

Original Article

Hypolocomotive and anxious behaviour in obese and type 2 diabetic rats: An experimental study

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ABSTRACT

Objectives: The objectives of the study are to examine the comparative effects of obesity and diabetes on locomotor activity and anxiety-like behaviour in Charles Foster male rats.

Materials and Methods: Eighteen male rats were divided into three groups: the Control group fed a normal pellet diet, the obese group fed a high-fat diet (HFD), and the diabetic group fed HFD followed by a single low dose of streptozotocin injection intraperitoneally (35 mg/kg bw). Open field test (OFT) and elevated plus maze (EPM) test were performed. The central latency time, ambulation, number of rearing, self-grooming, freezing time, and number of faecal boli released by each rat were the observed parameters in OFT. The number of entries into the open arm and total entries, duration and distance travelled in the open arm were the observed parameters in the EPM test. Data were analysed by one-way analysis of variance followed by Tukey's *post hoc* test for multiple comparisons.

Results: In OFT, there was a significant decrease in central latency time and ambulation in the obese and diabetic groups compared to the control group. In the obese and diabetic groups, there was a significant increase in freezing time compared to the control group. There was a significant increase in defecation in the obese and diabetic groups in comparison to the control group. In the EPM test, there was a significant decrease in the number of open arm entries, time spent in the open arm and distance travelled in the open arm in the obese and diabetic groups compared to the control group.

Conclusion: In conclusion, the present study shows that reduced motor activity and hypolocomotive behaviour in OFT are comparable in both the diabetic and obese groups. In addition, both groups exhibit comparable exploratory behaviour and anxiety-like traits in EPM.

Keywords: Diabetes, Elevated plus maze test, Obesity, Open field test, Streptozotocin

INTRODUCTION

Obesity and diabetes are twin epidemics and pose major challenges globally. The term 'diabesity' highlights the close relationship between obesity and diabetes; both are characterised by impaired insulin action.^[1] Higher appetite and a sedentary lifestyle are typically associated with obesity, and these factors can also contribute to mood swings, depression and low motivation.^[2] Diabetes is also linked to physical as well as psychological alterations, such as hypoactive behaviour and depressive symptoms.^[3,4] Diabetes-related acute hyperglycaemia and prolonged stress may affect mood and cognitive function, resulting in anxiety and depression, the most prevalent psychological disorders among patients with diabetes.^[5-7] Type 2 diabetes (T2Ds) induces

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hyperactivation of the hypothalamo-pituitary-adrenal axis and sympathetic nervous system, which can lead to increased secretion of cortisol, epinephrine and norepinephrine.^[8-10] Overproduction of cortisol can activate the fear system, leading to anxiety and depression through neurodegeneration in the hippocampal region.^[11,12] In addition, oxidative stress caused by reactive oxygen species in obesity and T2D may affect the neurotransmitter system or induce inflammation in the neuronal system.^[13] Chronic hyperglycaemia and insulin resistance can also alter the concentration of monoamines in the brain, which are associated with depression and anxious behaviour.^[14,15] In rodents, the open field test (OFT) and elevated plus maze (EPM) are widely used to assess locomotor activity, exploratory behaviour, depression-like behaviour and anxiety-related responses. OFT evaluates the locomotor activity and explorative nature, while EPM assesses anxiety-like behaviour.

Previous reports suggest that streptozotocin (STZ)-induced T2D rats spend more time in open arms in EPM and less time spent in the centre arena in OFT.^[16] Another study assessed timing behaviour and temporal memory in STZ-induced rats.^[17] In another study, anxiety- and aggression-related behavioural alterations were observed in diet-induced obese rats.^[18]

Behavioural changes in obese and STZ-induced diabetic rats have been reported previously. However, no comparative study has been conducted between the high-fat diet (HFD)-induced obese group and the low-dose STZ-treated T2D group. In the present study, we used the OFT and EPM tests to assess the behavioural changes associated with obesity and T2D in rats.

MATERIALS AND METHODS

Experimental animals

Male Charles Foster rats ($n = 18$), 11 weeks old with weighing 200 ± 50 g, were selected at the beginning of the experiment. All the experimental procedures were approved by the Institutional Animal Ethical Committee (IAEC), Institute of Medical Sciences, BHU, Varanasi (Letter no. Dean/2023/IAEC/6201, dated 10 August 2023). The rats were maintained under the standard conditions of an animal room with controlled temperatures ($23 \pm 2^\circ\text{C}$) and a 12-h light/dark cycle. Rats were provided *ad libitum* access to food and water. Control group rats were fed on the standard pellets, and the other two groups were fed on the HFD.^[19] All experiments were performed between 10:00 AM and 01:00 PM. Rats were randomly divided into three groups ($n = 6$ per group):

- Group Control: Fed upon standard rat pellets
- Group Obese: Fed upon HFD
- Group Diabetic: HFD + STZ.

Induction of T2D

Rats were given an HFD for 2 weeks, followed by an overnight fast before receiving a single low-dose intraperitoneal injection of STZ (35 mg/kg body weight) dissolved in 1 mL, 0.1 M sodium citrate buffer (pH = 4.5).^[19] T2D was confirmed by fasting blood glucose levels >250 mg/dL, measured 72 h after STZ injection using a glucometer (Dr. Morepen GlucoOne Blood Glucose Monitor, Model BG-03). Rat weight and STZ doses were measured using two different weighing devices: A digital weighing scale (Aliston, least count 0.1 g, weight limit 10 kg) for body weight and an analytical balance (Wensar, ISO 9001:2008 certified, least count 0.001 g) for STZ. The body weight of the rats in the diabetic group varied (216.2 ± 5.38 g), and the STZ dosage was administered based on individual body weight.

Behavioural tests

The OFT

The purpose of the OFT was to measure the rats' locomotor and exploratory activity, following the guidelines described in Buccafusco (2001).^[20] Using the maze-master video tracking programme, version 6.1.0, each rat was placed in the centre of an open field measuring $60\text{ cm} \times 60\text{ cm} \times 60\text{ cm}$, which was divided into 9 squares measuring $20\text{ cm} \times 20\text{ cm}$. The maze was linked to a computer running video tracking software (MazeMaster 6.1.0) for automated tracking analysis of the movements of the rat in their OFT. The central latency time (duration it took to enter the central zone), ambulation (total number of squares crossed), number of rearing (frequency of standing on hind limbs), number of grooming (frequency of self-cleaning behaviour), freezing time (length of time one was immobile) and defecation (number of fecal boli) released by each individual were the observed parameters.

EPM test

EPM was used to measure the anxiety-like behaviour of rats, protocol from the guideline.^[16] The setup was kept 50 cm above the floor. The EPM consisted of two open arms measuring $35\text{ cm} \times 5\text{ cm}$, two closed arms measuring $35\text{ cm} \times 5\text{ cm} \times 15\text{ cm}$ and a centre region measuring $5\text{ cm} \times 5\text{ cm}$. Recording was done by video tracking the MazeMaster software, as mentioned in OFT. The rat was positioned in the middle, and each rat was kept under observation for 5 min. The number of entries in the open arm and the total entries were recorded. Time spent and distance travelled in open arms were the observed parameters. An entry was counted when the four paws of the rat were placed in the respective arm.

Proper hygiene was maintained during both experiments. All trials were done for 3 consecutive days for each group between 10:00 am to 11:30 am.

Statistical analysis

IBM Statistical Package for the Social Sciences Statistics version 25 (International Business Machines Corporation, Armonk, New York, USA) was used for data analysis. All data sets passed the Shapiro–Wilk test for normality. Based on the results of the normality analysis, parametric tests were performed for statistical comparisons between groups. One-way analysis of variance followed by Tukey's *post hoc* test was used to assess multiple group comparisons. Data are presented as mean \pm standard error. A $P < 0.05$ was considered statistically significant.

RESULTS

Hypo-locomotor activity in the OFT

To evaluate locomotory and exploratory behaviour in control, obese and diabetic rats, the OFT was performed. Central latency time, ambulation, rearing, grooming, freezing time and defecation were among the behavioural parameters that were assessed. Both diabetic and obese rats exhibited longer freezing times and central latency times than control rats. The obese and diabetic groups showed significantly lower levels of ambulatory activity and rearing. Diabetic rats exhibited decreased grooming activity and a markedly increased faeces count.

Post hoc analysis indicated a significant decrease in central latency time in the obese and diabetic groups compared to the control group ($P = 0.01$ and 0.009 , respectively). However, the obese and diabetic groups showed comparable central latency times ($P = 0.995$). A significant decline in ambulation was observed in the obese group compared to the control group ($P = 0.003$). No significant differences were found in grooming and rearing behaviour ($P = 0.598$ and 0.983 , respectively). In both the obese and diabetic groups, freezing time was significantly increased compared to the control group ($P = 0.000$ and 0.001 , respectively). A significant increase in defecation was also observed in the obese and diabetic groups in comparison to the control group ($P = 0.000$ and 0.000 , respectively) [Figure 1].

Anxiolytic behaviour in the EPM test (EPM)

The EPM test was used to assess anxiety-like behaviour in diabetic, obese and control rats. Total arm entries, number of open arm entries, distance travelled, and time spent in the open arms were among the behavioural parameters assessed. Compared to control rats, both obese and diabetic rats exhibited higher anxiety-like behaviour by spending significantly less time in the open arms and making fewer open arm entries.

In the EPM test, there was a significant decrease in the number of open arm entries in the obese and diabetic

groups compared to the control group ($P = 0.008$ and 0.013 , respectively). No significant difference was observed between the obese and diabetic groups ($P = 0.968$). The total number of entries into both open and closed arms was comparable between the obese and diabetic groups in comparison to the control group. Both the obese and diabetic groups travelled significantly shorter distances in the open arms than the control group ($P = 0.000$ for both). The distance travelled in the open arms was similar between the obese and diabetic groups ($P = 0.088$). Furthermore, the obese and diabetic groups spent significantly less time in the open arms compared to control rats ($P = 0.000$ for both). No significant difference in time spent in the open arms was observed between the obese and diabetic groups ($P = 0.091$) [Figure 2].

DISCUSSION

The present study investigated the impact of obesity and diabetes on locomotor activity, exploratory behaviour, anxiety and depression like behaviour in rats using the OFT and EPM tests. The findings demonstrated that both the obese and diabetic groups exhibited significant behavioural alterations compared to the control group. A significant behavioural alteration in obese and diabetic groups was observed, which emphasises the severity of metabolic disorders on central nervous system functions. These findings conform with the emerging evidence that suggests obesity and diabetes are not only metabolic disorders but are also associated with neurobehavioural dysfunctions.

OFT

Obesity and diabetes significantly decrease locomotor activity and exploratory behaviour as observed in OFT, in terms of reduced central latency time and ambulation. Rats of the obese and diabetic groups spent less time in the central zone, indicating high thigmotaxis behaviour and suggesting reduced exploratory behaviour. STZ-treated rats in an earlier study demonstrated decreased exploration of the central square area in OFT.^[16] The reduced exploratory behaviour may be due to STZ-induced inhibition of the synthesis and release of serotonin (5-HT).^[21] The cerebral dysmetabolism associated with a longer duration of diabetes leads to anxiety. The obese and diabetic groups showed a significant increase in freezing time compared to the control group, with the obese group exhibiting the highest freezing time among all three. The increased freezing time in the diabetic group is indicative of a higher level of stress. Similar findings were reported in an earlier study.^[16] Increased oxidative stress in diabetes may damage the prefrontal cortex and trigger anxiety^[22] which explains the greater freezing behaviour observed in diabetic rats. However, obese rats showed the longest freezing time, possibly due to their lethargic nature and higher body weight. A significant increase in defecation

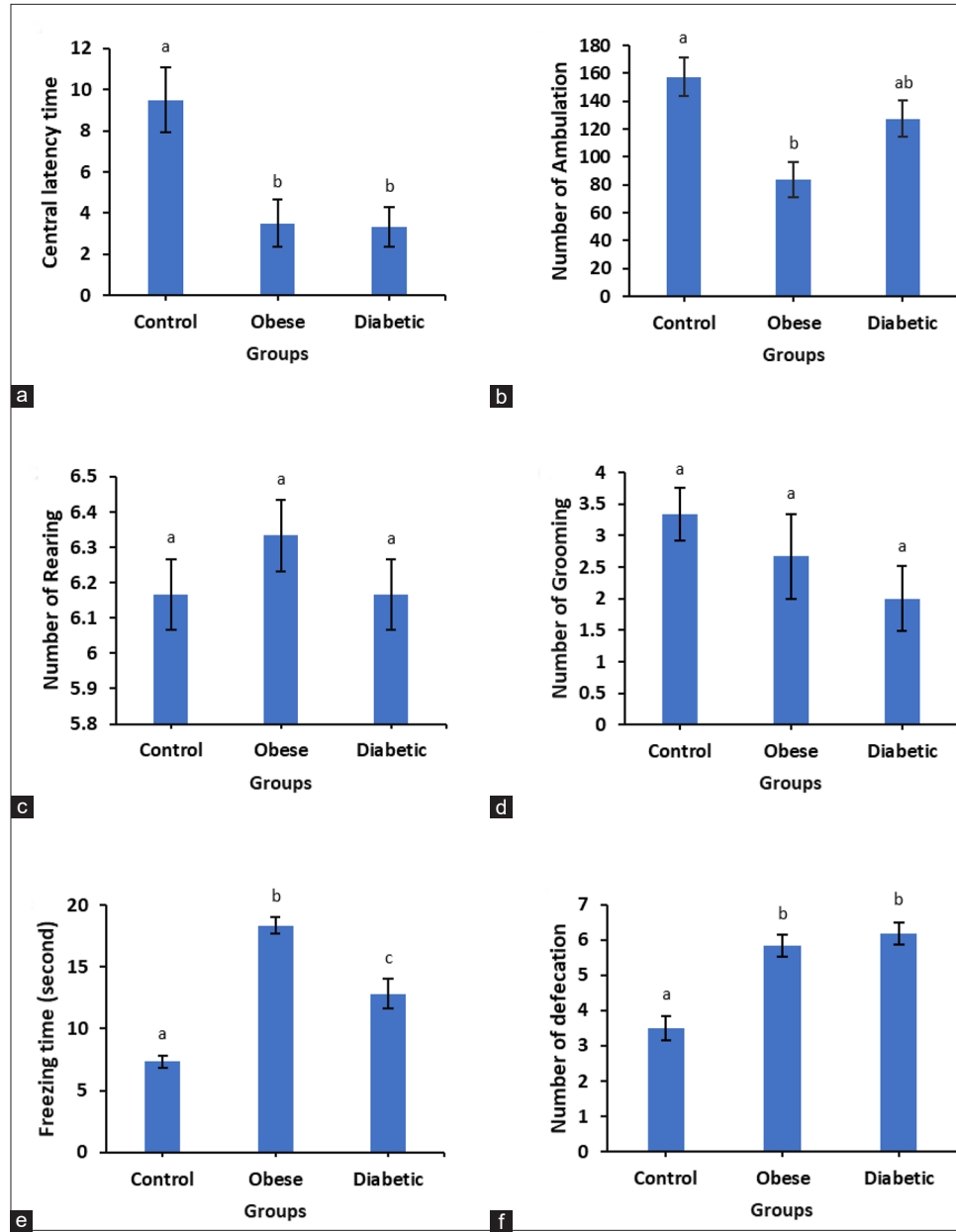


Figure 1: Locomotor activity and exploratory behavior in control, obese, and diabetic rats. Parameters assessed during the 5-minute open field test include: (a) latency to enter the central area, (b) number of ambulation, (c) number of rearings, (d) number of grooming episodes, (e) freezing duration, and (f) number of fecal boli. Data are presented as mean \pm SE ($n = 6$ per group). Bars with different superscript letters indicate statistically significant differences among groups ($P < 0.05$).

score of the diabetic group is likely due to fear and stress. Increased food intake can be another plausible explanation for it. Higher defecation score in diabetic mice has been reported previously.^[23] Self-grooming is a calming procedure for rodents and has been reported to reduce anxiety among rodents^[24] and in the current study, we did not observe any significant difference in grooming and rearing behaviour among the groups. Similarly, no difference was found

in ethological parameters.^[25] The number of faecal boli significantly increased in the obese group compared to the control group. In contrast to our findings, Grover *et al.* (2023) reported fewer faecal boli left by mice on an HFD compared to a chow diet.^[26] Some studies have also reported no direct correlation between defecation number and timidity.^[23] These variations in reporting may be attributed to differences in animal models and methods of investigation.

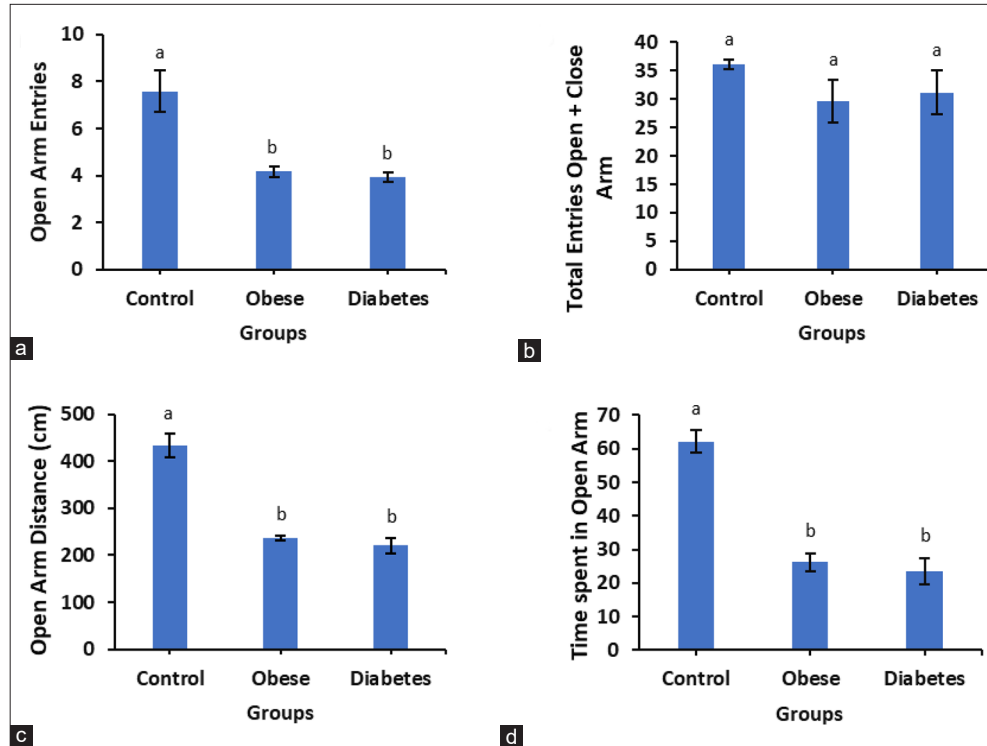


Figure 2: Anxiety-like behavior in control, obese, and diabetic rats assessed using the elevated plus maze test over a 5-minute session. The measured parameters include: (a) number of open arm entries, (b) total number of arm entries, (c) distance traveled in open arms, and (d) time spent in open arms (seconds). Data are presented as mean \pm SE ($n = 6$ per group). Bars with different superscript letters indicate statistically significant differences among groups ($P < 0.05$).

EPM test

In the EPM test, the obese and diabetic groups showed a significantly lower number of entries into the open arms compared to the control group, indicating highly anxiety-like behaviour. Anxiety levels appear to be equally elevated in both obese and diabetic groups, as no significant difference was found between them. The obese and diabetic group indicates that the anxiety level in both groups is high. It was also observed that the obese and diabetic group spent significantly less time in the open arm compared to the control group. Earlier studies observed that STZ-diabetic rats have a smaller number of entries and spend less time in the open arm in comparison to non-diabetic rats.^[27,28] The total number of entries into the open and closed arms was comparable across all groups, indicating that all groups were active. Our findings differ from a previous study, which suggested that the STZ group spent more time and had a greater number of entries in the open arm in comparison to the control group.^[16] This difference might be due to variations in the animal model, age, sex and experimental setups. The obese and diabetic group travelled significantly shorter distances in the open arm than the control group, indicating higher anxiety levels. Obese rats travelled approximately the same distance on the open arms as diabetic

rats. Hyperglycaemia and diabetic neuropathy are likely contributing factors to stress, which disturb the neuroendocrine system, leading to anxious behaviour in diabetic rats.^[29,30]

Additional behavioural tests, such as the forced swim test, the tail suspension test and the light-dark box, can be used to reinforce our findings and to confirm the behavioural changes associated with depression and anxiety. To evaluate motor activity, the Beam Walk Test, Grip Strength Test and Rotarod Test can be used. This work may be extended to compare the behavioural characteristics in obese and diabetic rats using the Morris water maze and Y-Maze to evaluate memory and cognitive capacities. Since the histological features of the hippocampal region were not examined in this study, we could not comment on the precise site of neurodegeneration or distinguish between the brain histopathology of the obese and diabetic groups. Future studies may incorporate the quantification of the cortisol levels and histological examination of the hippocampal region of the brain.

CONCLUSION

The result of our study demonstrates that diabetes and obesity exhibit significant alterations in behaviour, as evidenced by

hypolocomotive and anxious behaviour in OFT and EPM. These findings contribute to the evidence that metabolic disorders are associated with neurodegenerative changes in the central nervous system. This study provides a foundation for future research aiming to study neurobiological mechanisms underlying these behavioural changes and their correlation with structural changes in the brain.

Ethical approval: The research/study was approved by the Institutional Review Board at the Institutional Animal Ethical Committee (IAEC), Institute of Medical Sciences, BHU, approval number, Dean/2023/IAEC/6201, dated 10th August 2023.

Declaration of patient consent: Patient's consent is not required as there are no patients in this study.

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