Neurodegeneration in an animal model of Parkinson’s Disease

is exacerbated by a high fat diet

Jill K. Morris,¹ Gregory L. Bomhoff,¹ John A. Stanford,¹,³ and Paige C. Geiger¹,²
Department of Molecular & Integrative Physiology,¹ Landon Center on Aging,² and Kansas
Intellectual & Developmental Disabilities Research Center,³ University of Kansas Medical
Center, Kansas City, KS 66160

Running title: High fat diet affects neurodegeneration

Correspondence to: Paige C. Geiger
Department of Physiology, Mailstop 3051
University of Kansas Medical Center
3901 Rainbow Blvd., Kansas City, KS 66160
phone: 913-588-7408
email: pgeiger@kumc.edu
Abbreviations

6-OHDA, 6-hydroxydopamine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EDL, extensor digitorum longus; HF, high fat; HOMA-IR, homeostatic model assessment-insulin resistance; HPLC, high pressure liquid chromatography; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; T2D, Type 2 Diabetes
Abstract

Despite numerous clinical studies supporting a link between Type 2 Diabetes (T2D) and Parkinson’s disease (PD), the clinical literature remains equivocal. We therefore sought to address the relationship between insulin resistance and nigrostriatal dopamine (DA) in a preclinical animal model. High fat feeding in rodents is an established model of insulin resistance, characterized by increased adiposity, systemic oxidative stress, and hyperglycemia. We subjected rats to a normal chow or high fat diet for five weeks before infusing 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle. Our goal was to determine whether a high fat diet and the resulting peripheral insulin resistance would exacerbate 6-OHDA-induced nigrostriatal DA depletion. Prior to 6-OHDA infusion, animals on the high fat diet exhibited greater body weight, increased adiposity and impaired glucose tolerance. Two weeks after 6-OHDA, locomotor activity was tested and brain and muscle tissue was harvested. Locomotor activity did not differ between the groups nor did cholesterol levels or measures of muscle atrophy. High fat-fed animals exhibited higher homeostatic model assessment of insulin resistance (HOMA-IR) values and attenuated insulin-stimulated glucose uptake in fast-twitch muscle, indicating decreased insulin sensitivity. Animals in the high fat group also exhibited greater DA depletion in the substantia nigra and the striatum, which correlated with HOMA-IR and adiposity. Decreased phosphorylation of HSP27 and degradation of IκBα in the substantia nigra indicate increased tissue oxidative stress. These findings support the hypothesis that a diet high in fat and the resulting insulin resistance may lower the threshold for developing PD, at least following DA-specific toxin exposure.

Keywords: dopamine, diabetes, substantia nigra, striatum, insulin resistance
Introduction

Clinical studies suggest a link between Type 2 Diabetes (T2D) and Parkinson’s disease (PD) (30, 46), and between fat intake or adiposity and PD (1, 31, 34). Moreover, it was reported over forty years ago that greater than 50% of PD patients exhibit abnormal glucose tolerance (4, 10) or diabetes (36). Despite this information, very little is known regarding the relationship of these diseases and the impact of co-morbidity on their pathogenesis. T2D is estimated to impact 300 million individuals by 2025 (47) with the elderly at greatest risk (54), the population also at greatest risk for neurodegenerative diseases like PD. For these reasons, understanding the potential for T2D, obesity, high dietary fat intake, and insulin resistance to contribute to PD is critical. Although the exact cause of PD is unknown, various environmental factors such as aging, diet, and environmental toxin exposure have been implicated in contributing to its development (29, 34, 51). The idea that “multiple hits” play a role in PD degeneration is supported by the fact that 80% of dopamine (DA) producing neurons must be lost for symptoms to appear (50). While diabetes and PD do not invariably coincide, several studies suggest that obesity may potentiate neuronal dysfunction or even neurodegeneration (reviewed in (11)). High fat diet-induced insulin resistance could make DA neurons in the substantia nigra (SN), the origin of DA producing neurons that degenerate in PD, more susceptible to environmental insults.

While it is possible that a diet high in fat may contribute to the development of PD, much about this relationship remains unknown. In animal models, most studies have focused on the effect of obesity or high fat (HF) feeding on the mesolimbic DA pathway (17, 22, 23), which modulates response to reward and is likely affected in obese individuals. However, few studies have addressed this issue in the nigrostriatal DA pathway, which is involved in the production of movement and affected in PD. Although best known for its role in movement disorders, the nigrostriatal pathway has also been shown to play an important role in feeding behavior (41) and may also be affected by obesity or HF feeding. Although it is possible that HF feeding may
make DA neurons more vulnerable to environmental insults, such as neurotoxins, only one preclinical study has investigated the effect of a HF diet on DA neurodegeneration (15). These authors found that treatment with the neurotoxin MPTP (used to model PD) produced greater striatal DA depletion in HF-fed mice than in chow-fed controls.

We wanted to further characterize the effect of a high fat diet on toxin-induced nigrostriatal DA depletion using the 6-hydroxydopamine (6-OHDA) rat model of PD. Unlike MPTP, 6-OHDA may play a role as an endogenous neurotoxin (reviewed in (7)). Iron is abundant in the SN and can react in a fenton-type reaction with DA and hydrogen peroxide (produced extensively by monoamine oxidase during DA turnover) to produce 6-OHDA (43), which in turn can increase iron release from ferritin (35). This suggests that 6-OHDA may play an important role in perpetuating this damaging endogenous cycle. In addition, 6-OHDA is increased in the urine of patients treated with L-DOPA (3, 32), the most common and effective treatment of PD, and L-DOPA treatment in rodents increases 6-OHDA production in the striatum (9). To determine if HF-fed animals were indeed more sensitive to 6-OHDA mediated DA depletion, we administered equal amounts of 6-OHDA to HF-fed, insulin resistant rats and chow-fed controls. We observed that the HF diet group exhibited significantly greater levels of DA depletion in the SN, the origin nucleus of DA neurons in the nigrostriatal pathway, and the striatum, the termination point of this pathway. These results support an exacerbating role for dietary fat, and consequent insulin resistance, in vulnerability to toxin-induced nigrostriatal DA depletion.

Materials and Methods

Animals and Diet

Sixteen-month-old Fisher 344 rats were obtained from National Institutes on Aging colonies (Harlan). Rats were individually housed, maintained on a 12 hour light/dark cycle, and provided food and water ad libitum. Rats in the chow group (n=10) received normal chow (Harlan Teklad rodent diet 8604), while animals in the HF diet group (n=8) received a diet with
60% calories from fat. The composition of the HF diet has been described previously (26). During the seven weeks of the experiment, food intake was measured every 2-3 days. Body weight was measured weekly. Protocols for animal use were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee and adhered to the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Materials

Chemicals used in high pressure liquid chromatography (HPLC) (norepinephrine, DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and 3,4-Dihydroxybenzylamine) and D-(+)-Glucose were obtained from Sigma-Aldrich (St. Louis, MO). [14C]mannitol and 2-deoxy [1,2-3H] glucose were purchased from American Radiolabeled Chemicals (St. Louis, MO). Rat insulin ELISA kits were obtained from Alpco diagnostics (Salem, NH). Cholesterol E and LDL-C kits were purchased from Wako Diagnostics (Richmond, VA). Antibodies against actin were obtained from Abcam (Cambridge, MA). Anti-phospho-Hsp 27 and anti-Hsp 25 (the rodent homologue of Hsp27) were purchased from Stressgen (Victoria, BC, Canada), while IkBα antibody was purchased from Cell Signaling Technology (Beverly, MA).

Pre-lesion glucose tolerance analysis

Three days prior to surgery, animals were administered an intraperitoneal glucose tolerance test (IPGTT) as described previously (39). Animals were fasted overnight (~12 hours) at which time they were anesthetized with Nembutal (50mg/mL) at 1mL/kg body weight. To begin the test, an intraperitoneal injection of 60% of D-(+)-Glucose (Sigma) at 2g/kg body weight was given. Blood glucose was measured using a glucometer at 0, 15, 30, 60, 90, and 120 minutes following injection, and 400uL of tail blood was collected at each timepoint for measurement of serum insulin. Blood samples were placed on ice for 30 minutes and centrifuged for 1 hour at 3,000 × g and serum was aliquoted into fresh tubes for serum insulin analysis.

6-OHDA Infusion
Five weeks after beginning the diet, all animals received a unilateral 6-OHDA lesion in the medial forebrain bundle. This procedure was modified from previous studies published by our laboratory (39). Anesthesia was induced with 5% isoflurane. Anesthetized rats were placed in a stereotaxic frame and maintained at 3% isoflurane anesthesia during surgery. Animals were infused with 3μg 6-OHDA (4μL of 0.75 mg/mL 6-OHDA in 0.9% NaCl with 0.02% ascorbate) into the right medial forebrain bundle (stereotaxic coordinates with respect to bregma: M/L 1.3, A/P -4.4, and D/V -7.8). The infusion rate was 0.5μL/minute over a period of 8 minutes. The cannula was withdrawn 1 minute after infusion was completed. Animals were allowed to recover for two weeks post-surgery. After two weeks and prior to tissue harvest, animals were placed onto a force plate actometer (49) in a dark room for 30 minutes so that spontaneous locomotor activity could be measured.

**Glucose, insulin, and cholesterol measures**

Prior to sacrifice following an overnight (12 hour) fast, animals were anesthetized with sodium pentobarbital (60 mg/kg). Tail blood was harvested for fasting blood glucose and insulin measurements at the end of the experiment. Glucose measurements were made using a glucometer, while serum samples were collected as previously described (39) and analyzed using a rat insulin ELISA (Alpco). Serum samples were also analyzed for total cholesterol levels using a Cholesterol E kit (Wako diagnostics) and low density lipoprotein cholesterol (LDL-C) levels using an L-type LDL-C kit (Wako diagnostics). HOMA-IR was calculated for rats as described previously (12) using fasting glucose and fasting insulin values. This method has been validated in rodents and is consistent with other measures of insulin sensitivity (12, 53).

**Epidydmal fat and gastrocnemius muscle atrophy measures**

To measure fat accumulation, epidydymal fat was dissected and weighed. In addition, to analyze if any muscle atrophy occurred due to disuse of the contralateral muscle post-lesion, gastrocnemius muscles were carefully dissected tip to tip and weighed.

**Muscle incubations**
Soleus and extensor digitorum longus (EDL) muscles both ipsilateral and contralateral to the lesioned hemisphere were quickly dissected from anesthetized animals. Glucose transport was measured as previously described by our laboratory (26, 27) Each muscle was cut in half horizontally to avoid diffusion limitation and allow assessment of both basal and insulin-stimulated glucose transport. Both halves were placed into 2mL of Recovery buffer (8mM glucose, 32mM mannitol, and 0.01% BSA in KHB) for 30 minutes at 35°C. One muscle half (insulin-stimulated) was incubated in 8mM glucose, 32mM mannitol, 1mU/mL insulin, 0.01% BSA in KHB for 1 hour at 35°C while the other muscle half (basal) was retained in recovery buffer. Each muscle half was then rinsed in 40mM mannitol and 0.01% BSA in KHB for 10 minutes at 29°C. Finally, the insulin stimulated muscle half was placed into 4mM 2-[1,2-\textsuperscript{3}H]deoxyglucose (2-DG) (1.5 µCi/ml), 36mM, [\textsuperscript{14}C]mannitol (0.2 µCi/ml), 0.01%BSA in KHB containing 1mU/mL insulin at 29°C. The other half was placed into the same buffer without insulin. After 20 minutes both muscles were immediately removed, trimmed, and clamp-frozen. A gas phase of 95%O\textsubscript{2}/5%CO\textsubscript{2} was maintained during all incubations. Muscles were processed as previously described (56), and analyzed in a scintillation counter to determine intracellular 2-DG content (\textsuperscript{3}H dpm) and extracellular space (\textsuperscript{14}C dpm).

**HPLC-EC analysis of Whole Tissue Dopamine Content**

Following muscle harvest, brains were immediately removed and placed on an ice-cold brain block. Striatum and SN samples were dissected from each hemisphere, weighed, and frozen on dry ice to be processed for HPLC-EC and Western blot analysis. For HPLC-EC analysis, burnt citrate acetate mobile phase was added to samples from each tissue. For striatum tissue, 450μL of burnt mobile phase and 50μL DHBA (1e-6M) was added to each sample. Because the size of the SN is smaller than the striatum, SN samples were diluted in 250μL of burnt mobile phase and 50μL DHBA (1e-7M) for maximum analyte detection. Samples were then prepared and analyzed by HPLC as described previously (39).

**Western Immunoblotting**
For protein extraction, frozen pellets from SN samples that were processed for HPLC were diluted 15x in cell extraction buffer (Invitrogen) with protease inhibitor cocktail (500μL, Invitrogen), sodium fluoride (200mM), sodium orthovanadate (200mM) and phenylmethanesulphonylfluoride (200mM) added. Samples were placed on ice for 1 hour and vortexed every 15 minutes to allow for protein extraction. Samples were centrifuged at 3,000 x g at 4°C for 20 minutes before supernatants were collected. Protein concentration was determined using a Bradford assay. Bradford dye concentrate was diluted 5x in water to obtain working reagent and used to analyze samples in triplicate. Samples were diluted with HES buffer (20mM HEPES, 1mM EDTA, 250M Sucrose, pH 7.4) and reducing sample buffer (0.3M Tris-HCL, 5% SDS, 50% glycerol, 100mM dithiothreitol, Thermo Scientific) based on protein concentration to generate samples of the same concentration for analysis using SDS-PAGE.

Samples were run on 10% SDS-PAGE gels and transferred to nitrocellulose membranes at 200mA for 60 minutes. After membranes were blocked for 1 hour in 5% milk, they were incubated overnight with primary antibody at 4°C (1:1,000 dilution in 1% BSA). Secondary antibody was used at a dilution of 1:10,000 in 1% milk for 1 hour at RT and corresponded to the host primary antibody of interest. Films were scanned at high resolution and densitometry measurements were analyzed using Image J software. Repeated measurements were taken for each band of interest. Protein content was normalized to the loading control actin.

Statistical Analyses

Data for body weight and food intake were analyzed using two-way analyses of variance (ANOVA) with diet as the grouping variable and time as the repeated measure. Glucose transport and DA turnover were analyzed using one-way ANOVA with diet and side (ipsilesional vs contralesional) as grouping variables. Correlations were assessed using Pearson's method. All other data were analyzed using one-way ANOVA with diet as the grouping variable. Data were considered statistically significant at p≤ 0.05.
Results

Body weight and food intake

Analysis of body weight (Fig. 1A) yielded significant main effects for both group (F=7.91, p=0.01) and time (F=25.5, p<0.0001), as well as a significant interaction effect (F=16.57, p<0.0001). As expected, HF-fed animals gained more weight than chow-fed animals, and both groups lost weight post-lesion during the recovery period. Statistical analysis of food intake (Fig 1B) revealed significant main effects for group (F=16.15, p<0.001) and time (F=14.43, p<0.001), and a significant interaction effect (F=10.51, p<0.001, Fig. 1B). Initially, animals in the HF diet group consumed far more calories than chow-fed animals, although this difference became less pronounced after several weeks of feeding. Food intake dropped in both groups post-lesion but rebounded during the recovery period, and was virtually the same between groups post-lesion.

Systemic effects of HF feeding

An intraperitoneal glucose tolerance test (IPGTT) was performed on a subgroup of rats after 5 weeks of feeding, prior to the 6-OHDA lesion. Glucose measurements revealed a significant effect of group (F=4.84, p=0.04) and time (F=22.27, p<0.001; Fig. 2A). Glucose levels increased in response to the glucose bolus and HF-fed animals exhibited higher glucose values over the course of the test than chow fed animals. Over the course of the test, serum insulin measurements (Fig. 2B) revealed a strong trend for a difference between group (p=0.07), a significant effect of time (F=22.3, p<0.0001), and a significant interaction effect of group and time (F=2.73, p=0.03). Serum insulin levels increased in both groups in response to insulin, but remained high in HF-fed animals well after insulin levels had returned to normal in the chow-fed group at the end of the IPGTT. Analysis of serum for total cholesterol and LDL-C levels revealed no statistical difference between groups (Table 2).

When spontaneous locomotor activity was assessed 4 days prior to tissue harvest, total distance traveled did not differ significantly between the two groups (Table 2). This indicates no difference in activity level between groups. Fasting blood glucose and serum insulin were
measured on the last day of the experiment prior to tissue harvest and values are given in Table 2. HF diet-fed animals exhibited significantly higher fasting glucose levels compared to chow animals. (F=12.0, p=0.003). HF animals also exhibited a non-significant (p=0.07) trend toward greater fasting serum insulin levels compared to the chow group. To determine whether the lesion caused peripheral muscle atrophy, gastrocnemius muscles were dissected bilaterally tip-to-tip and weighed. Gastrocnemius muscle weights did not differ significantly between the ipsilesional and contralesional sides, nor did they differ significantly between the two groups (Table 2).

**Glucose uptake**

The majority of glucose uptake in the body occurs in skeletal muscle. Thus, to analyze the degree of insulin resistance, we assayed 2-deoxyglucose uptake into two different skeletal muscles: the EDL (fast-twitch glycolytic fiber type) and soleus (slow-twitch oxidative fiber type). Because there was no difference in glucose transport between muscles ipsilateral or contralateral to the lesion, muscles for each rat were pooled for these analyses. In the EDL, insulin exposure affected the diet groups differently: glucose uptake was greatly increased in chow-fed animals in response to insulin (Fig. 3A), but only a slight increase from basal was observed in HF animals. This led to a significant effect of insulin (F=18.4, p=0.0001) and a significant interaction between group and insulin (F=7.25, p=0.01). The decrease in insulin action due to HF feeding is characteristic of insulin resistance. Basal glucose uptake did not differ significantly between groups. In the soleus muscle, insulin significantly increased glucose uptake from basal in both groups (F=24.7, p<0.0001). No significant difference was observed for either basal or insulin stimulated glucose uptake between groups in the soleus (Fig. 3B).

**DA depletion**

In the SN, DA depletion was 48.8 ± 7.2% in the HF group compared to 27.8 ± 6.1% in the chow-fed group (Fig. 4A), a difference that was statistically significant (F=4.96, p=0.04). Likewise, striatal DA depletion was also significantly higher in the HF diet group (F=4.986,
p=0.04), averaging 28.3 ± 4.6% for chow animals and 49.0 ± 8.3% for HF animals (Fig. 4B).

Average analyte values for DA and DOPAC in each region are provided in Table 1.

**DA depletion and systemic effects of HF feeding**

When HOMA-IR was analyzed to take into account both fasting glucose and fasting insulin values (12, 53), HF animals exhibited values that were significantly greater than chow-fed animals (F=4.23, p=0.05), indicating decreased insulin sensitivity (Fig. 5A). Interestingly, we observed a significant positive correlation between this index of insulin resistance and DA depletion levels in both the SN (p=0.03, Fig. 5B) and striatum (p=0.02, Fig. 5C). Epidydymal fat mass was measured to determine differences in body fat composition and overall adiposity. As expected, HF rats exhibited far greater fat mass than chow fed rats (F=38.56, p<0.001); Fig. 5D). A significant positive correlation also existed between fat mass and DA depletion in the SN (p=0.04, Fig. 5E) and striatum (p=0.01, Fig. 5F).

**Dopamine turnover**

To estimate whether diet-induced differences in depletion levels affected DA metabolism, we analyzed the ratio of DOPAC to DA (a measure of DA turnover). In the SN of HF animals, DA turnover was increased in the lesioned hemisphere compared to the nonlesioned hemisphere, while chow animals exhibited a decrease in the lesioned hemisphere (Fig. 6A). This led to a significant main effect for group (F=10.9, p=0.002) and a significant interaction between group and hemisphere (F=9.97, p=0.004). In the striatum, there were no significant effects for hemisphere or group with regard to DA turnover (Fig 6B).

**Protein effects in the substantia nigra**

We measured activation of heat shock protein 27 (Hsp27) and protein levels of (IkBα) to indirectly assess oxidative stress in the SN. Hsp27 is activated by phosphorylation, and phosphorylated Hsp27 was significantly decreased in the HF-diet group compared to chow-fed controls (F=8.01, p=0.01). The stress kinase IKKβ is activated by cellular stress, such as
oxidative stress and insulin resistance (24, 57) and degrades the protein inhibitor I kappa B alpha (IkBα) when active. Thus, protein levels of IkBα can be used to gauge stress kinase activity (28). We observed a strong trend for decreased IkBα protein levels in HF fed rats compared to chow rats (F=4.02. p=0.056), indicating increased stress kinase activity.

**Discussion**

We report novel data here that HF-fed, insulin resistant rats exhibited enhanced nigrostriatal DA depletion following 6-OHDA. These changes occurred in the absence of altered cholesterol levels, diminished locomotor activity or muscle atrophy. Our results support an exacerbating role for dietary fat, and consequent insulin resistance, in vulnerability to toxin-induced nigrostriatal DA depletion. If 6-OHDA is produced endogenously even in small amounts, the increased vulnerability of DA neurons in response to HF diet-induced insulin resistance could put the nigrostriatal pathway at greater risk of damage during chronic HF feeding.

HF feeding in rodents has been previously characterized by our laboratory to cause weight gain, impaired insulin signaling, and glucose intolerance (26). In the current study, an IPGTT indicated that HF-fed animals were insulin resistant prior to 6-OHDA administration. Skeletal muscle accounts for the vast majority of glucose uptake in the body (18), and muscle glucose uptake decreases as a result of insulin resistance. Thus, to further analyze the extent of insulin resistance in these animals, glucose uptake was measured in two muscles: the EDL (fast-twitch glycolytic) and soleus (slow-twitch oxidative) muscles. In the EDL, insulin action was impaired with HF feeding: insulin exposure elicited a much greater increase in glucose transport in chow animals compared to HF-fed rats. However, in the soleus, insulin-stimulated glucose transport did not differ significantly between the two groups. This may be due to different metabolic adaptations to a HF diet between muscle types (27), or different levels of heat shock proteins, which protect tissues against oxidative stress (25).
The fact that HF-fed animals exhibited significantly greater DA depletion than chow-fed animals in both the SN and the striatum after 6-OHDA treatment supports a relationship between insulin resistance and PD. Neurons in the SN exist under a high oxidative load due to DA metabolism. Both enzymatic and non-enzymatic DA metabolism generates reactive oxygen species (37), and in this manner DA can cause both intracellular and extracellular damage to local neurons (55). The SN also has a very high iron content (48), which may further exacerbate oxidative damage by reacting with byproducts of DA metabolism to generate highly reactive radicals (16). Although the nature of DA neuron degeneration in PD is unclear, it is likely that reactive oxygen species play a role in early disease progression (reviewed in (33). It is possible that the HF diet may exacerbate further DA depletion in this region because it is already highly vulnerable to damage. Because neurons in the SN project to the striatum and release DA, it follows that DA depletion would be evident in this tissue as well.

Interestingly, DA depletion in both the SN and striatum correlated significantly with HOMA-IR values. HOMA-IR is a widely used assessment of beta cell function and insulin resistance that accounts for both fasting glucose and fasting insulin levels (53). As expected, HF-fed animals exhibited significantly higher epididymal fat weight compared to the chow group. HF feeding and increased adiposity have been shown to increase levels of free fatty acids (14), which are known to contribute to insulin resistance in skeletal muscle (8). Like HOMA-IR, epididymal fat content also correlated significantly with nigral and striatal DA depletion.

It is possible that increased oxidative stress contributes to SN vulnerability in the HF diet group. As an indirect measure of oxidative stress occurring in the SN, we measured activation (phosphorylation) of Hsp27 and protein levels of IkBα. We chose to analyze Hsp27 because, when active, it can protect against oxidative stress and mitochondrial complex I damage (19, 20), which occurs in PD (5). Hsp27 also binds and sequesters the stress kinase IkKβ, in turn decreasing its activity (42). We observed a significant decrease in protective Hsp27 activity (pHsp27) in the SN of HF-fed rats, indicating an impaired stress response in this group.
Because the stress kinase IkKβ degrades the downstream protein IkBα, analysis of IkBα levels is used as an indicator of IkKβ activity (26, 28). The HF group exhibited a strong trend for decreased IkBα protein levels, likely due to increased activity of IkKβ.

Altered DA turnover (indicated by the ratio of DOPAC to DA in whole tissue) is a measure of DA metabolism (2) and reflects a functional response to nigrostriatal degeneration. In the SN, DA turnover was greater in HF-fed animals compared to the chow fed animals. Previous studies have shown that DA turnover is dependent on the extent of DA depletion, with greater DA depletion resulting in increased turnover [42, 43]. In our study, DA turnover was significantly increased in the lesioned compared to nonlesioned SN in HF rats, the group that exhibited nearly 50% DA depletion. Increased DA turnover could be a compensatory mechanism for maintenance of normal synaptic DA levels following DA neuron loss (59), and in a progressive MPTP model, DA turnover increases with greater DA depletion (6). However, the chow group in our study actually exhibited decreased DA turnover in the lesioned vs. nonlesioned SN. It is possible that, with much lower (~30%) DA depletion, decreased turnover could also be a compensatory effect to keep existing DA in the synapse for an extended time period. It is clear that the low depletion level in chow animals was not sufficient to trigger an increase in DA turnover, as occurred in the HF group. In addition, there was no significant effect of group or hemisphere for this measure in the striatum. This suggests either that alterations occurring “upstream” in the SN occur first, or that the partial DA depletions in the chow-fed rats did not reach the threshold necessary to produce these compensatory effects in the terminal region.

Although our results support an exacerbating role for insulin resistance on toxin-induced DA depletion, other effects of a HF diet may also contribute. Increased inflammatory signaling, adipokine levels, oxidative or nitrostatative stress, mitochondrial dysfunction, and lipid metabolism have all been shown to occur with HF feeding (13, 27), reviewed in (52). Some of these
Peripheral effects, such as oxidative stress, also occur in the brain following HF feeding (21, 40, 58) and HF feeding increases cognitive impairment, tau deposition, MPTP vulnerability, and inflammation in the brain (15, 38, 44, 45). Although specific contributions of other HF diet effects cannot be ruled out, our observation of increased markers of insulin resistance in the absence of increased total cholesterol or LDL-C levels and a positive correlation between HOMA-IR and DA depletion support a role for insulin resistance in mediating increased toxin-induced DA depletion.

Perspectives and Significance

Our results suggest that a HF diet can increase 6-OHDA-induced DA depletion in the nigrostriatal pathway. This study supports the findings of a previous study reporting enhanced MPTP toxin-induced nigrostriatal DA depletion in mice following a HF diet (15) and extends these findings to a different model and species. Our novel findings regarding 6-OHDA are particularly of interest in light of the fact that 6-OHDA is likely produced endogenously by DA metabolism. These findings, which support a “multiple hit” hypothesis regarding insulin resistance and neurotoxin exposure in vulnerability to PD, warrant further investigation into the mechanisms by which DA depletion is increased by a HF diet and insulin resistance.
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Figure legends

Figure 1. Food intake and body weight. HF feeding affected body weight (A), and food intake (B). HF-fed animals weighed more than chow-fed rats, although food intake in the HF group was increased only initially and was affected to a greater extent by the lesion. Values are means ± SE for 8-10 rats per group. *p<0.05 Chow vs. HF.

Figure 2. Intraperitoneal glucose tolerance test (IPGTT). After an overnight (12 hour) fast, an intraperitoneal injection of 60% glucose was administered at 2g glucose/kg body weight. Insulin (A) and glucose (B) were measured in tail blood at six time points: 0, 15, 45, 60, 90, and 120 minutes after the glucose bolus (injection at t=0). The HF group exhibited significantly higher glucose values 90 and 120 minutes following the bolus. Serum insulin levels were significantly higher at the fasting (0) time point and 120 minutes post-bolus. *P≤0.05.

Figure 3. HF diet affects glucose transport in skeletal muscle. Glucose transport was measured to further characterize the effect of diet on peripheral insulin sensitivity. 2-deoxyglucose uptake was determined in EDL (A) and soleus (B) muscles incubated in the absence (black bars) or presence (gray bars) of 2mU/mL insulin. Insulin significantly increased glucose uptake in both the EDL and soleus muscles of all rats. In the EDL muscle, insulin stimulated glucose transport in HF rats was less than controls, indicating insulin resistance. Values are means ± SE for 10-12 muscles per group. *p<0.05 Basal vs. Insulin, #p<0.05 interaction effect between group and insulin.

Figure 4. Effects of HF diet and 6-OHDA on tissue DA content. DA content in the right (lesioned) striatum was divided by DA levels in left (nonlesioned) striatum to obtain a percent depletion for each rat. Percent depletion was calculated in two different tissues: the SN (A), and striatum (B). HF-fed animals exhibited significantly greater DA depletion than controls in both tissues. Values are means ± SE for 8-10 samples per group. *p<0.05 Chow vs. HF.

Figure 5. Systemic effects of HF feeding correlate with DA depletion. HF-fed rats exhibited a higher HOMA-IR value compared to chow fed rats (A), indicating impaired insulin sensitivity.
In all animals, there was a significant positive correlation between HOMA-IR and DA depletion levels in SN (B) and striatum (C). The HF diet group also exhibited higher epidydymal fat weight as expected (D). Like HOMA-IR, epidydymal fat also correlated significantly with DA depletion in both tissues (E, F). Values are means ± SE 8-10 rats per group. *p<0.05 Chow vs. HF.

**Figure 6. Effects of HF diet and 6-OHDA on DOPAC/DA ratio.** The ratio of DOPAC divided by DA was calculated as an estimate of DA turnover in the SN (A) and the striatum (B). In the SN, DA turnover was significantly increased in the lesioned hemisphere of HF-fed animals, but was decreased in the lesioned hemisphere of chow-fed animals. No difference between groups was observed in the striatum. Values are means ± SE for 8-10 samples per group. *p<0.05 chow vs. HF, #p<0.05 interaction effect between group and hemisphere.

**Figure 7. HF feeding decreases heat shock protein activation and IkBα protein levels.** Activation of Hsp25 (A) and IkBα expression (B) were analyzed in the SN. There was significantly less activation of Hsp25 in HF-fed animals compared to the chow group. The HF group also exhibited a strong trend (p=0.056) for a decrease in IkBα protein levels compared to chow rats. No difference between hemispheres was observed for either protein measure. Values are means ± SE for 5-7 samples per group. *p<0.05 Chow vs. HF.
Table 1

Mean (+/- S.E.M.) striatal DA and DOPAC values for experimental groups (ng/g)

<table>
<thead>
<tr>
<th>Region</th>
<th>Metabolite</th>
<th>Chow Nonlesion</th>
<th>Chow Lesion</th>
<th>High Fat Nonlesion</th>
<th>High Fat Lesion</th>
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<tbody>
<tr>
<td>Striatum</td>
<td>DA</td>
<td>8485 ± 2683</td>
<td>6026 ± 1905</td>
<td>11329 ± 3776</td>
<td>5918 ± 1972</td>
</tr>
<tr>
<td></td>
<td>DOPAC</td>
<td>1557 ± 492</td>
<td>1141 ± 360</td>
<td>1952 ± 650</td>
<td>1105 ± 368</td>
</tr>
<tr>
<td>Substantia</td>
<td>DA</td>
<td>629 ± 198</td>
<td>404 ± 127</td>
<td>647 ± 228</td>
<td>322 ± 113</td>
</tr>
<tr>
<td>Nigra</td>
<td>DOPAC</td>
<td>198 ± 62</td>
<td>88 ± 28</td>
<td>210 ± 74</td>
<td>163 ± 57</td>
</tr>
</tbody>
</table>
Table 2

Characteristics of Chow- and HF-fed rats

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>High Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>115 ± 2.4</td>
<td>127 ± 1.8</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>0.675 ± 0.204</td>
<td>1.26 ± 0.420</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>93.3 ± 4.77</td>
<td>90.0 ± 4.13</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>36.8 ± 2.2</td>
<td>30.2 ± 2.3</td>
</tr>
<tr>
<td>Distance Traveled (m)</td>
<td>44.28 ±3.6</td>
<td>45.27 ± 4.0</td>
</tr>
<tr>
<td>Ipsilateral Gastrocnemius wt (mg)</td>
<td>1955 ± 54.2</td>
<td>2005 ± 72.8</td>
</tr>
<tr>
<td>Contralateral Gastrocnemius wt (mg)</td>
<td>2045 ± 51.9</td>
<td>2017 ± 63.9</td>
</tr>
</tbody>
</table>
Figure 1

A. 

B. 

Lesion

Initial 1 2 3 4 5 6 7

Time (week)

Body Weight (g)

Food Intake (kcal / day)

Chow

High Fat

Lesion

Chow

High Fat
Figure 2

A.

B.

Glucose (mg/dL) vs. Time (min)

Insulin (ng/mL) vs. Time (min)

- Chow
- High Fat

* indicates statistical significance.
Figure 3
A. EDL Muscle

B. Soleus Muscle

Glucose Uptake (EDL) μMol / mL / 20min

Chow          High Fat

Glucose Uptake (Soleus) μMol / mL / 20min

Chow          High Fat

Basal

Insulin

Basal

Insulin

#  *

*  *

*  *
Figure 4

A. Substantia Nigra

B. Striatum

Percent DA depletion

- Chow
- High Fat

* Indicates significant difference.
Figure 5

A. HOMA-IR

B. Percent DA depletion (SN)

C. Percent DA depletion (STR)

D. Epidydimal Fat (mg)

E. Percent DA depletion (SN)

F. Percent DA depletion (STR)

Chow   High Fat

R = 0.532  p = 0.03
R = 0.552  p = 0.02
R = 0.482  p = 0.04
R = 0.612  p = 0.02
Figure 6

A. Substantia Nigra

B. Striatum

Chow          High Fat Chow          High Fat

DOPAC / DA

Nonlesion

Lesion

Nonlesion

Lesion
Figure 7

A.

Chow    High Fat

pHsp27 - 25 kD
Hsp27 - 25 kD

B.

Chow    High Fat

IkBα - 43 kD
Actin - 42 kD

pHsp27 - 25 kD
Hsp27 - 25 kD

*p = 0.056

pHsp27 / Hsp27

IkBα / Actin

Contra Ipsi    Contra Ipsi

Chow    High Fat

Contra Ipsi    Contra Ipsi

Chow    High Fat

Contra Ipsi    Contra Ipsi

Chow    High Fat