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The effects of metformin on high-fat diet-induced neuroinflammation and cognitive impairment

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**The effects of metformin on high-fat diet-induced neuroinflammation
and cognitive impairment**

An honors thesis presented to the
Department of Psychology,
State University of New York at Albany
in partial fulfillment of the requirements
for graduation with Honors in Psychology
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Abstract

Chronic high-fat feeding is associated with neuroinflammation, cognitive impairment, and anxiety-linked behaviors in rats. Metformin, a popular treatment for type II diabetes, has been shown to attenuate metabolic dysregulation and weight gain associated with an obesogenic diet. We demonstrated that HFD caused elevated fasting blood glucose, glucose intolerance, and increased body weight without cognitive impairment or anxiety as measured by novel object recognition and open field testing. Further, we demonstrated that metformin did not produce cognitive impairment, which was a concern associated with its chronic use. Further work will elucidate the impact of chronic HFD and metformin treatment on molecular markers of neuroinflammation and neural insulin resistance.

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Introduction

Diet-induced obesity is a major risk factor for the development of type II diabetes mellitus (T2DM), at least in part as a result of obesity-induced peripheral inflammation (Furukawa et al., 2017). Inflammation caused by a high-fat diet (HFD) are is not limited to the periphery; short- and long- term HFDs are associated with increased neuroinflammation (Posey et al., 2009; Sobesky et al., 2016). Pro-inflammatory neural changes contribute to the pathological impact of conditions such as Alzheimer's Dementia (AD) and T2DM (De Felice & Ferreira, 2014; Tarkowski et al., 2003). Inflammatory processes result in brain-wide changes to protein expression that can contribute to neurodegenerative pathologies across a variety of cell types, including microglia: the innate immune cells of the brain (Graeber et al., 2011). Dysregulation of cytokines such as interleukin (IL)-4, IL-10, IL-6 and tumor necrosis factor (TNF)-α is implicated in the pathology of T2DM through insulin-sensitive and insulin-insensitive pathways (DeFuria et al., 2013; O'Connor et al., 2007). Insulin resistance impairs phosphatidylinositol-3-kinase (PI3K) activity promoting cellular resistance to anti-inflammatory cytokines while obesity and metabolic dysregulation upregulate proinflammatory cytokines (DeFuria et al., 2013; O'Connor et al., 2007).

T2DM is a proinflammatory condition that often results in a phenomenon known as microglial priming. Microglia are capable of polarizing into an M1-like or an M2-like phenotype where they experience gross morphological changes to carry out proinflammatory or anti-inflammatory activities (Cherry et al., 2014). While surveilling (baseline-state) microglia in healthy rats display characteristics of an M2-skew, reducing the intensity of a response to a proinflammatory stimulus. In T2DM, an opposing phenomenon is seen, where microglia are more likely to polarize into an M1-like phenotype in response to proinflammatory stimuli. A

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commonly used marker of microglial polarization, ionized calcium binding adaptor protein (Iba1), is upregulated in response to both pro- and anti-inflammatory stimuli (Tanokashira et al. 2018; Yang et al., 2017). M1-like microglia express a variety of proteins that increase the production of reactive oxygen species (ROS) and promote the cytotoxic effects of microglia (Boche et al., 2013). In particular, the production of ROS by an oxidative burst requires the activity of the insulin-regulated Kv1.3 voltage gated potassium channel (Fordyce et al., 2005). Kv1.3 is expressed predominantly by microglia (and by neurons of the olfactory bulbs, which are known to be insulin responsive) (Fadool et al., 2000; Rangaraju et al., 2015). In T2DM and AD, ROS are produced more readily by microglia and this production is exacerbated by both insulin resistance and chronic inflammation (Butterfield et al., 2014). Taken together, these data suggest a potential role for Kv1.3 in cognitive impairment accompanying neuroinflammation (Rangaraju et al., 2015; Schilling & Eder, 2011) To date, there have been no studies that investigate the effects of chronic HFD exposure on the state of Kv1.3 phosphorylation in the rat. As Kv1.3 phosphorylation is associated with both insulin signaling and channel inhibition, impairments in the phosphorylation of Kv1.3 by insulin may be associated with the development of insulin resistance (Fadool et al., 2000). Further, as Kv1.3 is required for the cytotoxic capabilities of microglia, impairment in Kv1.3 phosphorylation may facilitate proinflammatory changes in the brain. Understanding the effects of diet-induced obesity and insulin resistance on Kv1.3 phosphorylation would provide invaluable insight into how insulin resistance can promote neuroinflammation.

A bidirectional relationship between inflammation and insulin resistance has been demonstrated in both the brain and the periphery through numerous mechanisms. Among them, overexpression of insulin degrading enzyme in both T2DM and AD results in the upregulation of

IL-1β and other proinflammatory cytokines (Mittal et al., 2016). Separately, impairment of 5'adenosine monophosphate-activated protein kinase (AMPK) in these disorders promotes overexpression of the proinflammatory transcription factor nuclear factor kappa beta (NFkβ) in response to proinflammatory stimuli (Peixoto et al., 2017). Overexpression of proinflammatory cytokines can further induce cellular insulin resistance in the periphery and the central nervous system (de la Monte, 2012). A major feature of cellular insulin resistance is a reduction in translocation of insulin-regulated glucose transporter four (GluT4) in response to insulin stimulation (Leto & Saltiel, 2012). This impairment is due to decreases in activity of protein kinase B (Akt)/PI3K pathway stemming from increased inhibitory phosphorylation of insulin receptor substrate-1 (IRS1) at serine site 307 (Danielsson et al., 2005; de la Monte, 2012). Further, AMPK activity, which normally promotes insulin sensitivity and inhibits neuroinflammation, is impaired in insulin resistant conditions (Ruderman et al., 2013). As brain insulin resistance and neural hyperglycemia are common in AD patients, researchers have begun referring to a subtype of AD as "type III diabetes" (Akter et al., 2011; de la Monte, 2009; Mullins & Kapogiannis, 2018).

Chronic exposure to a diabetogenic diet results in the development of metabolic abnormalities, and it also leads to the development of anxious and depressive behavior in tests like the elevated plus maze and open field test (Dutheil et al., 2016; Souza et al., 2007). (Sims-Robinson et al., 2016). Novel object recognition is a behavior task that relies on the innate preference rodents show for novel rather than familiar stimuli (d'Isa, Brambilla, and Fasano, 2014). Further, novel object testing often takes place in an open field apparatus, where on habituation to the maze, time spent in the center zone of the apparatus negatively correlates with anxiety (Feyissa et al., 2017). Not only are T2DM and AD associated with neuroinflammation,

insulin resistance, cognitive impairment and anxiety, but anxious symptoms are predictive of the conversion of human mild cognitive impairment to AD (de la Monte et al., 2009; Li et al., 2016; Li et al., 2019; Mah et al., 2015). As anxious symptoms in these conditions are potentially related to neuroinflammation via IL-6 and TNFa mediated pathways, it is likely that there is substantial mechanistic overlap between the anxiety symptoms of T2DM and AD (Fourrier et al., 2019; White et al., 2009).

Typical antidepressants are unsuccessful at ameliorating HFD induced anxiety and depression symptoms, but insulin sensitizing agents like metformin hydrochloride normalized anxious behaviors in HFD animals (Zemdegs et al., 2019). While metformin is commonly thought to prevent the development of peripheral insulin resistance through inhibition of hepatic gluconeogenesis, it is known to cross the blood brain barrier and can prevent neuronal insulin resistance in vitro (Gupta et al., 2011; Moreira, 2014). There is currently some debate about whether metformin is beneficial or detrimental to cognition, but evidence suggests that HFD-fed animals exposed to metformin will not experience the cognitive impairment associated with the diet (Moreira, 2014; Pintana et al., 2012).

Whether metformin can normalize diet-induced cognitive deficits and neuroinflammation remains to be tested. Hence, we sought to address whether chronic metformin administration concurrent with HFD can prevent the induction of obesity, neuroinflammation, and cognitive impairment commonly seen in this model. We measured cytokine expression: an additional question was whether chronic metformin would promote the expression of anti-inflammatory cytokines while inhibiting proinflammatory signaling. The effect of metformin on markers of neural insulin resistance, in the context of a HFD or control diet, will be assessed post mortem to determine the effect of metformin administration. We tested animals on both novel object

recognition and open field cognitive tasks to assess the impact of diet and treatment on cognitive function.

Methods

Animals

40 Wistar rats were purchased from Envigo (Indianapolis, IN). Rats were kept on a 12:12h light/dark cycle and given food and water *ad libitum.* Following their arrival, animals were pair housed, and allowed to acclimate for two days with no handling. On days 3-9 after their arrival, rats were handled daily for 10 minutes. After day 9, rats were weighed daily and handled for 5 minutes every other day. On day 10 after arrival, animals were split into four groups: high-fat diet/saline, high-fat diet/metformin, standard diet/saline, standard diet/metformin. Animals were on their respective diets until the end of the experiment on day 92. High-fat diet was purchased from Research Diets (New Brunswick, NJ; Catalog Number: D12492), and the standard Chow was purchased from LabSupplyTx (Fort Worth, Tx; Catalog Number: 5P75). Animals received daily intraperitoneal injections of either saline (Vehicle) or metformin (150mg/kg) from day 10 to day 87 in the facility, ending the day before testing began. Metformin was purchased from Sigma Aldrich (St. Louis, MO; Catalog Number: D150959) and metformin solutions (50mg/mL) were prepared weekly in sterile saline.

Testing Diabetic Status

Fasting blood glucose measurements were taken from tail prick blood samples between 1:00pm and 3:00pm with a *OneTouch® UltraMini®* glucometer after a four hour fast on day 88 of the experiment. After this, an IPGTT was performed, during which rats were challenged with 2g/kg of glucose. Glucose solutions (40-60%) were prepared in 1x phosphate buffered saline

(137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, and 2 mM KH_2PO_4: pH 7.4). Blood glucose was measured at 5, 15, 30, 60, and 120 minutes post-injection. The first cohort of animals was unable to undergo glucose tolerance testing because an early iteration of the timeline had glucose tolerance testing scheduled the day of euthanasias. There was a concern that this challenge would influence later molecular data. Data were analyzed by comparing area-under-the-curve for blood glucose across the 120 min period.

Open Field Testing

Open field testing was the day after the IPGTT. The open field apparatus was a 70cmx70cmx40cm arena with markings under the clear floor, visually dividing it into a grid containing 16 squares. Animals were placed in the center of the arena and allowed to explore freely for 5 minutes. Latency to leave the center zone and total time spent in the center (as defined by 50% of the animal's body being in the center zone) were measured.

Novel Object Recognition Testing

Novel object recognition testing took place over the next two days after open field testing, using the same apparatus. On day one, animals were trained to identify familiar objects by placing two identical objects in opposite corners of the apparatus. Rats were placed into the center of the apparatus and allowed to explore the objects for 5 minutes. On day two, one of the familiar objects from the previous day and a novel object were placed on opposite corners of the apparatus, and the side the novel object was placed on was counterbalanced across all subjects. Rats were again placed in the center of the apparatus and allowed to explore freely for 5 minutes. Object interactions were defined as touching and looking towards (Snout facing the direction of the object within 1 square of the object) the object, while rearing (Two paws off the ground,

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sniffing the air), standing on the object (at least three paws on the object), and grooming were excluded. Behavior coding was done in Solomon Coder. Discrimination indices compare the difference in time the animal spent exploring the novel versus familiar object to the total time exploring both objects, while recognition indices compare the time spent interacting with the familiar object on the training session to the time spent interacting with the familiar object on the testing session (d'Isa, Brambilla, & Fasano, 2014). The formulae for discrimination index and recognition index were as follows:

$$\text{Discrimination Index:} \frac{(\text{Time interacting with Novel Object}) - (\text{Time interacting with Familiar Object})}{(\text{Time interacting with Novel Object}) + (\text{Time interacting with Familiar Object})}$$

$$\text{Recognition Index:} \frac{(\text{Time interacting with Familiar Object, training}) - (\text{Time interacting with Familiar Object, testing})}{\text{Time interacting with Familiar Object, training}}$$

Euthanasia Procedures

Rats were deeply anesthetized with isoflurane and euthanized by rapid decapitation after a five hour fast. Whole brains were promptly removed and chilled in ice-cold 1xPBS for 5 minutes. Hippocampi and prefrontal cortices were removed and immediately stored on dry ice before being transferred to long-term storage at $-80_{o}C$. 4-6mL of trunk blood was collected and centrifuged at 11,000rpm for 7 minutes, and the blood plasma was separated from other fractions and frozen. Epididymal fat pads were removed and weighed.

Analysis

All behavior tests were coded using the open source video coding software Solomon Coder (Peter, 2019). All data were analyzed using two factor ANOVAs comparing diet and drug, unless otherwise stated, using IBM SPSS 26. *Post hoc* pairwise comparisons with tukey corrections

were used to explore the relationships between specific groups. Data are expressed as mean ±
standard error and *post hoc* analysis is presented as mean-difference ± standard error, unless
otherwise stated. Data points greater than 3 ± standard deviations from the mean without the
inclusion of the data point were removed as outliers; this caused the removal of one animal from
the fat pad weights, 2 animals from center zone interactions in open field analysis, 3 from latency
to leave the center, one from discrimination index, and two from the recognition index. All data
were normally distributed.

Results

Weight Gain

Weight gain was analyzed using a mixed repeated measures ANOVA comparing diet and
drug across time, as measured by the first weight measurement on day 8 and the weight
measurement on day 88 of the experiment. A main effect of diet ($F_{1,36}= 16.235$, $p < 0.05$), but
not of drug ($F_{1,36}= 3.654$, $p > 0.05$) was seen; an interaction effect was not seen ($F_{1,36}= 0.272$, p
>0.05). The high-fat/saline group (n=10) differed significantly from the standard chow/saline
group (n=10) (44.70 ± 13.890 grams, $p > 0.05$) and the standard chow/metformin group (n=10)
(58.35 ± 13.890 grams, $p > 0.05$); however, the high-fat/metformin (n=10) group did not
significantly differ from the high-fat/saline group (-23.90 ± 13.890 grams, $p > 0.05$), the standard
chow/saline group (20.80 ± 13.890 grams, $p > 0.05$) or standard chow/metformin group (34.45 ±
13.890 grams, $p > 0.05$). The standard chow/saline and standard chow/metformin groups did not
differ from each other (13.65 ± 13.890 grams, $p > 0.05$).

Epididymal Fats Pads

One animal had an undescended teste with a congenitally malformed epididymal fat pad and was excluded from the analysis. There were main effects of both diet ($F_{1,39}$= 25.552, $p <$ 0.05) and drug ($F_{1,39}$= 7.372, $p <$ 0.05), but no interaction effects were seen ($F_{1,39}$= 0.817, $p >$ 0.05). The high-fat/saline group (n=10) differed from both the standard chow/metformin group (n=10, 1.7973 ± 0.32302%, $p <$ 0.05) and the standard chow/saline group (n=9, 1.3798 ± 0.33187%, $p <$ 0.05). The high-fat/metformin group (n=10) differed from the standard chow/metformin group (0.9611 ± 0.32302%, $p <$ 0.05), but not from the high-fat/saline group (-0.8361 ± 0.32302%, $p >$ 0.05) or from the standard chow/saline group (0.5437 ± 0.33187%, $p >$ 0.05). There was no difference between the standard chow/saline and standard chow/metformin groups (0.4175 ± 0.33187%, $p >$ 0.05).

Glucose Tolerance Testing

Analysis of fasting blood glucose measurements yielded a main effect of diet ($F_{1,36}$= 19.597, $p <$ 0.05) and drug ($F_{1,36}$= 6.062, $p <$ 0.05), but no interaction effect ($F_{1,36}$= 1.672, $p >$ 0.05). The fasting blood glucose significantly differed between the high-fat/saline group (n=9), the standard chow/saline group (n=9) (25.56 ± 6.318 mg/dL, $p <$ 0.05), and the standard chow/metformin group (n=9) (30.78 ± 6.318 mg/dL, $p <$ 0.05). In contrast, the high-fat/metformin group (n=9) did not differ significantly from the high-fat/saline group (-16.78 ± 6.318 mg/dL, $p >$ 0.05), the standard chow/saline group (8.78 ± 6.318 mg/dL, $p >$ 0.05), or the standard chow/metformin group (14.00 ± 6.318 mg/dL, $p >$ 0.05). The standard chow/saline group did not differ from the standard chow/metformin group (5.22 ± 6.318 mg/dL, $p >$ 0.05).

Area under the curve analysis showed a main effect of diet ($F_{1,36}$= 9.682, $p < 0.05$), but failed to show a main effect of drug ($F_{1,36}$= 2.454, $p > 0.05$) or an interaction effect ($F_{1,36}$= 2.278, $p > 0.05$) between them. Mirroring the relationships of fasting blood glucose, the high-fat/saline group (n=9) differed from the standard chow/saline group (n=9) (10152.222 ± 3107.0488, $p < 0.05$) and the standard chow/metformin group (n=9) (10277.778 ± 3107.0488, $p < 0.05$), but not from the high-fat/metformin group (n=9) (6757.778 ± 3107.0488, $p < 0.05$). The high-fat/metformin group did not differ from the standard chow/saline group (3394.444 ± 3107.0488, $p > 0.05$) nor the standard chow/metformin group (3520.000 ± 3107.0488, $p > 0.05$), and the standard chow/saline group did not differ from the standard chow/metformin group (125.556 ± 3107.0488, $p > 0.05$).

Open Field Testing

No main effects of diet ($F_{1,38}$= 1.034, $p > 0.05$) or drug ($F_{1,38}$= 0.755, $p > 0.05$) were seen on total time in the center zone, nor was there an interaction effect ($F_{1,38}$= 0.556, $p > 0.05$). Standard chow/metformin animals (n=10) averaged 9.38 ± 7.57311 seconds in the center zone whereas standard chow/saline animals (n=9) spent an average of 9.6889 ± 7.89754 seconds. High-fat/metformin (n=10) animals spent an average of 10.0600 ± 4.75539 seconds in the center zone, and high-fat/saline (n=9) animals spent an average of 14.1111 ± 9.90309 seconds.

There was no main effect of diet ($F_{1,37}$= 3.724, $p > 0.05$) or drug ($F_{1,37}$= 0.679, $p > 0.05$) on latency to leave the center zone, nor was there an interaction effect ($F_{1,37}$= 0.050, $p > 0.05$). The standard chow/saline (n=8) group took an average of 4.4750 ± 0.90040 seconds to leave the center while the standard chow/metformin (n=9) group took an average of 4.2222 ± 0.83931 seconds. The high-fat/metformin (n=10) took an average of 4.9400 ± 1.37291 seconds to leave

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the center while high-fat saline took an average of 5.3800 ± 1.67451 seconds to leave the center zone.

Novel Object Recognition

An analysis of the total time exploring both objects indicated that there was no main effect of diet ($F_{1,39}= 2.549$, $p > 0.05$) or drug ($F_{1,39}= 0.006$, $p > 0.05$) nor an interaction effect ($F_{1,39}= 0.061$, $p > 0.05$). A comparison of the discrimination index yielded no effect of diet ($F_{1,39}= 0.281$, $p > 0.05$), drug ($F_{1,39}= 0.310$, $p > 0.05$), or the interaction of diet and drug ($F_{1,39}= 0.643$, $p > 0.05$). A comparison of the recognition index yielded no main effect of diet ($F_{1,38}= 0.169$, $p > 0.05$) or drug ($F_{1,38}= 0.117$, $p > 0.05$), and no interaction effect was observed ($F_{1,38}= 0.454$, $p > 0.05$).

Discussion

The high fat/saline group gained significantly more weight than both the standard chow/saline and the standard/chow metformin group. Further, the high fat/saline group's epididymal fat pads were a significantly greater proportion of their total body weight than either standard chow group. As the high fat/metformin group did not differ from any group in post hoc comparisons of total body weight, it appears that metformin was able to partially attenuate the effect of HFD on total body weight. As the high fat/metformin group had significantly larger relative fat pad weights than the standard chow/metformin group, and an effect of metformin was seen in the two factor ANOVA, metformin may have conferred the partial protection against HFD-induced weight gain through modulating proportions of fat in the periphery. This effect was not large enough to produce a reversal of obesity or a statistically significant interaction.

This indicates that metformin administration to animals fed an obesogenic diet was able to produce an intermediate phenotype, in which obesity was partially, but not wholly, attenuated.

The success of the diet in promoting obesity and its comorbidities were further highlighted by the increases in both fasting blood glucose and the area under the curve in response to an IPGTT. This relationship was mirrored by differences in blood glucose, where HFD animals injected with metformin did not significantly differ from any other group, while HFD animals injected with saline differed from animals fed a standard chow, regardless of drug condition. This was further highlighted by differences in the area under the glucose tolerance curve, where the HFD/saline group demonstrated an impairment in glucose metabolism while neither standard chow group had impaired glucose clearance. The HFD/metformin group again served as an intermediate, in that they did not significantly differ from any group. This implies that, in addition to partially attenuating the weight gain associated with HFD, it also partially attenuates the development of glucose intolerance.

As the fasting blood glucose and glucose intolerance seen in the high fat diet groups were not severe enough to be indicative of fully-developed diabetes, these groups were likely prediabetic, as their fasting blood glucose was lower than 200 mg/dL (Meng et al., 2017). Given that only the high fat/saline group had a significantly higher blood glucose than the standard chow groups, the high fat/metformin group was likely progressing into a prediabetic state.

In open field testing, no difference in center zone interactions were detected between any groups. This indicates that the high fat diet was unable to produce anxiety-like behaviors in our animals, nor did metformin have any effect on anxiety. Further, there were no differences in the latency to leave the center zone, their starting zone, indicating that all animals left the initial zone

at about the same rate. As HFD was unable to cause the expected increase in anxiety-related behaviors, this could result from the length of the diet. As there is data suggesting that moderate short-term high fat diets (of around 5 weeks) and a moderate-term diets (of around 12 weeks) produce a reduction or no difference in anxiety when compared to control animals, it is possible that our diet length was insufficient to produce changes anxiety related behaviors (Sweeney et al., 2017). Metformin may only be able to reduce anxiety when animals are exposed to excess stress, so it is possible that metformin didn't influence anxiety in our study because there weren't increases in anxiety to attenuate.

In novel object recognition testing, we saw no differences in total time spent interacting with both objects across the groups. This indicates that our discrimination or recognition indices were not influenced by differences in total time exploring the objects. Further, as the two indices yielded average values greater than zero for each group, it is indicative of preference towards the novel object. As no group differences were seen in novel object testing HFD nor metformin was unable to influence memory in our novel object task. In part, this may result from the long intertrial interval as compared to some other diet-induced cognitive impairment studies (Rollins et al., 2019). Further, the diet may not have been long enough to produce cognitive impairment, as animals on an intermediate length diet (6 weeks) have previously shown no difference in novel object score when compared to controls (Gainey et al., 2017). We were able to demonstrate that metformin did not produce cognitive impairment, alleviating concerns that chronic metformin treatment harms cognitive functioning.

As later work on this project will elucidate the effects of chronic HFD feeding on neuroinflammation and markers of insulin resistance, we will be able to establish some of the consequences of long-term HFD feeding. Further, as the rats stopped receiving daily injections

of metformin on the day of the IPGTT, we will be able to assess the molecular consequences of

chronic metformin treatment in the absence of the acute effects of the drug. These data will

provide us with a more complete understanding of the effects of metformin on markers of

neuroinflammation in the hippocampus.

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Appendix

Acronyms

T2DM   Type II diabetes mellitus

HFD   High-fat diet

AD   Alzheimer's Dementia

IL   Interleukin

TNF   Tumor necrosis factor

Iba1   Ionized calcium binding adaptor protein

ROS   Reactive oxygen species

Kv1.3 Kv1.3 voltage gated potassium channel

AMPK   5' Adenosine monophosphate-activated protein kinase

NFkβ   Nuclear factor kappa beta

GluT4 Glucose transporter four

IRS-1 Insulin receptor substrate 1

Akt   Protein kinase B

PI3K   Phosphotidylinositol-3-kinase

Figures
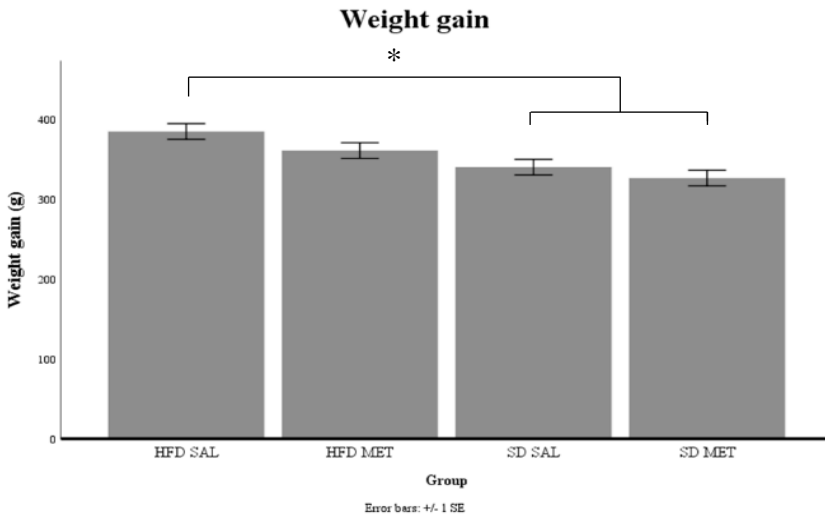
Figure 1: Animals were weighed daily throughout the experiment. Weights on day 8 of the experiment were compared with weights on day 88 of the experiment. ($p < 0.05$). Error bars: ± Standard error*
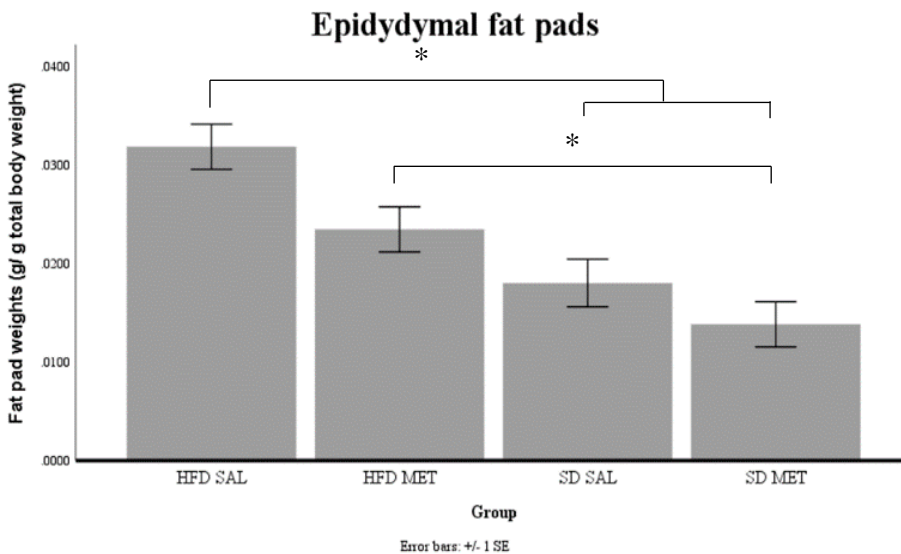
Figure 2: On euthanasia, epididymal fat pads were excised and immediately weighed. The proportion of fat pad weight to total weight was calculated. ($p < 0.05$), Error bars: ± Standard error*
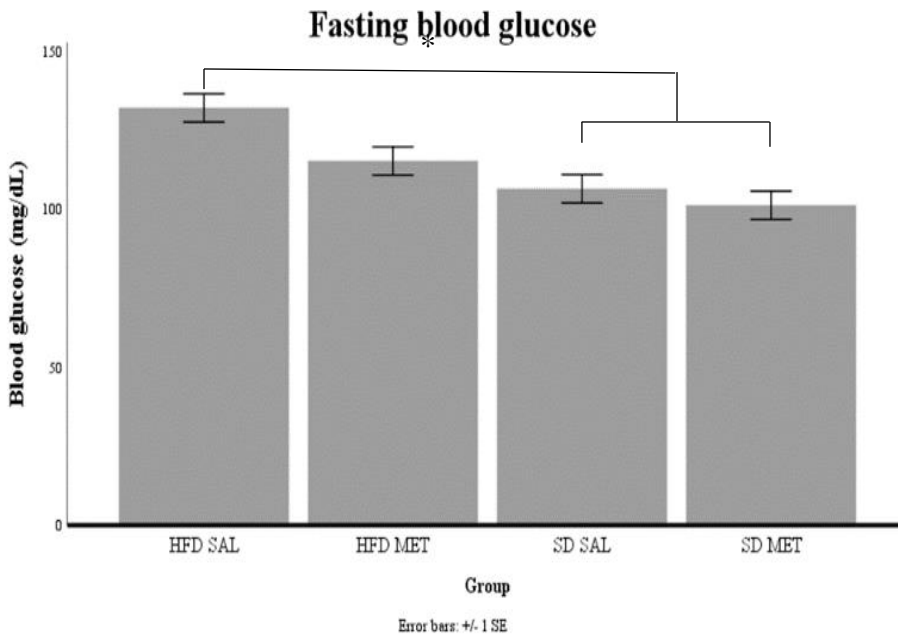
Figure 3: Blood glucose measurements were taken from a tail prick after a four hour fast. (* p < 0.05), Error bars: ± Standard error
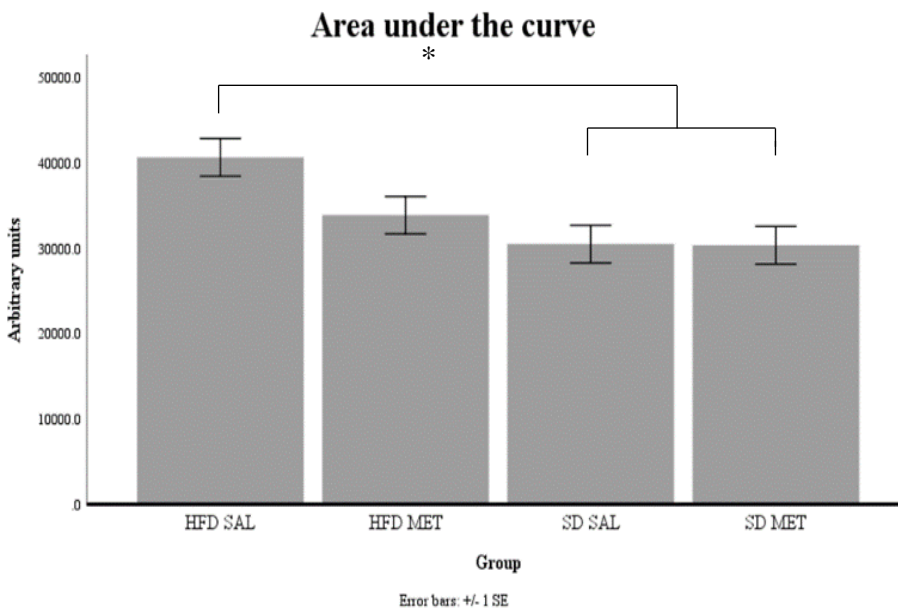
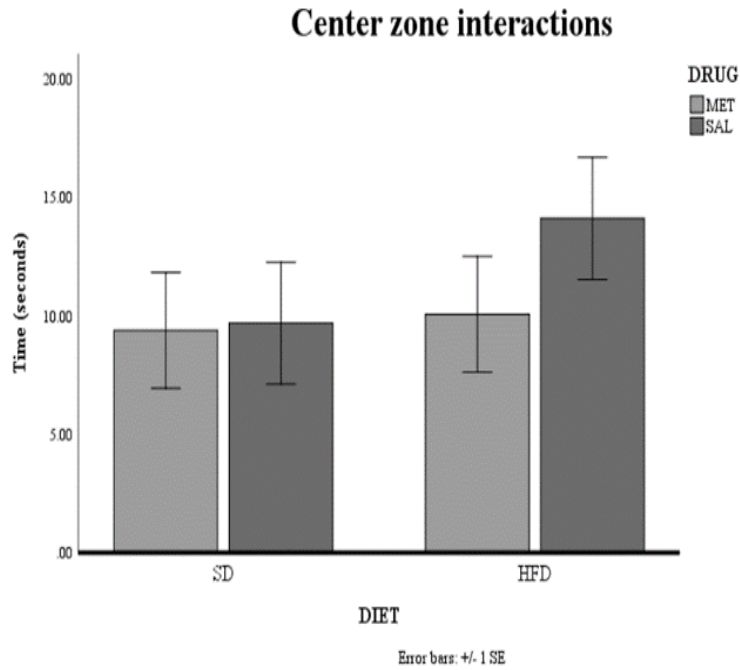*Figure 4: Area under the glucose tolerance curve. (* p < 0.05), Error bars: ± Standard error*

Figure 5: Time spent in the center zone. Error bars: ± Standard error
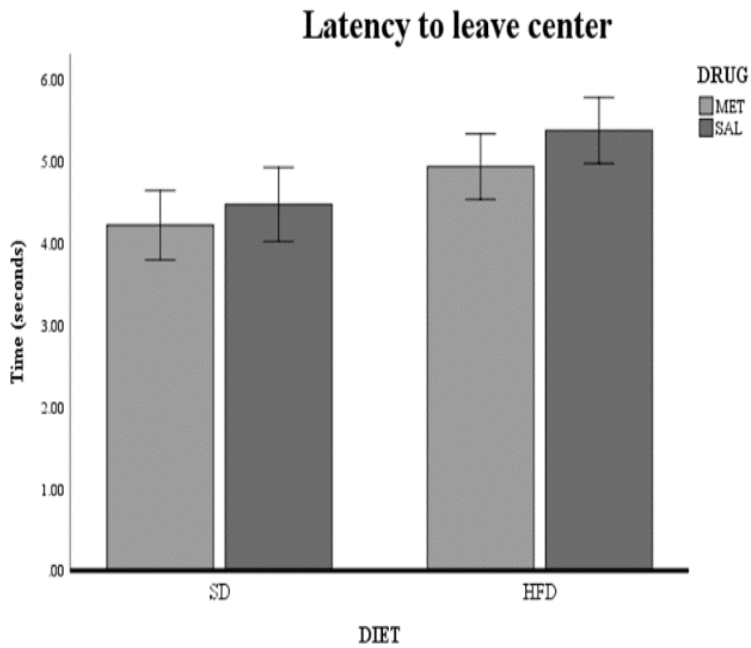
Figure 6: Latency to leave the center zone. Error bars: ± Standard error

Recognition index

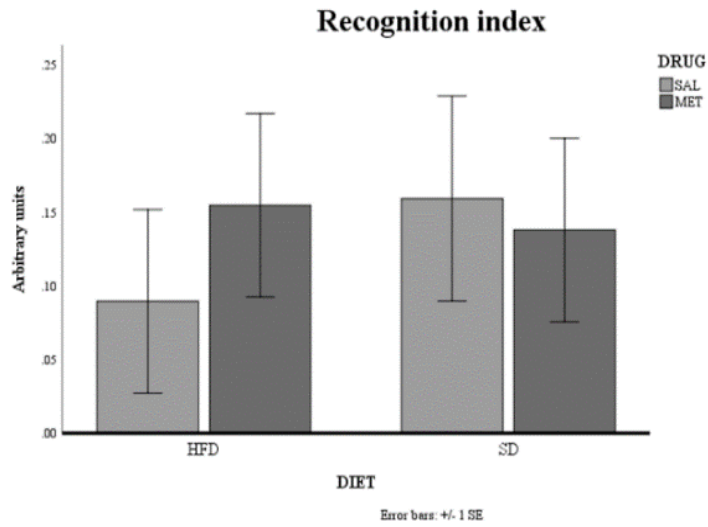*Figure 7: Discrimination index scores. Error bars: ± Standard error. Chance level is 0. HFD/Saline (n=10), HFD/Metformin (n=10), Standard Diet/Saline (n=9), Standard Diet/Metformin (n=10)*
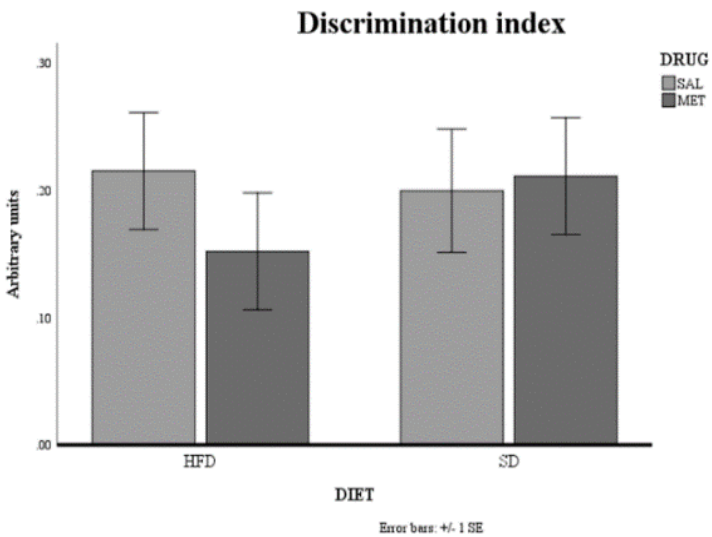

Discrimination index

Figure 8:Recognition index scores. Error bars: ± Standard error. Chance level is 0. HFD/Saline (n=10), HFD/Metformin (n=10), Standard Diet/Saline (n=8), Standard Diet/Metformin (n=10)