WISTAR ALBINO RATS. J Popul Ther Clin Pharmacol. 2023;30(19):223–32.
31. Blunn CT. The age of rats at sexual maturity as determined by their genetic constitution. Anat Rec. 1939;74(2):199–213.
32. Makori Arika W, Kibiti CM, Njagi JM, Ngugi MP. Effects of DCM leaf Extract of Gnidia glauca (Fresen) on Locomotor Activity, Anxiety and Exploration-Like Behaviors in High Fat Diet-Induced Obese Rats. bioRxiv. 2019;786103.
33. Neelotpol S, Rezwan R, Shahriar M. Chemical analysis of calabash chalk and its effect on locomotor activities and behavior in Swiss albino mice. Heliyon. 2023;9(3).
34. Owhorji B, Okon U, Nwankwo A, Osim E. Chronic consumption of calabash chalk diet impairs locomotor activities and social behaviour in Swiss white Cd-1 mice. Heliyon. 2019;5(6).
35. Jarrar B, Al-Doaiss A, Shati A, Al-Kahtani M, Jarrar Q. Behavioural alterations induced by chronic exposure to 10 nm silicon dioxide nanoparticles. IET nanobiotechnology. 2021;15(2):221–35.
36. Ramos A, Pereira E, Martins GC, Wehrmeister TD, Izídio GS. Integrating the open field, elevated plus maze and light/dark box to assess different types of emotional behaviors in one single trial. Behav Brain Res. 2008;193(2):277–88.
37. Denson TF, Spanovic M, Miller N. Cognitive appraisals and emotions predict cortisol and immune responses: a meta-analysis of acute laboratory social stressors and emotion inductions. Psychol Bull. 2009;135(6):823.
38. Jankord R, Herman JP. Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. Ann N Y Acad Sci. 2008;1148(1):64–73.
39. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, et al. PPARγ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes. 2001;50(9):2094–9.
40. Várhelyi ZP, Kálmán J, Oláh Z, Ivitz E V., Fodor EK, Sántha M, et al. Adiponectin receptors are less sensitive to stress in a transgenic mouse model of Alzheimer's disease. Front Neurosci. 2017;11(APR):1–13.
41. Fallo F, Scarda A, Sonino N, Paoletta A, Boscaro M, Pagano C, et al. Effect of glucocorticoids on adiponectin: a study in healthy subjects and in Cushing's syndrome. Eur J Endocrinol. 2004;150(3):339–44.
42. Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R. Hormonal regulation of adiponectin gene expression in 3T3-L1 adipocytes. Biochem Biophys Res Commun. 2002;290(3):1084–9.
43. Coque L, Mukherjee S, Cao J-L, Spencer S, Marvin M, Falcon E, et al. Specific role of VTA dopamine neuronal firing rates and morphology in the reversal of anxiety-related, but not depression-related behavior in the ClockΔ19 mouse model of mania. Neuropsychopharmacology. 2011;36(7):1478–88.
44. Brischoux F, Chakraborty S, Brierley DI, Ungless MA. Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. Proc Natl Acad Sci. 2009;106(12):4894–9.
45. Anstrom KK, Woodward DJ. Restraint increases dopaminergic burst firing in awake rats. Neuropsychopharmacology. 2005;30(10):1832–40.