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Review Article

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Understanding The Intense Effects of Caloric Restriction and Overfeeding on Zebrafish (Danio rerio) Behaviour and Biochemical Processes, Along with Their Implications For Offspring

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ABSTRACT
Zebrafish offer powerful molecular and genetic manipulation tools, enabling researchers to investigate the mechanisms linking obesity to CNS dysfunction. Studies on the relationship between food intake and neurobehavioral outcomes, particularly in the context of obesity, hold great promise in advancing our understanding of this complex issue and its potential generational impacts. This study design allows for a comprehensive investigation into the effects of different feeding regimens on various aspects in the control, overfed, 12-h, and 24-h fasting groups. The comprehensive assessment of physical, biochemical, behavioural, and neurochemical parameters in zebrafish provides a robust understanding of how different feeding regimens impact various aspects of their biology. These caloric restriction habits positively influenced the health and behaviour of offspring compared with the overfed group. We agreed on a powerful approach to understanding the complex interplay between diet, brain function, and behaviour. According to an imitation study, excessive food consumption contributes to further diseases.

Keywords: Behavioural, Fasting, F1 Generation, Overfed, Neurotransmitter, Zebrafish

Introduction
Dietary restriction (DR), intermittent fasting (IF), and calorie restriction (CR) are three commonly researched fasting techniques, each with their own approaches and potential benefits [1]. CR involves reducing calorie intake while maintaining essential nutrient intake. It typically aims to reduce calorie intake to about 20%–40% of ad libitum consumption, which is the amount of food an individual eats when allowed to eat as much as they want [2]. Intermittent fasting (IF) has recently attracted significant attention owing to its potential health benefits and flexibility in implementation. IF typically involves cycling between periods of eating and fasting, and there are various approaches to it, including the one you described. One popular method is the 16/8 method, in which individuals fast for 16 h each day and consume all their calories within an 8-hour window. During fasting, they typically consume only water, tea, or coffee without adding calories [3]. Numerous scientific studies have investigated the potential effects of intermittent fasting (IF) on various health outcomes, and their findings have revealed several potential benefits. The findings indicated that fasting contributed to an extended lifespan and prevented a variety of health disparities, such as cardiovascular illnesses, kidney disorders, various types of cancer, and brain and diabetes [4]. Obesity is indeed a significant and complex global health issue with multifaceted causes and consequences. The prevalence of obesity has been steadily increasing worldwide and will continue to increase in the future. The 2021 Nutrients Review, predicts that the prevalence of obesity will approach 50% of the global population by 2030 if current trends persist [5,6].

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The behavioural apparatus of the T-maze is commonly used in neuroscience research to assess spatial learning and memory in various animal models, including fish and rodents. A maze typically consists of a T-shaped runway with a starting area and two arms extending from it, forming a T shape [7-9]. The T-maze was used to evaluate the effects of medications and pharmacological interventions on animal behaviour [10,11]. To employ the T-maze to assess the effects of drugs or other interventions on animal models' learning, memory, or other behavioral processes. Additionally, the T-maze was adapted to study the social behaviors and interactions of zebrafish. Earlier studies conducted experiments using the T-maze to assess how factors such as food rewards or exposure to conspecifics influence zebrafish behavior in a social context [12]. Moreover, pharmacological studies can explore the influence of drugs on social interactions and behaviors in zebrafish models, providing insights into the neurochemical basis of social behaviours. The second test, the Novel Tank Task (NTT), is one of the most commonly used assays to assess anxiety-like behaviour in zebrafish. This task involves placing a zebrafish in a novel tank environment and quantifying its behaviour, such as time spent at the bottom (thigmotaxis), erratic movements, and exploring the tank's vertical and horizontal dimensions. Changes in these behaviours can indicate changes in anxiety levels or stress responses. [13]. The study by (Tan et al., 2022) likely describes experiments in which zebrafish were subjected to conditioning paradigms to assess anxiety-like behaviours and the effects of medication. By observing changes in behaviour before and after treatment, researchers can evaluate the efficacy of medications in modulating anxiety-related responses in zebrafish models.

To demonstrate that zebrafish fed different high-fat diet regimens experienced increased subcutaneous fat content, body weight, body mass index (BMI), fasting blood glucose levels, and cholesterol and triglyceride levels. These findings mirror observations in mammalian models and highlight the utility of the zebrafish as a model organism for studying metabolic disorders and obesity-related complications [14]. The active avoidance test will be used to investigate the impact of a high-fat diet on zebrafish behavior. This test assesses an animal's ability to learn and avoid aversive stimuli in an operant conditioning paradigm. [15]. Effects of food on outcomes Preliminary clinical studies have shown that fasting may affect neurotransmitter levels. Fasting, or intermittent fasting, influences neurotransmitter production and release, potentially affecting mood, cognition, and behavior [16]. Moreover, these neurotransmitters are believed to control the behavior and nervous system activity [17]. We considered this using one of the best animal models. Likewise, zebrafish contain 75% of human genes (external source) and also have a mouth, two eyes, a brain, spinal cord, gut, pancreas, liver, bile ducts, kidney, esophagus, heart, ear, nose, muscle, blood, bone, cartilage, and teeth. Humans and zebrafish share many genes and essential pathways for developing these traits. Therefore, evidence may not apply to larvae because they lack a developed central nervous system. The current investigation assessed the effects of modification to overfeeding and fasting on various behavioral, physiological, and neurological endpoints in future zebrafish generations.

Material and Methods

Animal Husbandry

A wild-type, 3-6-month-old, short-fin phenotypic zebrafish (Danio rerio) was obtained from Asia's largest aquarium shop in Chennai, Tamil Nadu. Maintaining proper water quality is crucial for fish health, growth, and behavior. Regular water testing and appropriate adjustments should ensure optimal conditions [18]. The Department of Biochemistry and Biotechnology conducted the experiments at the Faculty of Science, Annamalai University. Using genetically heterogeneous zebrafish from a survival lab to more accurately reflect natural populations. This approach can help reduce the impact of random genetic drift on inherited features, a common concern in experimental biology.

Experimental Diet

An experimental setup involved four dietary groups to evaluate the impact of diet on morphological and behavioural characteristics in zebrafish. The experiment involved feeding and fasting the fish daily according to the nutritional groups.

Group I: Control (fed twice per day)
Group II: overfed (fed five per day)
Group III: 12hrs fasting (fed twice per day)
Group IV: 24hrs fasting (fed once per day)

The composition of the proximate diet was determined based on the following factors: moisture, crude protein, crude fat, crude fibre, carbohydrate, and ash. In the event of fish mortality, the deceased fish were promptly removed from the tank and preserved at -80˚C freezer for subsequent histological studies [19].

Morphological Measurement

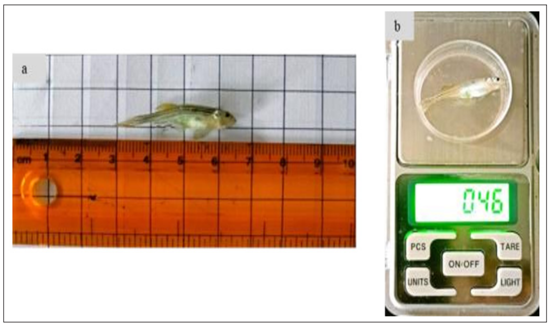

Figure 1: (a) This part of the figure probably shows the measurement of the height or length of the zebrafish at different time points during the study. This may demonstrate how the height of the fish changes over time or across different dietary groups. (b) This part of the figure likely illustrates the weight paradigm of the zebrafish. The results may depict the weight measurements of the fish over time or variations in weight among the different dietary groups.

This experimental design allowed us to assess how different diets affect growth (measured by weight and length), body composition (evaluated through BMI), and overall body weight fluctuations in the zebrafish population throughout the study. Laterally photographed images can provide additional insights into visible morphological changes that may have occurred [14]. The zebrafish used in this study underwent weight and length measurements, and the results are shown in Fig.1 (a) (b). BMI was calculated by dividing body weight (g) by the square of body length (cm2) [20].

Behavioural Observation

T-maze

Zebrafish require food incentives or stimuli from conspecifics to complete the T-maze test. [12]. It was made from a sheet of clear acrylic glass (Figure 2). The maze comprised three arms: a 50 cm x 10 cm long arm, a 20 cm x 10 cm short arm, and a 10 cm × 10 cm starting box at the base of the stem. The two short sides on the left and right were covered with green and red sleeves. The transfer latency was used to calculate how long the fish took to reach the deeper compartment (TL). A stimulus that rewards food (green arm) or punishes stress (red arm) is the basis of the T-maze test in zebrafish. Williams (2002) measured the time taken and the number of entries made by each arm of a T-maze in adult zebrafish [12]. The aim of this study was to develop a protocol that efficiently evaluates learning and memory function in zebrafish using a combination of the T-maze and passive avoidance test principles. This is the development of a quick and reliable method for assessing cognitive abilities in adult zebrafish, which could have implications for further neuroscience or behavioral studies [21].

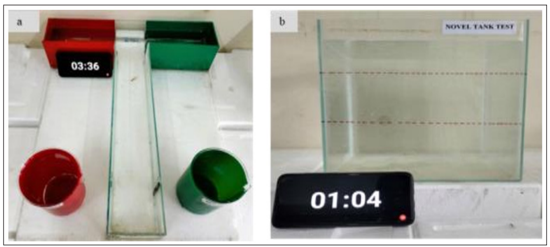

Figure 2: (a) This paradigm involves using a T-maze setup to assess learning and memory in zebrafish (b) The NTT is used to assess anxiety and cognitive behaviour in zebrafish. This involves placing the fish in a novel tank environment.

NTT

The Novel Tank Test (NTT) was used to assess anxiety-like behaviours in zebrafish, as described by Blaser and Rosemberg (2012). Here is a breakdown of the key components and methods described [13]. Rectangular glass aquariums with the same dimensions and features as the experimental aquariums were used for the NTT (Figure 2 (b). The aquariums are likely divided into two zones: a top zone and a bottom zone. The behavioural analysis was performed using a Canon EOS 1500D 24.1 digital camera. The fish were removed from the test aquarium for five minutes. During this time, the top and bottom components of the test tank are separated. The amount of time the fish spent in each zone (top and bottom) and the number of entries into each zone is assessed.

Biochemical Markers

The passage describes a biochemical study where fundamental values were assessed by analyzing homogenized tissue samples from subjects under different conditions, namely fasting and overfeeding. We allowed these samples to sit at room temperature in dry test tubes for 40 minutes before the assessment. We separated the sample after centrifuging it at 2000 rpm for 10 minutes. Then it was stored in a refrigerator at -20°C until the assays were completed, as outlined in the study by Emami et al. The tissue samples underwent a quantitative assessment using a photometric assay, specifically utilizing the Roche/ Hitachi Cobas C 111 system from Roche, USA. This methodological detail indicates a rigorous approach to biochemical analysis, utilizing established equipment and procedures to ensure accuracy and consistency in the results obtained [22].

Brain Tissue Extraction

The passage describes a procedure for homogenizing brain tissue weighing 1.55 mg. The tissue was homogenized for 1 minute in 0.1 ml of HCl-butanol solution. This solution was prepared by mixing 0.85 ml of 37% HCl with n-butanol to make a final volume of 1 liter. The homogenization was conducted using a glass homogenizer made from a miniature centrifuge tube with a volume of 1.5 ml. After homogenization, the total volume was found to be 0.105 ml, taking into account the volume of the tissue itself (1 mg of tissue corresponds to 0.001 ml). After centrifugation at 2000 g for 10 minutes, a volume of 0.08 milliliters of the supernatant phase was carefully transferred into an Eppendorf tube with a volume of 1.5 ml. To this, 0.2 milliliters of heptane (for spectroscopy) and 0.025 milliliters of HCl 0.1 M were added. We then centrifuged the tube under the same conditions as before to separate the two phases. This step was conducted after 10 minutes of vigorous shaking. We carefully disposed of the organic phase that covered the top of the tube after centrifugation. It is worth noting that all steps were carried out at a temperature of 0°C, likely to maintain the integrity of the samples and to prevent any unwanted reactions or degradation of the compounds being analyzed. We conducted all assays using spectrophotometric analysis, evaluating different wavelengths for each specific neurotransmitter assay.

F1 Generation

The description outlines a method for breeding fish, particularly ensuring the safety of the eggs from being consumed; see Figure 3. This method involves using a breeding tank divided into male and female sections, likely to facilitate controlled mating between specific individuals. The removable divider enables male and female fish to be separated or reunited during breeding. Additionally, the net in the bottom zone shields the eggs from ingestion, guaranteeing successful breeding and hatching. The breeding method described involves collecting and preserving the eggs for fertilization while maintaining temperature control below 28°C. After hatching, typically at five days postfertilization, the fish are fed exclusively with artemia and rotifers before being transferred to a growth tank. This feeding regimen is likely to provide essential nutrients for the fish's early development. We keep each experimental group for three months to allow the fish to mature into adults. This timeframe ensures the fish reach sexual maturity and are ready for further experiments or studies.

Statistical Analysis

In addition to traditional statistical methods like ANOVA and Tukey's test, a mathematical learning technique known as least mean ± SD was utilized. This technique likely involves calculating the mean ± standard deviation for each group and using mathematical models to assess the probability ($P<0.05$ value) of a correct response as an expression of the variable groups.

Results

Behavioural Evaluation

T-maze

Zebrafish adults were trained one-on-one in testing tanks see Figure 2 (a). We evaluated spatial alternation learning and memory in zebrafish for five minutes in this experiment. We evaluated the proportion of accurate responses at the food reward (green sleeves) and punishment (red sleeves) choice step. Green sleeves, associated with food reward, and red sleeves, associated with punishment, represented the stimuli presented to the zebrafish. Zebrafish made choices between the food reward and punishment stimuli. We used One-way ANOVA for multiple comparisons and DMRT multiple comparison tests for post hoc comparisons. A significant level of P < 0.05 is utilized to convey the values. In contrast, the zebrafish that were overfed and fasted on opposite sides of the tank had a pattern of wise decisions resembling those shown in earlier tests. The analysis evaluated the time spent and entries made in response to green (+) and red (-) sleeves. Time spent and entries could indicate the zebrafish's learning and memory behaviour.

Spending Time in The Green Sleeve

We evaluated the fish based on how long they spent in the green sleeve of the T-maze. The highest amount of time that fish could spend in the green sleeve was 12 hours, 3.7±0.28 (p<0.05), 24 hours, 3.8±0.42 (p<0.05), and the control, 3.5±0.27 (p<0.05). Thus, they showcased food as a prize for spending too much time. On the other hand, inadequate time for overfeeding (2.9±0.22) and their generation (2.8± 0.24; p<0.05), as shown in (fig.3, a), were reported.

Spending Time in The Red Sleeve

We looked at the red sleeve side, where the overfed group spent the most time (3.8±0.29; p<0.05), and their generation (3.9±0.30; p<0.05) occupied the punishment side. In comparison, the control groups spent less time in the red sleeves (3.4±0.26; p<0.05), and the 12fasting group spent 2.9±0.022 (p<0.005), 3±0.23 (p<0.05), and 24 hours were 3±0.23 (p<0.05) Expressed on figure 3(b).

Number of Entries in The Green Sleeves

Fish spent a maximum of 12 hours, 5±2.10 (p<0.05), and 24 hours, 6±0.46 (p<0.05) in the green sleeves to learn and remember food reward sleeves. Conversely, the overfed groups spent less time in the green sleeves (3±0.23 (p<0.05)), as shown in Figure 3(c).

Number of Entries in The Red Sleeves

A maximum of 7±2.94 (p<0.05) of the entrants in the red sleeves were overfed, and their progeny also received 7±2.94 (p<0.005) of extra time to spend in the red sleeves of punishment. Figure 3 (d) shows that there were fewer entries in the red sleeves for the fasting group (3±0.23; p<0.05) and the control group (5±0.38; p<0.05).

Novel Tank Test (NNT)

This NTT was used to compute anxiety and cognitive tests. The amount of time spent in the top and bottom zones and the total number of entries to record typical fish, with the top zone requiring the most time and the bottom zone requiring less time. Therefore, we computed anxiety flaws in this behavior. Maximum time spent in the top (+) and bottom (-) zones was prompted by reduced anxiety, whereas less time was spent in the top (-) and bottom zone (+) when measured anxiety increased. Additionally, the bottom zone produces planned anxiety-like behavior.

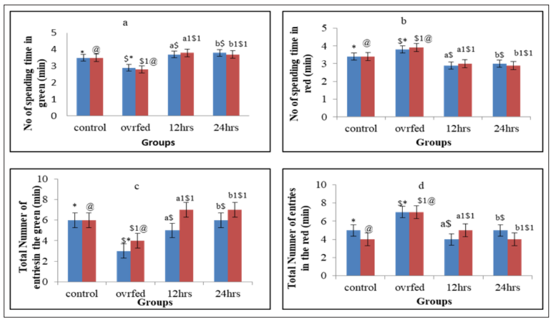

Figure 3: The graph presentation compares the overfeeding groups with the control and fasting groups and F1. The maze apparatus uses green and red sleeves in (a,b) to show the values of evaluated and spent entries. (c,d) The number of entries in the green and red sleeves was assessed. Every value was reported as mean + SD with a significance level of less than p < 0.05.

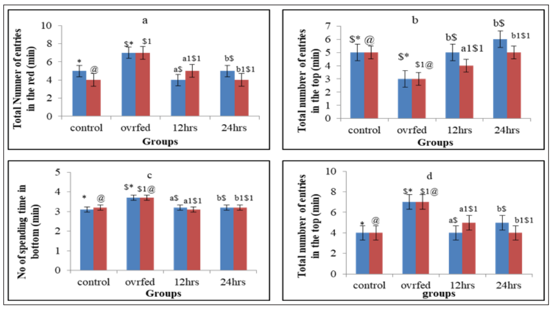

Figure 4: The graphical [presentation compared overfeeding with control and fasting groups along their F1(a,b) NTT test graphs show F1 and the parent. Restraint, fasting as a contrast to overfeeding (a,b) The duration of time in the upper and lower zones was assessed. (c,d) Display the number of entries in the top and bottom zones, respectively, from the control, overfed, 12-hour, and 24-hour fasting groups. The data were presented as mean ± SD, with a significance threshold of less than P<0.05.

Total Number of Time Spend in The Top Zone

We observed less anxious behaviour in the fasting groups with a maximum time of 12 hours (3.7±0.28; p<0.05) and 24 hours (3.8±0.29; p<0.05) in the top zone. Additionally, after having enough time, they were overfed 3±0.23 (p<0.05), and their progeny 2.9±0.22 (p<0.05) were assessed see figure 4 (a).

Total Number of Time Spend in The Bottom Zone

Figure 4 (b) shows the highest amount of time that could be spent in the overfed groups, which was 3.7±0.28 (p<0.05), compared to the fasting groups, which had 3.2±0.24 (p<0.05) and 3.4±0.26 (p<0.05). Thus, poor group fish performance is caused by overfeeding.

Number of Entries in The Top Zone

We enter the majority of the time in the top zone, which is 12 hours at 5±0.29 (p<0.05) and 24 hours at 6±0.46 (p<0.05). On the other hand, overfed were enough time to spend 3±0.23 (p<0.05) refer to figure 4 (c) Lastly, we discovered that the best entries had lower anxiety behaviour evaluations.

Number of Entries in The Bottom Zone

Fish in the overfed group had more entries (7±2.94; $p<0.05$). While there were less entries in the 24 hour group (5±0.38; $p<0.05$) and the 12 hour group (4±0.30; $p<0.05$) see figure 4 (d) the correlation fasting group had more entries (maximum) to investigate potential anxiety-related defects.

Physical of Body Measurement

We compare the body weight before and after the evaluation. Fish were found to have changed morphologically and in terms of body weight. For instance, the control baseline weight was 0.50±0.02 to 0.53±0.04, and the fish's length was 3.5±0.27, which led to a BMI level of 0.041±0.04 to indicate an increase in body weight. Initially, the 12-hour fasting groups had a body weight of 0.55±0.05 after being assessed at 0.52±0.04 as length 3.6±0.27 and a BMI level that was lowered to 0.037±0.04 to achieve the same outcome for their offspring. The next 24hour fasting group experienced a reduction in body weight to 0.51±0.02 as length 3.7±0.28 and a BMI level of 0.033±0.03, both of which are statistically significant at less than $p<0.05$. Table 1 show that although this finding indicates a modest difference in their F1 generation, their observations were identical to those of their parents. In contrast, the duration of 3.2±0.25 was significantly longer for the overfed, increasing from 0.53±0.03 to 0.57±0.04, with a BMI level 0.052±0.05 higher than all other levels, and a significant level of $p<0.06$, indicating the same observation results as the F1 generation. Compared to fasting groups, overfeeding results in negative feedback on morphology and impacts the F1 generation. Our findings show that the BMI levels of the obese groups decreased, whereas the levels of the overfed group considerably increased. Additionally, these effects were passed on to the next generation.

Biochemical Assessment

A summary of the effects on multiple biochemical markers in the different groups following a 28-days fasting and overfeeding endurance exercise regimen. Compared to the control, 12-hour, and 24-hour fasting groups, the overfed groups had an increase in glucose levels of 97±7.43 $p<0.05$ and an increase in F1 production of 101±7.73 $p<0.05$, respectively. We concluded that extra sugar causes unfavorable biochemical markers based on these findings. Plasma AST and ALT activity were significantly elevated ($P<0.05$) in overfed groups but significantly lower ($P<0.04$) in fasting groups. There were no significant changes made to their F1 generation. Table 2 shows how endurance training sessions influenced the plasma lipid profile of each group. The majorities of biochemical markers had abnormal values and were evaluated as an excess eating habit that impacts all types of diseases. However, the overfed group showed higher TG, cholesterol, HDL, and LDL than the control and fasting groups. Notably, their contrast levels were substantially higher than those of the group that fasted for 12 hours straight. The F1 generation ratio did not significantly differ between the groups. Protein levels in the control, 12- and 24-hour fasting groups varied modestly ($p<0.04$ and $p<0.05$), which was consistent with their F1generation. In the meantime, the overfed group and their F1 generation group considerably reduced their protein profile. Thus, the fasting group's values were shown to be consistent and beneficial. On the other hand, it is noteworthy that the biochemical values were highly impacted by surplus feed and its subsequent development.

Evaluation of Neurotransmitter

Table 3 summarises our analysis of the neurotransmitter levels in the brains of the control, overfed, 12- and 24hour fasting group fish. The analysed values clearly show that the neurotransmitter levels of dopamine (0.158±0.018), serotonin (0.173±0.015), adrenaline (0.32±0.02), and nor-epinephrine (0.173±0.013) appeared to be gradually reduced in the overfed groups, and their F1 generation was evident. Conversely, while F1 production was similar in the control, 12-hour, and 24-hour fasting groups, neurotransmitter levels were elevated; all values are reported less than $p<0.05$, accordingly; table 2 briefly explains these groups' results. Compared to overfed fishes, we discover that the fasting groups had an increase in neurotransmitters overall. The high concentrations of neurotropic factor, which can repair damaged neurons and prevent neurodegenerative disease, may cause the steady state rise in neurotransmitter levels in the fasting group [23].

Brain Histology

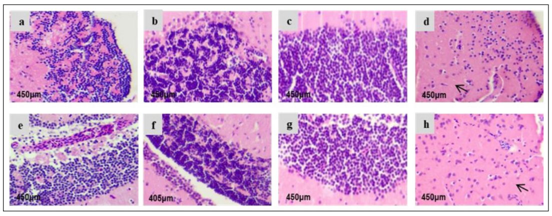

Figure 5: Zebrafish brain morphology carry out by 40X magnification after being overfed and fasted. (a,e) No anomalies were discovered in the neuronal and neurophil architecture of the control zebrafish's brain tissues. (b,c,f and g) The basal dendrites in the 12 and 24 hour and their F1showed a high density of neutrophil infiltration; (d,h) arrow mark showed long brain space were overfed, and their offspring decreased the granular neutrophil spine density.

The number of neutrophil facets in the reciprocal control of the immunological and neurological systems First, there is debate over the function of neutrophils as a widespread source of catecholamine and acetylcholine, as well as the role played by these neurotransmitters in neutrophil activity. Furthermore, the infiltration of neutrophils in the brain system during inflammatory responses points to these immune cells as novel targets for the therapy of neurological, neurodegenerative, and central infectious illnesses. Determining the function of neuron-neutrophil communication in the pathophysiology of viral, inflammatory, and neurological illnesses requires multidisciplinary research, including that of immunologists and neuroscientists. At the same time, several of the earliest research studies analyzed brain cell damage or lower to indicate responsibility for memory, cognition, neurotransmitters, and anxiety, respectively. This present study was a histological observation that exposed neutrophil-dense changes in the overfed and their F1 generation see Figure 5 (d,h). Moreover, when compared to control as (a, e), 12hrs (b,f), and 24hrs fasting (c,d), they showed similar changes in these groups. Likewise, it's not a problem for behavioral and neurotransmitter consequences. These neutrophil densities reduced in the overfed groups were outcomes abnormal responsibilities showed, which may be one of the reasons for structural changes. It looks like Figure 5 clearly outcomes their brain morphology.

Discussion

To assist in zebrafish conditional training, we present a modified T-maze technique in this work that has several advantages, including weekly testing and training duration, a T-maze instrument, and reliability. We specifically designed our modified T-maze with an optimised electric shock at the left arm and a green colour signal at the right. Previous studies [24] also included a colour cue at the bottom of the labyrinth, three side sleeves, or one arm of the T-maze. [25]. In our T-maze experiment, we used green sleeves as a food reward and red sleeves as a sort of punishment. In contrast to earlier research that used electric shock, our goal was to make the fish fearful by punishing them with churning water. Remarkably, in spite of this unorthodox method, the fish never touched the red sleeves, especially the ones with the glass rod in them. More specifically, about 90% of our research findings support this observation. A previous study found that participants preferred zebrafish over yellow in a colour preference test. In the current study, fasting groups favoured green over red and spent more time in the red compartment throughout training sessions. We were able to verify that zebrafish preferred red to green, even though they were unable to recall the red compartment from the memory test. [26]. However, in our current study, the control, 12, and 24 fasting groups spent the most time in green sleeves, while the overfed and their generation did not like living and traveling in green; they showed different results in the fasting groups. As shown in figure 6(a), the fasting groups' green sleeves had more entries than the overfed groups. These results indicate that food rewards help zebrafish learn and memorise spatial cues. Animal research has successfully used food incentives as a positive motivator for behavioural tasks. Food reward preference is a simple behavioural test that demonstrates the appetitive behaviour of zebrafish [27]. While we expected colour preference to translate into a learning paradigm, there are limitations to our zebrafish colour-sleeve learning methods. It appears that the time and quantity of repeats of the experiment were insufficient to highlight the favouredolor. [26]. Research indicates that generations pass on the beneficial effects of fasting on memory. Overfed groups have shown diminished memory and learning capacities. These results point to a possible connection between memory performance and nutritional conditions, as well as their intergenerational effects. To fully comprehend the impacts on cognitive capacities that have been reported in the setting of fasting and overfeeding, more investigation into the underlying mechanisms is necessary.

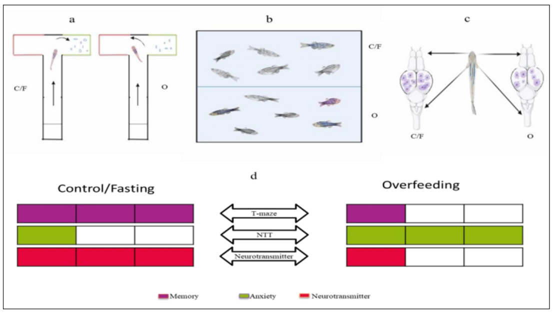

Figure 6: Illustrates the overall expression of our research (control/fasting (C/F), overfeeding (O). a) T-maze was exposed to the outcomes of control and fasting groups; fish were traveled in the green arm of food reward sleeves, and another was overfed to spend in the red arm of punishment arm. (b) NTT results showed C/F fish spent in the top zone and opposite of overfed fish groups. (c) The brain neutrophils accumulated in C/F groups and, at the same time, reduced overfed groups. Lastly, the C/F groups had better-interrogated memory and neurotransmitter levels compared to the overfed groups. This was seen in differences in anxiety levels between the groups, with higher levels of anxiety seen in the overfed groups and lower levels of anxiety seen in the C/F groups.

Researchers widely use the NTT to assess zebrafish activity that mimics anxiety [28-30]. Previous research that compared the two tasks found a strong cross-test association in vivo and similar sensitivity to anxiety-like states in Zebrafish [31]. We focused on swimming patterns in both top and bottom positions that exhibit anxiety-like behavior. The length of time in a fresh tank at the bottom (↑ time= =↑ anxiety), the top half (↑ time= =↑ anxiety), or the top third (↑ time= =↑ anxiety) are several analysis interpretations of anxiety in the NTT. According to studies conducted by Egan et al. 2009, Gerlai et al. 2000, Rosemberg et al. 2012, and Parker et al. 2012, there are several interpretations of anxiety in the NTT. Present research of Zebrafish from the control and fasting groups, as well as their offspring, showed evidence of spending a considerable amount of time at the top zone [32-35]. This behaviour suggests a calmer or less worried state because it is suggestive of the lack of anxiety-related behaviours. On the other hand, Zebrafish that were made obese by forcefeeding them an obesogenic diet showed altered behavioral responses, especially those related to anxiety. When given high-fat diets, both larval and adult specimens showed signs of anxiety, suggesting a possible connection between dietinduced obesity and anxiety-related behaviours in Zebrafish [36,37]. This is one of the findings from our most recent study. Conversely, the bottom zone showed the largest amount of time spent there as well as the inclination toward entrances, whereas the top zone and entries showed less time spent for overfed individuals and their offspring (see Figure 6,b). It was decided to raise the behavioral variables in order to look at the activation of the central monoaminergic circuitry and confirm past findings [38,39]. The findings of our current study on overfed individuals and their generation support the emergence of anxiety-like behaviors. For obese individuals, we suggested a negative feedback mechanism in which an excess feeds this experimental study strategy.

For this study, we created a model of overfed and fasting zebrafish. We noted hepatosteatosis, hypertriglyceridemia, and higher BMI in overfed zebrafish. These results suggest testing new drugs on the DIO (dietinduced obesity) zebrafish model. This can also help find promising drug targets for treating obesity in people [14]. Our study revealed that while the BMI rates were lower in the 12,24-hour fasting groups, the overfed fish populations were higher. We indicate that prior research has shown that high-fat diets (HFDs) elevate body mass index (BMI), the main indicator of obesity [40]. The second of these earlier investigations confirmed our findings, demonstrating that a short period of a high-fat diet affected parameters linked to obesity and metabolic diseases by promoting higher body weight and belly length in zebrafish. Changes in sociability, hostility, anxiety, and memory are also present. The results we obtained showed an obesogenic effect and modified a wide variety of behaviours, supporting the use of zebrafish as a special alternative model organism to evaluate the neurobehavioral implications of obesity in addition to the prior rodent models. The zebrafish model's high degree of face validity is demonstrated by these findings, as behaviours were assessed through well-characterized activities and mirrored those observed in humans and other animals [36]. The fasting group mostly exhibits a protective response against obesity and subsequent illnesses as compared to the overfed individuals. Zebrafish with higher visceral and subcutaneous fat content (which is associated with longer abdomens) and BMI also had higher blood glucose, cholesterol, and triglyceride levels, according to an HFD study that employed chicken egg yolks. But only after four weeks, not after two, did the fish on the diet exhibit a rise in body weight [41]. Our current investigation is actually what we were learning, However, the overfeeding of this generation led to elevated biochemical levels of glucose, cholesterol, triglycerides, protein, HDL, LDL, liver enzymes, ALT, and AST.

Additionally, their increased body weight had an effect on the morphological consequences of their brains

According to Meguro et al. 2019, zebrafish fed maize oil or lard show changes in parameters related to metabolic health and obesity. Similar to this, feeding zebrafish too much artemia, a fat-rich live food, raises their BMI and triglyceride levels, causing hepatic steatosis and increased fat buildup [14]. This is an excellent example of a research study that focuses on a specific phenomenon. Obesity and/or fat consumption lead to neurobehavioral alterations in both humans and rats, including reductions in cognitive performance. Researchers have reported similar findings in both humans and rats [42,43]. In particular, cognitive decline in mammals is linked to high-fat diets (HFDs) and the ability of the hippocampus to experience fatrelated problems. Some of the things that HFD does to the central nervous system are lower neurogenesis, lower expression of memory-related genes, impaired synaptic plasticity, insulin resistance, and changes in the shape of the brain. We received strong recommendations to complete this assignment earlier [44-46]. Dyslipidemia, frequently observed in obesity, is characterized by increased flow of free fatty acids, rising total cholesterol, low levels of high-density lipoprotein cholesterol, and increased levels of low-density lipoprotein [47,48]. In fact, the comprehensive research report indicates that fatty acid metabolism, cholesterol efflux, and triglyceride metabolism are closely related. The diagnosis of illnesses and liver damage compromising the liver's structural integrity is commonly accomplished by liver assays that measure AST and ALT activity [49,50].

Our study's elevated levels of these markers are due to the fatty liver resulting from HFD. HFD changed liver enzyme levels, hepatocyte degeneration, and lipid metabolism, according to several research [51]. The plasma AST and ALT activity of the fasted groups in this study returned to normal levels, suggesting a decrease in fatty liver. The antioxidant capability of the fasting approach could potentially lead to a drop in liver damage. The previous study revealed that overfed individuals had significantly elevated blood glucose levels. Generation groups were also informed that oxidative stress is caused by biochemical indicators and that this leads to insulin resistance in the liver, hormones, and behavior. Additionally, they displayed elevated triglyceride, cholesterol, glucose, and visceral adiposity levels. By altering the amounts of Y neuropeptide in fat people's brains, triglycerides can penetrate the blood-brain barrier and cause central leptin resistance [52]. Consequently, this could potentially exacerbate nervous tendencies [53]. Mice exhibit anxiolytic-like effects due to the high degree of conserved leptin found in fish and humans [54]. Strong anxiety-like behaviors are displayed by zebrafish knockouts [55]. We discovered that blood glucose levels and all biochemical parameters were aberrant as a result of excessive feeding behavior and that fasting duration was significantly reduced. We attributed this to the elevated adiponectin level and insulin sensitivity.

Once the neurotransmitter levels of the overfed and fasting fish were analyzed, it was discovered that the overfed fish had lower levels than the fish in the 12,24-hour fasting group. Research on serotonin and norepinephrine has demonstrated that fasting increases the brain's availability of serotonin and tryptophan [56,57]. This implies that the adult fish brains experienced a significant increase in monoamines following their fast. Increased neurotransmitter levels during fasting have been connected to improved synaptic plasticity in terms of memory for any number of reasons, including abnormal behavior. Several of the early studies that describe the effects of food restriction on brain plasticity also showed an increase in neurotrophic factors, including brain-derived neurotrophic factor (BDNF) [58]. We corroborate our findings with research that indicates fasting enhances learning and short-term memory retention, as well as dramatically improves memory function [59]. Yes, our present data supports this, yet as compared to overfed groups, the neurotransmitters in the fasting group showed beneficial behavioural changes (see above Figure 6, d). BDNF has a critical role in memory and neuroprotection [60]. Down-regulation plays a crucial role and expresses itself in the hippocampus and cortex, which may impact abnormalities in behaviour linked to depression and anxiety [15]. This study also raises the idea that intermittent fasting may indirectly improve the brain by improving insulin sensitivity, which may help prevent neurological disorders and delay the onset of other diseases Researchers found a connection between the lard-induced memory impairment in zebrafish and the alterations in genes that control neuronal function, oxidative response, and blood-brain barrier integrity. This demonstrates that a high-fat diet retains the pathophysiology of memory impairment. Therefore, in order to minimize memory loss and its associated behavioral repercussions, we suggested that the current study focuses on higher-level neurotransmitters [61]. Accordingly, there is a link between obesity and overweight and human aggression, anxiety, and depression [62,63].

Furthermore, we discovered that, in the past decade, there has been a greater emphasis on neutrophil migration and its role during inflammation in our assessment of the histology results from previous investigations. Thanks to developments in real-time imaging and the application of novel model systems, we now have a better knowledge of neutrophil behavior in ill or damaged tissues. The article talks about the different tissue signals that tell neutrophils to move forward or backwards in response to injury or infection. It also talks about what these pathways mean for human disease [64]. In the current study, we found that minor variations in neutrophil cell density after 12 and 24 hours of fasting did not affect any markers. As shown in Figure 6 (c), the overfed and their F1 generation showed poor accumulation of neutrophil cells. Consequently, our findings demonstrate a change in the ensuing behavioral and physiological consequences. Thus, brain-derived neurotrophic factor (BDNF) is a neurotrophin that is vital to the development and flexibility of the central nervous system (CNS). It is now recognized to be important in regulating food consumption.

Conclusion

The findings revealed that zebrafish overeating behavior, body weight effects, and biochemical fluctuation were significantly negative feedback, which led to deterioration in learning and memory. It is important to note that the details of these effects would depend on the parameters examined in the study, as well as the length and severity of calorie restriction and overfeeding. The results of this type of research could have an impact on our understanding of how parental nutrition influences the health of offspring in vertebrates, including humans. The cross-generational effects of dietary decisions may shed light on the longer-term effects of dietary practices on subsequent generations. Future studies might examine the traumatic impacts of compulsive eating on the body's chemistry and how it affects the next generation. Researchers may also look at how eating excessively impacts psychological wellness and illness associated with obesity while limiting dietary intake to short-term intermittent fasting's possible advantageous effects on quality of life, which are recognized to be important in regulating food consumption.

Declaration

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Conflicts of Interest

The authors have no relevant financial or nonfinancial interests to disclose.

Ethics Approval

This study was performed in department of biochemistry and biotechnology at Annamalai University in Tamilnadu, India. It is part of my PhD work an exemption from ethics approval Annamalai University CPCSEA for Zebrafish, where there is no deception of participants and no sensitive topics or materials are presented to participants. Consent to participate Informed consent was obtained from all participants in the study.

Consent for Publication

Participants consented to the publication of their anonymized data in an open-access repository

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