**Journal of Positive Sciences (JPS)** **eISSN: 2582-9351, https://positive-sciences.com** **JPS. 2022 Nov; 2(6): 1-6**

**Article info** **Research Article**
**Received on: 21.10.2022**
**Accepted on: 27.11.2022**
**Published on: 30.11.2022**
doi: https://doi.org/10.52688/ASP54839

# Impaired Memory and Cognitive Performance in Zucker Rats with Insulin Resistance and Obesity

Layla Abd-Al-Sattar Sadiq Laylani [1, *]

[1] Northern Technical University, Mosul, Iraq

[*] doctor.layla@ntu.edu.iq

## ABSTRACT

Studies on the underlying pathophysiological alterations in type 2 diabetes have made considerable use of the genetically obese Zucker rat. Both obese and lean Zucker rats had their brainpower measured using a variable-interval delayed alternation test. Obese rats were no worse than control rats at learning the alternation rule and at shorter intervals, but their performance at longer intervals, which requires the hippocampus, was impaired. The insulin-sensitive glucose transporter and its receptor are both expressed at similar levels in the brains of obese rats, but there is a decrease in the plasma membrane form of the GLUT4 in the hippocampus. These results are in line with those from studies involving humans, and they indicate that the hippocampus and related tissues are more susceptible to the negative effects of diabetes mellitus. In this study have been tested the Cognitive Performance and Impaired Memory in Zucker rats.

**Keywords:** Zucker rats, type 2 diabetes mellitus, obesity, cognition, memory

## INTRODUCTION

Disorders in glucose metabolism and impaired insulin receptor signaling lead to the complications of insulin resistance and type 2 diabetes, such as peripheral neuropathy, retinopathy, cardiovascular disease, and renal failure. DM2 is characterized by cognitive decline, particularly in elderly people, who may have memory loss and poor executive function. IR is linked to age-related cognitive impairment regardless of the presence or absence of type 2 diabetes. Clues to possible biological mediators of the cognitive deficits can be gleaned from the fact that Several brain areas, including those implicated in cognition, are physiologically vulnerable to hyperinsulinemia because of their association with abnormalities in glucose metabolism and insulin signaling (e.g., frontal lobes, hippocampus). Animal models for studying the association between poor uncorrelation and cognition are severely lacking. Diabetic rats treated with streptozotocin (STZ) have trouble with both spatial and nonspatial memory and learning. While it is true that this paradigm is "helpful for examining the implications of chronic hyperglycemia," it cannot be utilized to study either type 1 or type 2 diabetes because "it's endocrinological features do not properly replicate either type 2 or type 1 diabetes." Insulin resistance (IR), glucose intolerance (GI), obesity, and endocrinological features of DM2 have been observed in Zucker fa/fa rats, a rodent model of DM2; however, these rats have not been put through a battery of demanding mental tests, and results from tests of GI and spatial memory have been inconsistent. Obese Zucker rats have been found to have electrical deficits in the hippocampus, a key location for learning and memory, suggesting that this model might helpful for investigating the causes of cognitive issues in individuals with similar disorders. The amount of time between trials in a go/no-go delayed alternation task was changed. We examined cognitive performance in obese and lean Zucker rats, an animal model of IR (VIDA). Several diverse processes involving various parts of the brain are necessary for a successful performance, although these processes are not always tightly coupled. The ability to learn the simple response-alternation rule is specifically decreased in rats with frontal lobe injuries. However, rats with lesions in their hippocampi are still able to learn the rule and show no impairments at all in their performance during brief inter-trial periods (ITIs). However, as their use increases, their efficiency decreases. Cognitive Illusions that test one's capacity to retain detailed information. Dietary fat modification is only one of several factors that might affect how well you do on this assignment. Therefore, the VIDA task may be used to evaluate neurocognitive performance in Zucker rats and ascertain whether or not this strain can successfully replicate cognitive alterations related with IR and obesity in people. As a preliminary step in identifying physiological markers of altered cognition, we investigated the effects of insulin and glucose on the expression of insulin receptors and the translocation of these receptors in the hippocampus. To measure insulin receptor activity, scientists analyze levels of GLUT4, a glucose transporter that responds to insulin.

***Corresponding author**
Layla Abd-Al-Sattar Sadiq Laylani,
Northern Technical University, Mosul, Iraq
e-mail: doctor.layla@ntu.edu.iq

# METHOD

## SUBJECTS

Rats were either lean (FA/?) controls or obese (fa/fa) Zucker rats. The Aleppo University animal ethics committee authorized the procedure, and animals were brought in at the age of 5 months. However, the animals were kept in quarantine for an additional 8 weeks before the behavioral testing started (body weight-lean: 226 17g and obese: 28820g). Involvement of Rats in the experiment in separate cages made of wire mesh and provided with access to water at all times. According to the protocol, the usual fare of the laboratory canteen was provided.

## Machines and Methods

There are more in-depth explanations of the VIDA exam available online. All trials were conducted in a soundproof room, with one retractable lever Skinner boxes operated by a computer, and a 3-watt lamp set in the middle of the ceiling to the right of a central feeder. Rats were handled often and restricted to 60% of their ad libitum food intake for two weeks prior to training. Later, the rats were trained using a CRF regimen, in which they were rewarded for pushing the lever with food.

Over the course of 2 days, trainees participated in daily 30- minute CRF sessions until reaching a response rate of 80. At the end of each training session, the rats were given a single,

45-mg Noyes food pellet as a reward for pressing the lever. Rats were given their regular diets of normal chow after each session and then returned to their cages. One day after reaching criteria in CRF training, testing with VIDA was begun. There were 24 total trials in each testing session, 12 of which were reinforced (go) and 12 of which were not. The go and no-go trials lasted 20 seconds each, and the lever was always there. When the trials were considered a "go," each leverpress resulted in a 45-mg Noyes food pellet being generated, but in the "no-go" trials, leverpresses yielded no such reward. An arbitrary ITI during which the lever was retracted separated the go and no-go trials. A total of 12 successful and 12 unsuccessful attempts were made in each session. Each ITI lasted either 0 or 5 seconds, 10 or 40 seconds, or 80 seconds, and happened twice after successful trials and twice after unsuccessful trials, for a total of four ITIs each session. Every session started with a go try, but the ITI order was different each time. For 15 days in a row, testers met with products at regular intervals.

## EVALUATION OF BIOCHEMICAL VARIABLES

Trunk blood was collected from rats who had been fasting overnight and were still on their food restriction program one week after the testing sessions had concluded. As was previously mentioned, plasma glucose was analyzed using the glucose oxidase technique. In order to measure insulin, leptin, adiponectin, and corticosterone in the plasma, RIAs were carried out as per the manufacturers' instructions. We used the glycerol phosphate oxidase trinder reaction to determine the triacylglycerol levels in the plasma in accordance with the protocol provided by the manufacturer.

## METHODS of IMMUNOBLOTTING

During the slaughtering process, we swiftly removed the hippocampus and separated fractions containing the hippocampal membrane. Proteins were resolved on 12% SDS-PAGE gels, transferred to nitrocellulose membranes, and then blocked in Tris-buffered saline containing 12% non- fat dry milk for 60 minutes. Primary antisera against GLUT4 or IR- were incubated with NC membranes in TBS/6% non fat dry milk, and the resulting bands were developed using enhanced chemiluminescence reagents per the manufacturer's instructions. With the help of the MCID image analysis system, we calculated the micro densitometry of autoradiographic pictures (Imaging Research Inc.).

## MANIPULATION of INFORMATION and STATISTICAL MODELLING

There are a few standard methods to visualize data from the VIDA job, the most frequent being the latency to the first leverpress and the go/no-go latency ratio. At each ITI, performance was measured by comparing the mean latency to initial leverpress during go trials to that during no-go trials; a lower latency ratio indicates better performance. In order to simplify the data for statistical analysis, we compressed it across days and ITIs. To evaluate The rats' learning capacity was evaluated by averaging ITI-0 data throughout five lines of 3 days for each animal. Only the last three days of testing were analyzed to see how extending the time between go and no-go trials affected participants' ability to remember what happened in the task (Block 5). Based on these averages, we know that ITI-40 and ITI-80 are very long intervals, whereas ITI-5 and ITI-10 are very short intervals. To account for heteroscedastic data distribution, an autoregressive covariance structure was created in SAS for Windows to conduct repeated-measures ANOVAs comparing interaction effects between genotype, time (block), and ITI, and the other way around.

*Corresponding author
Layla Abd-Al-Sattar Sadiq Laylani,
Northern Technical University, Mosul, Iraq
e-mail: doctor.layla@ntu.edu.iq

The model was divided into sub-groups depending on latency, and ANOVAs were run to further examine the main influence of genotype when significant interactions were identified. We compared the outcomes of RIA and Western blotting using one-way analysis of variance.

## RESULTS

## OBJECTS

Heavy Zucker rats weighed more. (342 ± 4 vs. 518 ± 2g) fasting plasma glucose levels were significantly elevated (124± 2 vs. 186 ± 18 mg/dl), insulin (1.51 ± 0.12 vs. 20.6 ± 4.6 ng/µl), leptin (4.70 + 0.83 vs. 37.11 + 3.50 ng/µl), adiponectin (4.2 + 0.1 vs. 7.2 ± 0.3 µg/ml), corticosterone (17.5 ± 2.4 vs. 31.5 ± 3.3 µg/dl), and triacylglycerols (71 + 3 vs. 626 ± 105 mg/dl) level in comparison to the slimmer controls (all ps<.001; data are M ± SEM; n= 7/ blood-related genotype and n= 19 for weight).

## PSYCHOLOGY and METHODS of BEHAVIOURAL TRAINING

There was no correlation between the rat's body mass and its performance on the CRF regimen used to teach the leverpress response. At the end of training, both groups had attained the criteria, but the slim rats did so in 4.3 days on average (t <1) whereas the obese rats needed 3.9 days. These results are consistent with what would be expected from normally developed adult rats subjected to the same training.

## ADAPTIVE LEARNING THROUGH INTERMITTENT PRACTICE

Examining the rats' behavior at ITI-0, when there was no interval between the go and no-go trials, allowed us to gauge how well they could acquire the fundamental alternation rule (Figure 1 A). The performance of both obese rats and lean significantly increased with time (in 3 day blocks), reaching an asymptotic level by Block 4. It was seen as a shorter delay before the initial lever push in the go trials compared to the no-go trials, as well as a lower go/no-go latency ratio (all ps< 0.001). It was shown that the learning rates were equivalent between the two genotypes (Prevent Genealogical Interactions in the GO, F (4, 151) = 1.63, p = 0.20; no-go, F (4, 151) = 0.30, p = 0.87; and ratio, F (4, 151) = 1.58, p =0.18). Researchers also compared how long it took fat and lean rats to do their first lever push in go and no-go trials, with the hope that a delay might reveal a lack of physical competence related to obesity or a lack of motivation to complete the job. Both the "go" and "no-go" trials showed no significant difference between genotypes F (1, 152) =2.32, p= 0.13 and F(1, 152) =1.11, p= 0.29, respectively). Data demonstrate that thin and obese rats learn the alternation rule at similar rates, suggesting that they share certain motivational-performance qualities.

## MEMORY

After animals reached asymptotic performance at ITI-0 in Block 5, the impacts of adding a memory requirement were assessed by gradually increasing ITIs (Figure 1B). ITT increases had a negative effect on performance regardless of genotype, with go/no-go ratios rising across the board, F (2,78) = 112.30, p < 0.0001. The latency to first lever press in the no-go trials decreased significantly with increasing ITI from 6.26 0.36 s to 9.23 0.63 s [ITI-0] [for short delay] ,2.39 0.29 s [for long delay], M+SEM, F [2, 77] =72.55, p 0.0001), suggesting that this effect was caused by the participants becoming more accustomed to the task. The mean standard error of the mean (M SE) time until the first lever press for lean and obese rats was 1.10 0.13 seconds (ITI-0) and 1.25 0.17 seconds (short delay) and 1.53 0.13 seconds , respectively (for the go trials; (F [2, 77] =2.50, p = 0.09). Overall performance did not differ by genotype (F(1, 37) =2.76, p = 0.10), still, a substantial relationship between genotype and delay was seen. (F (2, 75) =5.49, p =0.06). At both ITI-0 and the short delay, the go/no-go latency ratios were comparable across lean and fat rats F (1, 37) =0.33, p = 0.56 and (F (1, 37) = 0.08, p = 0.78, respectively). However, the decision to proceed or not was only made after a lengthy wait.

*Corresponding author
Layla Abd-Al-Sattar Sadiq Laylani,
Northern Technical University, Mosul, Iraq
e-mail: doctor.layla@ntu.edu.iq

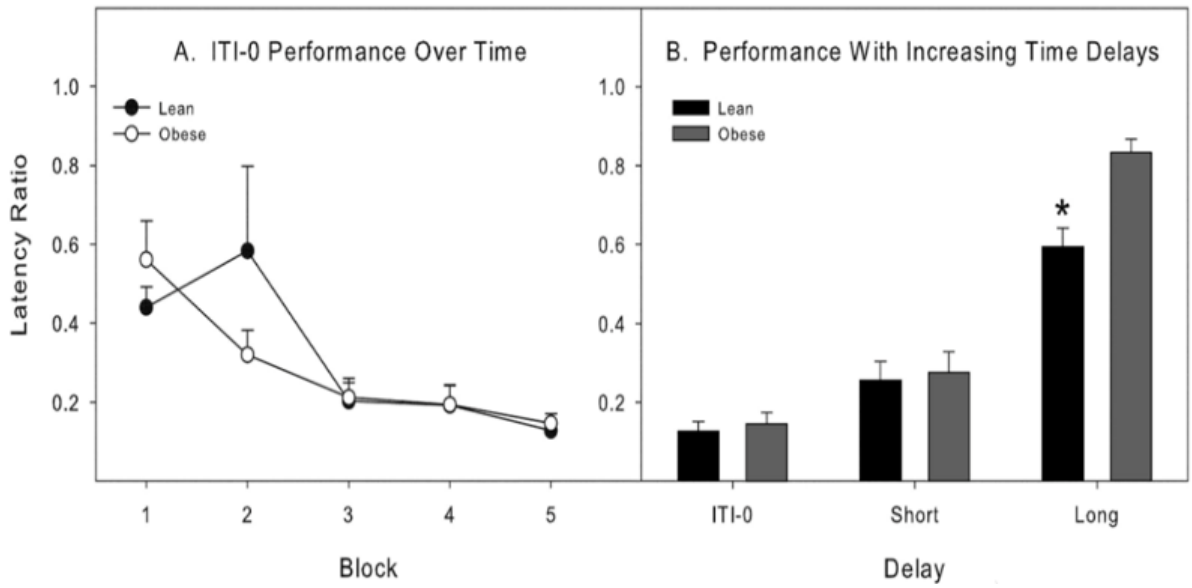


**Figure 1: Obese Zucker and Lean rats learned the basic alternation rule over the course of five 3-day blocks on the delayed alternation task with a variable interval between trials at inter-trial interval (ITI)-0. Data (M SEM, n 25/genotype) showing the delay-to-go/no-go ratio. Comparison of immediate (ITI-0), short (6, 9 s), and long (30, 70 s) delays in VIDA memory tests for lean and overweight Zucker rats. Go/no-go delay ratios are shown as data (M ± SEM, n=25/genotype). Observed differences between genotypes and the lengthy delay alone are shown with an asterisk if they are statistically significant ($p < 0.001$).**

The go trials, $F(0, 37) = 0.002$, $p = 0.1$ (2.53 ± 0.18 vs. 2.51± 0.18 s; M ± SEM for vs. obese lean), However, it was noticeably shorter in the obese rats than in the slim rats, $F(1,37) = 6.67$, $p = 0.01$ (2.95 ± 0.36 vs. 1.84 ± 0.22 s; M ± SEM for obese vs. lean).

## WESTERN BLOTTING ANALYSIS of GLUT4 and INSULIN RECEPTOR

Western blotting with subunit of the IR-specific primary antisera revealed the presence of IR- in the hippocampus of both obese rats lean (Figure 2B, 2A). Rats of both lean and obese body types show similar levels of expression for the insulin-sensitive glucose transporter (Figure 2C and 2D). In the hippocampus, the connection of GLUT4 with the plasma membrane was considerably lower in obese Zucker rats than in their lean counterparts. These results are consistent with the deleterious effects of diabetes on peripheral organs and provide credence to the hypothesis that deficits in central nervous system insulin receptor signalling may lead to impairments in the cognitive abilities of overweight rats, which rely on the hippocampus.

## DISCUSSION

In order to analyze different aspects of learning and memory independently, this research examined Zucker rats' performance on a behavioral task. These findings show that obese Zucker rats have a selective memory deficit that does not affect other elements of task performance. Decreases in GLUT4 levels in the hippocampus plasma membrane and increases in plasma insulin, leptin, and corticosterone in obese rats are suggestive of a role for neuroendocrine and neurochemical alterations in abnormalities in hippocampal- dependent behavior. Differences in performance on the VIDA task between obese and lean rats were limited to the longer ITIs, suggesting the capacity for long-term planning and short-term multitasking, which the prefrontal brain controls and which are required to learn the basic rule of response alternation were unaffected by obesity. Memory impairment linked to hippocampal dysfunction is shown by the selective deterioration at longer ITIs. In previous research with brain-injured rats, which is exactly the range within which the obese rats were affected in our study. Fatigue or lack of desire are not plausible causes of the impairment shown in rats. Throughout the duration of the both achieved equivalent results at the 0 and short ITIs in the VIDA task. Therefore, it seems that the overweight rats were both motivated and able to complete the job. Research into longer ITIs, which disproportionately impacted obese rats, also showed that their initial lever press latencies were comparable to lean rats during go trials but much shorter for no-go trials. It seems to reason that if the fat rats are weary or less eager to perform, their response latencies will increase. Keep in mind that in every session of testing, the ITIs were assigned at random; this ensured that the rats would not be able to predict when the ITIs would be longer, which might have an impact on their incentive to perform.

It may be argued, however, that the shorter latencies on the no-go trials at the longer ITIs in the overweight rats are suggestive of higher motivation in these animals. On the other hand, if this line of thinking is right, we should also observe them. It seems unlikely that the poor performance of fat rats is due to an underlying defect in inhibitory control processes, although response perseveration may have had a role. An impaired ability to regulate one's responses would have been seen across the board, not only during the extended ITIs. Extensive ITIs in obese rats have been linked to a lack of discrimination between go and no-go trials, worse memory for events surrounding a prior response, and an overall bias toward completing the rewarded lever press

*Corresponding author
Layla Abd-Al-Sattar Sadiq Laylani,
Northern Technical University, Mosul, Iraq
e-mail: doctor.layla@ntu.edu.iq

response and learned. The Zucker rat is used to illustrate the effects of insulin resistance (IR), a disease linked to a more rapid decrease in brain function with age. When compared to young adults, normal elderly rats perform poorly on the VIDA's tests of both strategic learning and memory. The increased vulnerability of the hippocampus to IR was demonstrated by impaired memory but preserved strategic ability in obese Zucker rats. Although IR has been shown to have negative effects on cognition, the extent to which these effects compound with age-related deterioration remains unknown. A wider range of cognitive impairments may be seen in older obese Zucker rats compared to the young rats employed in this investigation (who were all about 6 months old). Further research into the generalizability of the present results might benefit .Consistent with other studies that revealed reduced Results from the Morris Water maze have been reported as normal in Zucker ZDF rats, despite the fact that Zucker rats and db/db mice, both of which are obese, have been shown to have reduced spatial memory. Our Zucker rats were presumably a little older, substantially bigger, despite there being no potential for Zucker and Zucker to be dissimilar As compared to the ZDF rats, these rats exhibited much greater amounts of glucose in their blood used in the study. Li and coworkers discovered that the two mouse strains differed in their normal LTP performance. slices of rat hippocampi from ZDF animals, indicating that the structure was in good working order. We also describe a wide variety of biochemical changes in the hippocampus that are consistent with the kind of memory loss seen here. The weight of the evidence is currently in favor

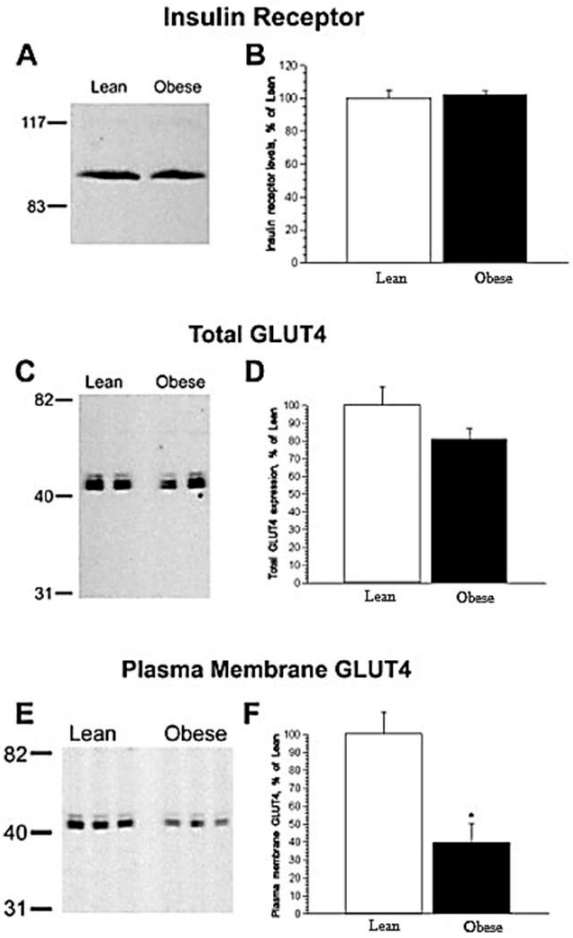


**Figure 2: GLUT4 and Insulin receptor protein expression in the hippocampus plasma membrane fraction as determined by immunoblotting**

Separated hippocampus plasma membranes from normal- weight and overfed Zucker rats (19 ug protein/lane) were probed with antisera specific for the subunit of the insulin receptor, and a single protein of roughly 95 kDa was identified (1:1000; Santa Cruz Biotechnology). Strong positivity for insulin receptors was observed in the hippocampus of both lean Zucker and obese rats. Using GLUT4 antisera, we separated and identified in the hippocampus was the same in both fat and trim Zucker rats. Obese Zucker rats' hippocampal plasma membranes showed significantly decreased GLUT4 immunoreactive levels compared to those of lean controls. On the left, you may see values that are relevant to the (*p Molar mass (in kDa) alone. Mean and standard deviation data for GLUT4 immunoreactivity in lean control rats are presented. hippocampal dysfunction and altered hippocampal biomarkers in Zucker rats with obesity. Owing to the widespread acceptance of long-term potentiation (LTP) as a cellular model of memory and learning, it is possible that LTP anomalies in the hippocampus are to blame for the lack of proper behavior in obese Zucker rats. To what extent the leptin receptor (Ob-Rb) mutant is responsible for the LTP deficits in the obese Zucker rat is an open question. Data suggesting that signaling from leptin receptors may increase N-methyl-D-aspartate receptors in the hippocampus and dentate gyrus, and leptin receptors in these regions, are involved in synaptic transmission. and CA1/CA3 hippocampus fields, all point to a key role for the faulty Ob- Rb receptor. The cognitive deficits found in this rat model are not the only ones seen in the endocrine and neuroendocrine systems. Researchers have speculated that worse memory in the obese Zucker

*Corresponding author
Layla Abd-Al-Sattar Sadiq Laylani,
Northern Technical University, Mosul, Iraq
e-mail: doctor.layla@ntu.edu.iq

rats was caused in part by IR and/or aberrant insulin receptor signaling. learning all point to a possible role for the insulin receptor in cognition. is a hallmark of insulin receptor activation, as shown in normally functioning mice when they are given with glucose from the periphery. Current findings reveal that sustained increases in plasma glucose do not promote hippocampal plasma membrane GLUT4 translocation, which is consistent with a prior study of diminished hippocampus insulin receptor activation in Zucker rats with obesity. Memory loss in obese Zucker rats has been linked to alterations in GLUT4 transporter translocation, which may affect neuronal glucose uptake, but it is not clear if these alterations are causal or merely a marker for dysfunctional insulin receptor signaling, which may affect cognition via events downstream to the insulin receptor. The trafficking of neuronal glucose transporters, absorption of glucose, and use of glucose all play important functions that need attention. Are all impaired in STZ-diabetic rats with insulin deficiency. The intriguing notion that reduced insulin receptor activation is a shared trait that leads to cognitive impairment in both the obese Zucker rat and the STZ-diabetic rat is based on the observation that both models of diabetes result in similar abnormalities in cognitive function. Although aging is a primary role in cognitive decline, additional metabolic abnormalities, such as those shown in our obese animals (increases in plasma cortisol and triacylglycerides), may also contribute to the memory difficulties associated with advancing years. Finally, we show that the fa/fa Zucker rat is a valuable model for investigating the factors that contribute to cognitive impairment in patients with IR and/or DM2. Similar to what has been shown in humans, fa/fa Zucker rats performed poorly on the VIDA memory subtest, suggesting that the hippocampus and associated structures are more susceptible to the negative effects of diabetes mellitus. Due to the high prevalence of endocrine and neuroendocrine problems, including leptin resistance, in people with type 2 diabetes, the Zucker rat provides a valuable model for investigating the intricacies of these changes in neural circuitry as they relate to cognition.

## CONCLUSION

Characterizing macromorphological changes owing to brain damage and how they are connected to metabolic and functional changes in OZRs is the primary contribution of this study. It is also possible that astrogliosis and microglia activation are involved in protecting the brain. Neuronal microenvironment. By halting the onset of neurodegenerative processes that might ultimately lead to dementia, these findings may be useful in the management of MetS progression.

## REFERENCES

[1] Yokoi, Norihide, et al. "A novel rat model of type 2 diabetes: the Zucker fatty diabetes mellitus ZFDM rat." Journal of diabetes research 2013 (2013).
[2] Fontana, Luis, et al. "Bifidobacterium breve CNCM I-4035, Lactobacillus paracasei CNCM I-4034 and Lactobacillus rhamnosus CNCM I-4036 Modulate Macrophage Gene Expression and Ameliorate Damage Markers in the Liver of Zucker-Lepr fa/fa Rats." Nutrients 13.1 (2021): 202.
[3] Wu-Peng, X. Sharon, et al. "Phenotype of the obese Koletsky (f) rat due to Tyr763Stop mutation in the extracellular domain of the leptin receptor (Lepr): evidence for deficient plasma-to-CSF transport of leptin in both the Zucker and Koletsky obese rat." Diabetes 46.3 (1997): 513-518.
[4] Plaza-Díaz, Julio, et al. "Gene expression profiling in the intestinal mucosa of obese rats administered probiotic bacteria." Scientific data 4.1 (2017): 1-10.
[5] Seeley, R. J., et al. "Intraventricular leptin reduces food intake and body weight of lean rats but not obese Zucker rats." Hormone and Metabolic Research 28.12 (1996): 664-668.
[6] Drotningsvik, Aslaug, et al. "Dietary fish protein hydrolysates containing bioactive motifs affect serum and adipose tissue fatty acid compositions, serum lipids, postprandial glucose regulation and growth in obese Zucker fa/fa rats." British Journal of Nutrition 116.8 (2016): 1336-1345.
[7] Wang, Tianlun, et al. "Responses of lean and obese Zucker rats to centrally administered leptin." Physiology & behavior 65.2 (1998): 333-341.
[8] Drotningsvik, Aslaug, et al. "A low dietary intake of cod protein is sufficient to increase growth, improve serum and tissue fatty acid compositions, and lower serum postprandial glucose and fasting non-esterified fatty acid concentrations in obese Zucker fa/fa rats." European journal of nutrition 54.7 (2015): 1151-1160.
[9] Korner, Judith, et al. "Leptin regulation of Agrp and Npy mRNA in the rat hypothalamus." Journal of neuroendocrinology 13.11 (2001): 959-966.
[10] Grauballe, M. B., et al. "Blockade of RAGE in Zucker obese rats with experimental periodontitis." Journal of periodontal research 52.1 (2017): 97-106.
[11] Feresin, Rafaela G., et al. "Effects of obesity on bone mass and quality in ovariectomized female Zucker rats." Journal of obesity 2014 (2014).
[12] Misawa, Eriko, et al. "Administration of phytosterols isolated from Aloe vera gel reduce visceral fat mass and improve hyperglycemia in Zucker diabetic fatty (ZDF) rats." Obesity research & clinical practice 2.4 (2008):239-245.

*Corresponding author
Layla Abd-Al-Sattar Sadiq Laylani,
Northern Technical University, Mosul, Iraq
e-mail: doctor.layla@ntu.edu.iq