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*Research Article*

# Rosuvastatin ameliorates anxiety but impairs skeletal muscle performance by malondialdehyde and calcium depletion in high fat diet-fed swiss albino mice

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**Keywords:**

Rosuvastatin, HFD, Anxiety, Muscle strength, Elevated Plus Maze, Wire Hanging Test

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**ABSTRACT**

**Background**: The high intake of high calorie, high fat diets (HFD) with an associated sedentary lifestyle has been linked with a number of neurobehavioral and neuromuscular disorders. This study aimed at investigating the effect of a lipid lowering drug - Rosuvastatin (ROS) on anxiety-like behavior and muscle strength in HFD-fed mice.

**Methods:** The animals were grouped into four (n=5); Group 1 (normal chow and water *ad libitum*); Group 2 (HFD *ad libitum*); Group 3 (HFD + ROS); Group 4 (HFD for 5 weeks then ROS for 1 week). Thereafter, mice were subjected to elevated plus maze (EPM) test and wire hanging test (WHT). Animals were then killed and brain samples homogenized and assayed for neurotransmitters and antioxidants. The blood samples were assayed for calcium, uric acid and Malondialdehyde (MDA).

**Results:** The HFD significantly ($p<0.05$) heightened anxiety in the mice which was ameliorated by ROS. Muscle strength was however decreased with ROS. Brain levels of dopamine and serotonin were not significantly affected ($p<0.05$) by ROS likewise superoxide dismutase (SOD) and Catalase. Serum calcium and MDA were significantly reduced by ROS.

**Conclusions:** High fat diet induced anxiety in the animals and improved muscle endurance on exertion. Rosuvastatin ameliorated the anxiety but reduced muscle strength and the proposed mechanism is suppression of MDA and Calcium functions respectively.

## 1. Introduction

The modern man is termed homo sedentarius because of his predominantly sedentary lifestyle. This lifestyle together with the global rise in consumption of westernized diet -rich in sugar and fats, has resulted in a myriad of cardiovascular, neurological and neurodegenerative ailments (Levin, 2014; Kopp 2019). The adoption of high fat diet (HFD) for induction of obesity, hyperglycemia and hyperlipidemia in experimental animals (especially mice) is gaining traction in recent literatures (Li *et al.,* 2020). Our previous studies revealed that chronic HFD administration can cause hyperglycemia, heightened anxiety and memory impairment in mice (Abi *et al.,* 2020; Abi *et al.,* 2022). High fat diet causes these, by some proposed mechanisms, such as the promotion of proinflammatory markers like IL-1, IL-6 and TNF-α; activation of toll-like receptors and activation of protein-C causing inhibition of insulin-PI3K-Akt signaling (Duan *et al.,* 2018; Wali *et al.,* 2020). Lipid lowering agents like the statins have been used to normalize hyperlipidemia; especially the lowering of harmful cholesterols like trigycerides and low density lipoprotein cholesterol (Cheng, 2004; Di Stasi, 2010). Some studies have shown benefits of statins in ameliorating a few neuropsychiatry ailments even though their effects on muscle strength proved counter effective (O'Niel *et al.,* 2012; Parker and Thompson, 2012). This research sought to investigate the role of rosuvastatin (ROS) in skeletal muscle performance and on anxiety, especially because literatures regarding these are limited.

## 2. Materials and Methods

### 2.1. Animal Management

A total of twenty albino mice aged 7-8 weeks weighing 30-40g were used for this experiment. They were housed in propylene cages in the animal house of the College of Health sciences Benue State University, Makurdi. They were kept under an environmental temperature of 23 ± 2$^{o}$C; humidity, 55 ± 15% and 12 h light/dark cycle. They were randomly divided into four groups of five animals each as follows: Group I - (normal chow plus water *ad libitum* serving as control); group II (HFD plus water *ad libitum* ); group III (HFD plus ROS 0.4mg/kg p.o) and group IV (HFD followed by 1 week of ROS 0.4mg/kg p.o). The HFD administration lasted for 35 days.

### 2.2. Ethical Approval

This study complied with the approved procedures of the Basic Science Research Ethics Committee of the College of Health Sciences, Benue State University, Makurdi, Nigeria which is in line with the guideline of the Institute for Laboratory Animal Research, USA (NRC 1996).

### 2.3. Drugs and Reagents

Rosuvastatin (Manufactured by Micro Labs Ltd India; Batch No.: TBBH0034; Exp. Date: 12/2024) was obtained from a local pharmacy in Makurdi. Accu check strips were purchased from a local pharmaceutical store and Glucometer was supplied by LaVida Hospital Makurdi. Ketamine Hydrochloride (Manufactured by Jawa International Ltd. Nigeria; Batch No.: 2322099; Exp. Date: 05/2025) was purchased from a local store. All reagents for serum calcium, uric acid were supplied and carried out by Evidence Research Lab Ltd, Makurdi. The reagents and procedure for brain neurotransmitters were supplied and carried out by GLMS Laboratories, Ibadan.

### 2.4 High Fat Diet Composition

The high fat diet was composed according to the protocol in our previous research (Abi *et al.,* 2020) with a total caloric content of 5340kcal/kg comprising of soy oil, tallow and normal chow.

### 2.5 Wire Hang Test (WHT)

Wire hang test is a simple test that is used to assess muscle performance in small rodents. It is best suited to measure skeletal muscle coordination and endurance (Hoffman and Winder, 2016). The protocol used was according to Jansone *et al.* (2016).The set up was constructed using a stainless steel wire (90 cm length, 2.5 mm in diameter), fixed horizontally between two vertical supports and 60 cm above a soft padded surface. The WHT was carried out on day 35 for groups 1-3 and day 42 for group 4. The mice were placed individually on the wire and assisted to grasp the central position of the wire with their forepaws. The latency to fall on the soft pad was measured; a maximum latency of 120 second was allowed for each mouse after which the animal was released. An average latency of three

consecutive trials was recorded for each animal and the longest duration was the value used for evaluation; with a resting interval of 3 minutes in between attempts.

**2.6. Elevated Plus Maze (EPM)**

The EPM is a cross-shaped apparatus used to measure anxiety behaviour in experimental animals. It was constructed from plywood and consists of two open and closed arms. The dimension of each of the open arms was 25cm x 5cm while that of the enclosed arms was 25cm x 5cm x 15 cm. These whole set up stood on a 55 cm stand above the floor (Abi *et al.,* 2022). On day 35 group 1-3 were assessed and on day 42 group 4 was assessed. Each mouse was placed at the edge of the open arm and allowed to freely explore the apparatus. The time spent in the open arm(s) before entering the closed arm with all four paws (transfer latency) was recorded for 5minutes. Ethanol (70%) was used to clean the maze after each trial to obliterate olfactory cues.

**2.7. Craniotomy and Sample Collections**

After running the animals through the outlined mazes they were anesthetized with 87mg/kg ketamine i.p (Van Pelt, 1977). Thereafter, trunk blood was collected in universal sample bottles and whole brains were removed after craniotomy and gently homogenized with sodium phosphate buffer. After centrifuging, supernatants were collected and frozen immediately at -20˚C for further analysis.

**2.8. Brain Dopamine and Serotonin Assay**

Assays for dopamine and serotonin were done using High precision liquid chromatography (HPLC) of the supernatants from the homogenized centrifuged brain sample as described by Chatterjee and Gerlai (2009). This method was carried out using a BAS 460 MICROBORE-HPLC system with electrochemical detection together with a Uniget C-18 reverse phase microbore column as the stationary phase.

**2.9. Serum Calcium Measurement**

Serum calcium was measured using the arsenazo III method as described by Jeffrey *et al.* (2007). The arsenazo III dye method is an improvement on the 0-cresolphthalein complexone dye method (Janssen and Helbing, 1991). All reagents were of analytical grade (Sigma Aldrich). The reactions of the dye and calcium happens in an acidic condition producing a blue-black coloration which is measured spectrophotometrically at wavelength of 660nm.

**2.10. Serum Uric Acid Measurement**

Serum Uric acid was measured using uric acid assay kit from Sigma Aldrich and was done according to manufacturers specifications. The test is based on the principle of coupled enzyme reaction resulting in a colorimetric (570nm)/flourometric ($\lambda_{ex}$ = 535/ $\lambda_{em}$ = 587nm) product proportional to concentration of uric acid present in blood.

**2.11. Estimation of Lipid Peroxidation (Malondialdehyde quantification)**

Lipid peroxidation by quantification of MDA using HPLC technique as described by Domijan *et al*. (2014) was used. Separation was done on an analytical column, 125.0 × 4.0 mm, particle size 5 µm equipped with a guard column (4.0 × 4.0 mm, 5 µm). The flow rate was set at 1.0 mL/min. The FL detector wavelengths were set for excitation (λex) at 527 nm and emission (λem) at 551 nm.

**2.12. Brain Catalase (CAT) and Superoxide Dismutase (SOD) Assay**

All kits were of analytical grade from Sigma Aldrich. The SOD activity was measured by in direct method according to the manufacturers specification, which is based on xanthine-oxidase and color reagent.

The measurement of CAT was based on the measurement of $H_2O_2$ left after the activity of CAT in the sample. The CAT converts $H_2O_2$ to water and oxygen. An aliquot of the reaction mix was assayed for the amount of hydrogen peroxide by a colorimetric method at a wavelength of 520nm.

**2.13 Statistical Analysis**

Results were presented as Mean ± SEM.
Differences between two groups was determined using Independent T test, while difference between more than two groups was determined using ANOVA followed by Tukey post hoc test.
Differences were considered significant when $P < 0.05$. Data were analyzed using SPSS version 20.0 software. (International Business Machine Corporation)

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## 3. Results

### 3.1. Effect of HFD and Rosuvastatin on Body Weight

Figure 1 shows the effect of HFD and Rosuvastatin on body weight across the groups. The change in body weights were not significantly increased ($p>0.05$)

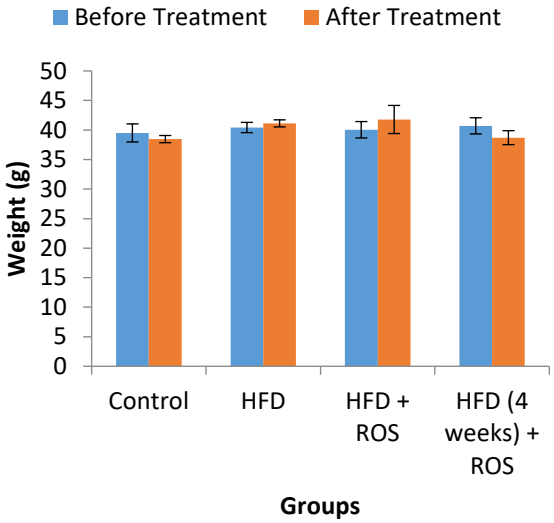


**Fig. 1 Weight difference between the groups before and after the experiment.**
There was no significant ($P>0.05$)weight difference across the groups (n=5 per group)

### 3.2. Effect of HFD and Rosuvastatin on Transfer Latency (TL) in the EPM Test

Figure 2 shows the effect of HFD and Rosuvastatin on transfer latency in the various groups. The HFD and HFD (4 weeks) + ROS groups had significantly reduced TL ($p<0.05$) compared to the other groups. This reduced TL is an evidence of anxiety characterized by heightened light aversion. The HFD+ROS group shows amelioration of this anxiety expression.

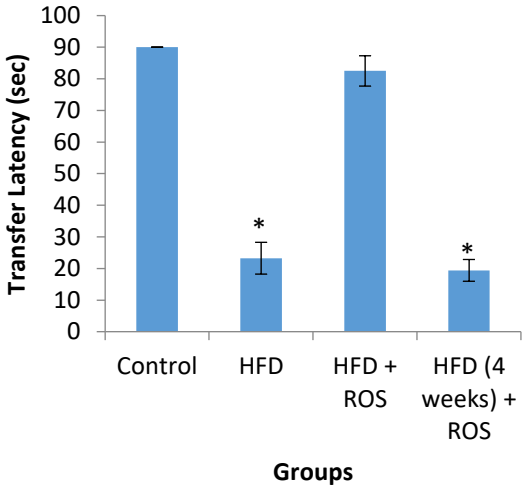


**Fig. 2 Transfer Latency Across the Groups**
Group 2 and 4 had a statistically significant reduced transfer latency compared to the control
*indicates statistical significance at $p<0.05$ (n=5 per group)

### 3.3. Effect of HFD and Rosuvastatin on Wire Hanging Time (WHT)

Figure 3 shows the effect of HFD and Rosuvastatin on WHT across the groups. The HFD+ROS group had a significantly reduced WHT ($p<0.05$) compared to the other groups. This indicates reduced muscle strength. The HFD group had a more enhanced muscular endurance on the wire hanging test.

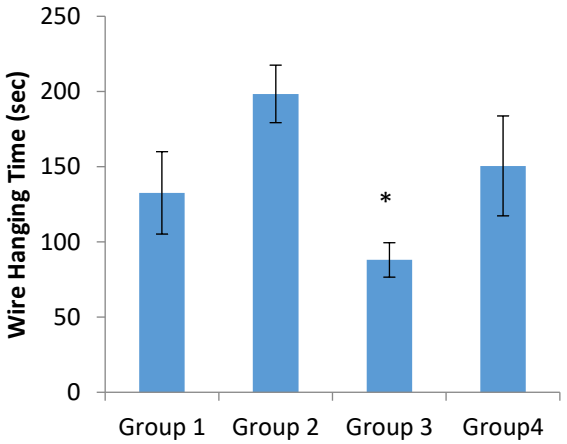


**Fig. 3 Wire Hanging Time Across the Groups.**
The hanging time in the ROS group was significantly lower compared to the other groups.
*indicates significance at $p<0.05$ (n=5 per group)

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### 3.4. Effect of HFD and Rosuvastatin on Brain Serotonin Levels

Figure 4 shows that HFD significantly elevated the brain serotonin levels ($p<0.05$) compared to the other groups. The HFD+ROS and HFD (4 weeks) + ROS groups had a reduced brain serotonin level comparable to the HFD group.

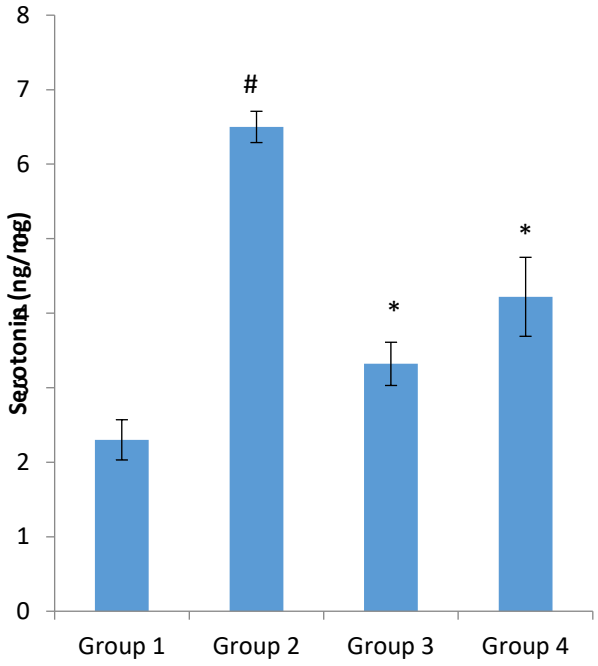


**Fig. 4 Brain Serotonin Levels Across the Groups**
The serotonin levels were significantly elevated in HFD group compared to control and other groups (n=5 per group).
*indicates significant decrease compared to HFD group; #indicates significant increase ($p<0.05$)

### 3.5. Effect of HFD and Rosuvastatin on Brain Dopamine Levels

Figure 5 shows that HFD and HFD+ROS groups had significant elevation in brain dopamine levels ($P<0.05$) compared to the other groups. The HFD (4 weeks) + ROS group had a significant reduction in brain dopamine.

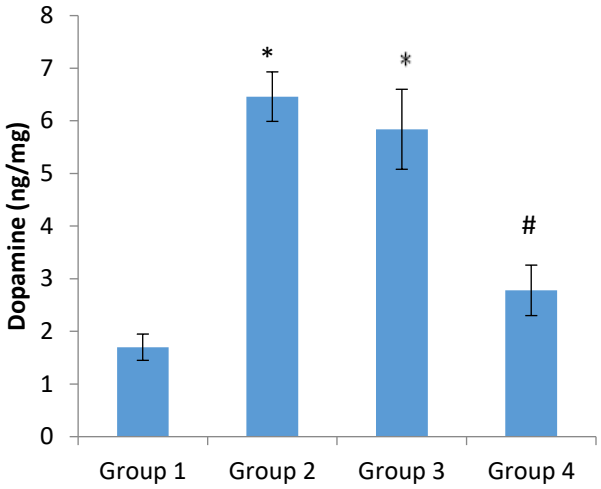


**Fig. 5 Brain Dopamine Level Across the Groups**
The HFD and HFD plus ROS groups had a significant elevation in dopamine levels compared to the other groups (n=5 per group).
*indicates significant elevation; # indicates significant reduction compared to HFD ($p<0.05$)

### 3.6. Effect of HFD and Rosuvastatin on Brain Super Oxide Dismutase (SOD)

Figure 6 shows no significant change in brain SOD concentration across the various groups ($P<0.05$).

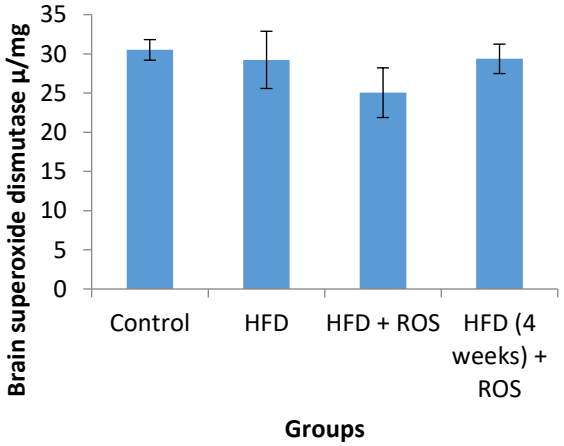


**Fig. 6 Brain SOD Levels Across the Groups**
There was no statistically significant change in brain SOD levels across the various groups (n=5 per group).

### 3.7. Effect of HFD and Rosuvastatin on Brain Catalase (CAT)

Figure 7 shows that HFD (4 weeks) + ROS group had a significantly reduced brain levels of CAT (P<0.05) compared to the other groups. The HFD and HFD+ROS group had no statistically significant difference compared to the control.

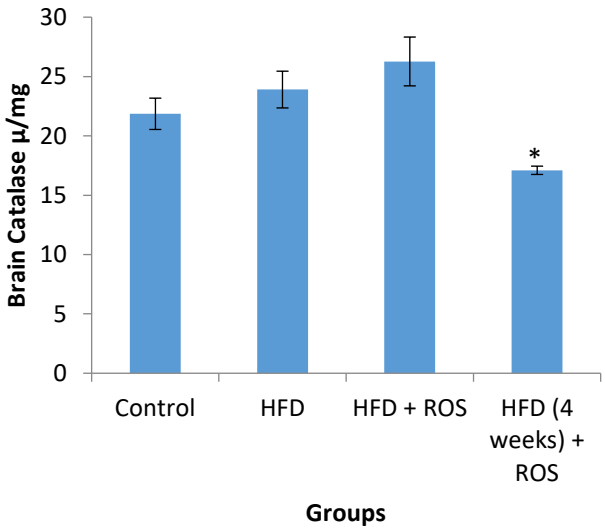


**Fig. 7 Brain CAT Levels Across the Groups**
The animals in Group 4 had a statistically significant reduction in brain CAT levels compared to the other groups (n=5 per group)
*indicates statistically significant reduction (p<0.05)

### 3.8. Effect of HFD and Rosuvastatin on Serum Calcium

Figure 8 shows that HFD + ROS group had a significantly reduced serum calcium concentration (P<0.05) compared to the other groups. The HFD and HFD (4 weeks) + ROS groups had no statistically significant difference compared to the control.

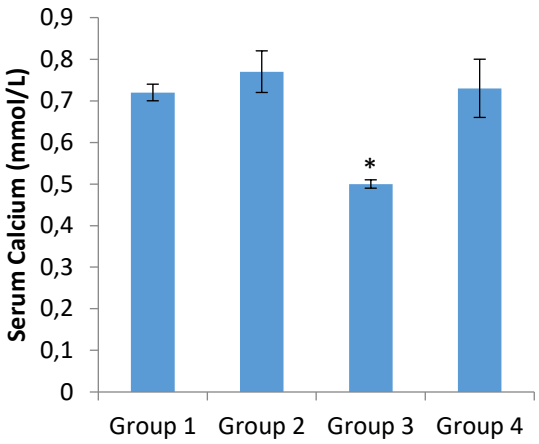


**Fig. 8 Serum Calcium Levels Across the Groups**
Serum calcium levels in HFD+ROS group was significantly lower compared to the other groups (n=5 per group)
*indicates statistical significance p<0.05

### Effect of HFD and Rosuvastatin on Serum Uric Acid

Figure 9 shows that HFD and HFD + ROS group had a significantly reduced serum concentration of uric acid (P<0.05) compared to the other groups. The HFD (4 weeks) + ROS group had no statistically significant difference compared to the control.

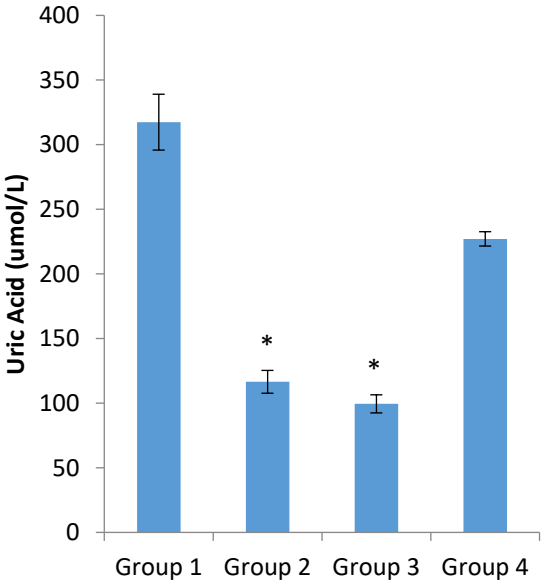


**Fig. 9 Serum Uric Acid Levels Across the Groups**
The HFD and HFD+ROS groups had a significantly lower serum calcium levels compared to control group (n=5 per group). *indicates significant decrease p<0.05

### 3.10. Effect of HFD and Rosuvastatin on Serum Malondialdehyde (MDA)

Figure 10 shows that HFD group had a significantly elevated serum MDA level ($P<0.05$) compared to the other groups. This indicates a significant level of lipid peroxidation. The HFD +ROS and HFD (4 weeks) + ROS groups had no statistically significant difference compared to the control.

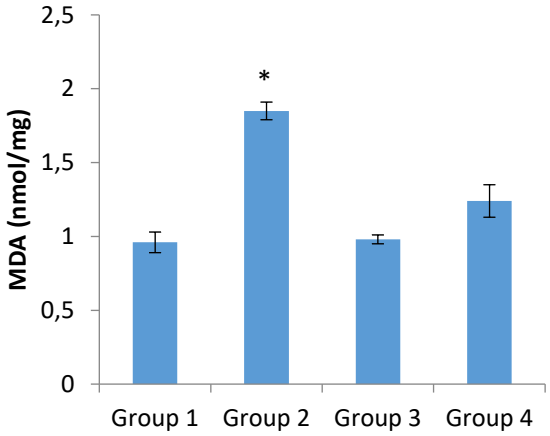


**Fig. 10 Serum MDA Levels Across the Groups**
The HFD group had a significantly elevated levels of MDA compared to the other groups (n=5 per group). *indicates significant elevation (p<0/05)

## 4. Discussion

In this study HFD resulted in a weight increase (Fig. 1) though not statistically significant which agrees with previous studies that have attributed HFD to hedonic feeding and weight gain (Abi *et al.*, 2022). The chronic administration of HFD has been used to model anxiety-like behavior in some studies (Dutheil *et al.*, 2016; Yong *et al.*, 2022) which has also been reproduced in this study. The transfer latency in the EPM was found to be significantly lower in the group fed with HFD when compared to the control (Fig. 2). The lesser time spent in the open arm is an indication of anxiety due to light aversion. Co-administration of ROS and HFD was found to significantly ameliorate this anxiety-like response - the mice spent longer time in the open arm when compared with the HFD fed group. Hyperlipidemia has been proven to be an independent risk factor for development of anxiety (Hu *et al.*, 2014; Huang *et al.*, 2017). Statins like ROS used in this study have been found to be beneficial in reducing the development of anxiety (O'Neil *et al.*, 2012; Emre-Aydingoz *et al.*, 2022). Our study proposes that a 5 week administration of HFD can induce anxiety via toxic cholesterol mechanism and possibly increased noradrenergic activity (Wang and Li, 2022) which was reversed by ROS.

The WHT test in this study demonstrated clearly that HFD could be beneficial in skeletal muscle endurance exercise. Conversely, ROS impaired muscle performance. The mice fed on HFD had a longer WHT when compared with the control and ROS treated group. This finding agrees with a previous study which showed that short term administration of HFD does not impede muscle protein synthesis despite intramuscular lipid accumulation (Satoru *et al.*, 2021). We propose that the intramuscular accumulation of fats in HFD fed animals is a rich reserve for calorie in aerobic exercise instead of glycogen breakdown (Burke, 2015). On the other hand mice that received ROS were noticed to have significantly impaired muscle strength as demonstrated by a significantly shorter WHT compared to the control and HFD groups (Fig. 3). Previous studies agree with this finding in that ROS was noticed to cause statin-induced myopathy via multifactorial mechanisms like: oxidative phosphorylation, impaired signal transduction, cell trafficking, gene transcription and structural protein formation (Urso *et al.*, 2005; Di Stasi, 2010). This study also proposes that ROS caused depletion in sarcolemmal lipids resulting in quick calorie exhaustion during strenuous exercise (Klopstock, 2008).

The effect on the brain neurotransmitters by HFD is also obvious in this study. Brain serotonin level was significantly elevated in this study (Fig. 4) with a significant reversal effect by ROS. A recent study revealed an enhancement of serotonin in the brains of rats fed with HFD for 12 weeks predominantly in the hippocampus (Haleem and Mahmood, 2021). This is contrary to several studies in the past that attributed the behavioural disorders seen in HFD-fed animals, to reduced brain serotonin (Kim *et al.*, 2013; Huq *et al.*, 2021). Duration of HFD administration and other factors like physical exercise, sex and age are possible determinants of the varying findings. The pleiotropic effect of ROS can be attributed to the normalizing of serotonin (Kasowski *et al.*, 2021).

Brain dopamine (DA) level was significantly elevated by HFD without any significant reversal effect by ROS. It was however noted that temporary stoppage of the HFD and introduction of ROS normalized the brain dopamine levels (Fig 5). This study supports the fact that HFD induces overeating by stimulating DA production from the ventral tegmental area; a neurotransmitter that has been widely implicated in reward and incentive motivation (Bassareo and Chiara, 1999). Though another study showed that depending on the time and duration of exposure to HFD, it could result in unpredictable DA activity (Reyes, 2012; Kim *et al.*, 2013).

High fat diet did not significantly alter the brains levels of SOD (Fig. 6) and CAT (Fig. 7). Rosuvastatin also did not give any significant alteration as well, except for the reduction in brain CAT after 1 week of ROS following stoppage of HFD. This implies that HFD induces oxidative stress with concomitant elevation in CAT which was restored when withdrawn and ROS administered (Okada *et al.*, 2013).

The serum calcium levels of the ROS group was found to be significantly lower (Fig.8) than the other groups. This further explains the reason for the poor skeletal muscle performance in the WHT in addition to the already established mechanisms (Urso *et al.*, 2005; Di Stasi, 2010; CTTC, 2022). A previous study found that statins could increase intracellular calcium (Hienke, 2004).The signifies a concomitant fall in extracellular calcium causing altered excitation contraction coupling.

The serum levels of uric acid was significantly lower (Fig. 9) in the HFD and HFD+ROS group. Uric acid has been known to have both deleterious and protective effects depending on whether it is acting as a proinflammatory agent or an antioxidant (El-Ridi and Tallima; 2017). Some studies corroborate our findings on the uric acid lowering potential of statins (Derosa *at al.*, 2016; Lin *et al.*, 2023).

Lipid peroxidation was induced by HFD as shown by significant elevation in serum MDA (Fig. 10) which was ameliorated by co-administration of ROS. Abundant evidence abounds in literatures about the role of high fat diet in causing lipid peroxidation and oxidative damage to cells just as found in our study (Beltowski *et al.*, 2000; Dhibi *et al.*, 2011; Haggag *et al.*, 2014; Hazzaa *et al.*, 2020). Administration of ROS was found to significantly ameliorate this effect by reducing the MDA levels. This finding is also corroborated by previous works (Zinellu *et al.*, 2019; Nishikido *et al.*, 2016). This may possibly be responsible for the anxiolytic effect seen with ROS intervention group in the EPM (Fig. 2) trial. Malondialdehyde has been implicated in development of anxiety in previous studies (Bal *et al.*, 2012; Islam *et al.*, 2014; Liu *et al.*, 2018). We therefore propose that ROS improved anxiety-like behavior in mice by inhibiting the deleterious effect of MDA and lipid peroxidation induced by HFD.

## 5. Conclusion

This study modeled anxiety-like behavior in mice using HFD. Rosuvastatin was co-administered to assess its effect on HFD fed mice. The result showed that ROS ameliorated the anxiety in the HFD fed animals but failed to improve muscle strength. Reduction in MDA levels is the proposed mechanism by which anxiolysis was achieved with ROS. Conversely, the HFD enhanced muscle strength in the WHT unlike ROS that significantly impaired this function. It is also proposed that significant depletion in serum calcium by ROS is strongly alluded as the cause of the muscle weakness and fatigue.

## Recommendation

Further research will be needed to understand molecular mechanisms behind these findings and possibly human studies.

## Acknowledgements

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## Conflicts of Interests

We declare that we have no conflicts of interest.

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