The emergence of insulin resistance following a chronic high-fat diet regimen coincides with an increase in the reinforcing effects of nicotine in a sex-dependent manner

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ABSTRACT

The present study assessed the sex-dependent effects of insulin resistance on the reinforcing effects of nicotine. Female and male rats received a chronic high-fat diet (HFD) or regular diet (RD) for 8 weeks. A subset of rats then received vehicle or a dose of streptozotocin (STZ; 25 mg/kg) that induces insulin resistance. To assess insulin resistance, glucose levels were measured 15, 30, 60, 120, and 180 min after an insulin injection (0.75 U/kg). Nine days later, the rats were given extended access to intravenous self-administration (IVSA) of nicotine (0.015, 0.03, 0.06 mg/kg) in an operant box where they consumed their respective diet ad libitum and performed responses for water deliveries. Each nicotine dose was delivered for 4 days with 3 intermittent days of abstinence in their home cage. The day after the last IVSA session, physical signs were compared following administration of mecamylamine (3.0 mg/kg) to precipitate nicotine withdrawal. The results revealed that there were no changes in insulin resistance or nicotine intake in HFD alone rats regardless of sex. Insulin resistance was observed in HFD-fed rats that received STZ, and the magnitude of this effect was greater in males versus females. Our major finding was that nicotine intake was greater among HFD + STZ female rats as compared to males. Lastly, the physical signs of withdrawal were similar across all groups. Our results suggest that females diagnosed with disorders that disrupt insulin signaling, such as diabetes may be at risk of greater vulnerability to nicotine use due to enhanced reinforcing effects of this drug.

1. Introduction

Individuals that develop diabetes are more prone to tobacco use and have a harder time quitting smoking as compared to their non-diabetic counterparts (Bishop et al., 2009; Willi et al., 2007; Will et al., 2001). It is generally believed that diabetic and/or obese individuals use tobacco products as a tool to reduce appetite (Chen et al., 2012) and control weight gain (White et al., 2007, 2012). Unfortunately, the combination of diabetes, obesity, and tobacco use results in compounded health outcomes, including increased rates of cardiovascular disease, cancer, and stroke (Al-Delaimy et al., 2002; Lycett et al., 2015; Śliwińska-Mossoń and Milnerowicz, 2017; Winhusen et al., 2019). Some recent clinical reports have also indicated that tobacco use may increase the likelihood of developing insulin resistance associated with Type 2 diabetes (Brath et al., 2019; Maddatu et al., 2017). To understand the cross vulnerabilities between diabetes and tobacco use, basic science approaches are needed to understand the underlying mechanisms by which nicotine use is enhanced in persons with diabetes.

With regard to sex differences, accumulating evidence suggests that tobacco use is greater in diabetic patients that are female. For example, tobacco use rates are four times higher among women diagnosed with diabetes as compared to non-diabetic women (Radzeviciene and Ostrauskas, 2018). Additionally, the latter report revealed that smoking cessation rates are at least 6 times lower in diabetic versus non-diabetic women. Subsequent reports have suggested that the lower quit rates among women with diabetes may be related to a greater fear of weight gain and stronger symptoms of anxiety and depression during abstinence as compared to men with diabetes (Al-Delaimy et al., 2002).
Although these reports suggest that nicotine use is greater among women with diabetes, the role of insulin in enhancing vulnerability to nicotine use in women has not been elucidated.

Pre-clinical rodent studies have used a chronic high-fat diet (HFD) regimen to study obesity and induce insulin resistance (Johnson et al., 2019; Richardson et al., 2014). To induce insulin resistance more rapidly and reliably, some laboratories use a combination of chronic HFD feeding with administration of a low dose of streptozotocin (STZ), a drug that destroys the insulin producing β cells of the pancreas (Mansor et al., 2013). This regimen has been shown to reliably produce insulin resistance, as evidenced by an increase in blood glucose, hepatic triglycerides, and plasma lipid levels as early as 21 days after initiating a HFD regimen and administration of STZ (Barrière et al., 2018; Mugalian et al., 2019; Mansor et al., 2013). Thus, the HFD + STZ model in rodents mimics the trajectory of Type 2 diabetes in humans that results from chronic HFD feeding and disruptions in pancreatic β-cell function and insulin resistance.

Prior work has revealed that hypoinsulinemia produced by STZ administration enhances intravenous self-administration (IVSA; Cruz et al., 2019; O’Dell et al., 2014) and conditioned place preference (CPP; Pipkin et al., 2017; Ibias et al., 2018) produced by nicotine. Other studies have shown that CPP produced by nicotine is only observed among insulin resistant rats that received a chronic HFD regimen (Richardson et al., 2014). The present study expands prior work by comparing sex differences in nicotine IVSA following a treatment regimen that induces insulin resistance. We utilized a model involving extended access to IVSA of escalating doses of nicotine separated by 3-day periods of drug abstinence. Concomitant changes in food and water intake and changes in body weight were also assessed during the IVSA sessions. At the end of the nicotine IVSA regimen, physical signs of withdrawal were assessed across treatment groups. We hypothesized that insulin resistance would enhance the reinforcing effects of nicotine, and that this effect would be greater in female versus male rats. This is based on previous work showing that disruptions in insulin signaling increase the reinforcing effects of nicotine in male rats (Cruz et al., 2019). Also, the reinforcing effects of nicotine are greater among healthy female rats as compared to males as assessed in IVSA procedures (Flores et al., 2016, 2019).

2. Materials and methods

2.1. Subjects

Female and male Wistar rats (N = 65 total) were bred from an outbred stock of animals (Envigo Inc, USA). All rats were housed in a humidity- and temperature-controlled vivarium using a 12 h light/dark cycle with lights off at 6:00 p.m. Rats had ad libitum access to a regular diet (RD) of standard laboratory chow or a HFD regimen. All rats were handled for 3–5 days prior to the start of the experiment. The animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals. Our procedures were approved by the Institutional Animal Care and Use Committee at The University of Texas at El Paso.

2.2. Experimental design

The present study compared nicotine IVSA following a RD or a HFD regimen for 8 weeks in female (RD + Vehicle n = 7; HFD + Vehicle n = 6) and male (RD + Vehicle n = 9; HFD + Vehicle n = 7) rats. Separate groups then received administration of a low dose of STZ (25 mg/kg, SC) in female (RD + STZ n = 9; HFD + STZ n = 5) and male (RD + STZ n = 7; HFD + STZ n = 6) rats. Three days later, the rats were tested for insulin resistance, as described below. Food intake, water responses, and body weight were recorded during nicotine IVSA. At the end of IVSA, physical signs of withdrawal were assessed. The inset below depicts our experimental timeline.

2.3. Drugs

(−) Nicotine hydrogen tartrate salt and mecamylamine were obtained from the NIDA Drug Supply Program (Research Triangle, Bethesda, MD). Nicotine and mecamylamine were dissolved in 0.9% sterile saline (pH 7.4). STZ was purchased from Millipore Sigma (St. Louis, MO) and was dissolved in a heparin solution (0.1 M Na citrate, pH 6.0). Fresh solutions of STZ were delivered subcutaneously within 15 min of preparation for each animal. Brevidal® sodium was purchased from Henry Schein (Melville, NY) and was dissolved in 0.9% sterile saline (pH 7.4). Cefazolin® antibiotic was purchased from Millipore Sigma (St. Louis, MO) and was dissolved in a heparin solution (Henry Schein, Melville, NY). Insulin was obtained from Humulin® (Indianapolis, IN) and was dissolved in 0.9% sterile saline (pH 7.4).

2.4. Feeding regimen and insulin resistance

The feeding regimen consisted of either a RD (3.1 kcal/g, 17% kcal from fat) or a HFD (5.1 kcal/g, 60% kcal from fat) purchased from Envigo Teklad (Madison, WI, Catalogue number: TD.06414). Rats were given ad libitum of their respective diet throughout the entire experimental timeline. The food was stored at 4 °C and replenished each day at 11 a.m. when the food and the body weights were recorded. On Day 57 of the feeding regimen, the rats received administration of STZ (25 mg/kg, SC). Three days later, the rats were tested for insulin resistance following a 16-h food deprivation period. The rats received a challenge injection of insulin (0.75 U/kg; IP) and glucose levels were assessed during baseline and 15, 30, 60, 120, and 180 min after insulin administration. Fasting glucose levels were assessed using a glucose meter calibrated for rodent blood with a lancet to prick the tip of the tail to extract a small drop of blood that was placed on test strips that were purchased from AlphaTRAK® (Abbott Park, IL). Their respective food regimen was replaced immediately after the insulin resistance test.

2.5. IVSA

The day after the insulin resistance test, the rats were placed into operant chambers where they were given access to a feeding cup that contained their respective diet. The rats were allowed to perform nose-poke responses for water on a fixed ratio-1 schedule of reinforcement to
obtain administration of 0.1 mL aliquots of water into an adjacent metal dipper cup. All rats reached stable levels of water responding prior to catheter surgery on Day 65 of the feeding regimen.

The rats were anesthetized using an isoflurane/oxygen vapor mixture (1–3%) and were then prepared with IV catheters into the jugular vein, as previously described (Cruz et al., 2019). After surgery, the rats recovered during Days 65–69 in their home cage with access to their respective food regimen and water ad libitum. The catheters were flushed daily (0.2 mLs) with an antibiotic solution containing Cefazolin® and heparin (30 USP units/mL). On Days 70–85, the rats were given extended (23-h) access to nicotine IVSA using an escalating dose regimen. The rats were allowed to respond for increasing doses of nicotine (0.015 then 0.03 and then 0.06 mg/kg/0.1 mLs/infusion). Each dose was available for 4 consecutive days with 3 intervening days of forced abstinence in their home cage prior to receiving access to the next higher dose of nicotine. The forced abstinence period was used to assess the motivational effects of nicotine during withdrawal (e.g., after nicotine access was discontinued for 3 days). During IVSA, each response on the active lever delivered a 1-s infusion of nicotine followed by a 20-s timeout period where responses were recorded but had no consequences. The chambers also contained an inactive lever where responses were recorded but had no scheduled consequences. The nicotine solutions were prepared fresh every day and were adjusted to the rats’ body weight from the previous day. Our IVSA regimen was based on previous work in our laboratory using 23-h access to nicotine IVSA in female and male rats (Flores et al., 2016; Uribe et al., 2019). At the end of IVSA testing, catheter patency was verified using a 0.1 mL IV infusion of the short-acting barbiturate Brevital® sodium (10 mg/mL). Three rats were eliminated from the study due to catheter malfunctions.

2.6. Physical signs of withdrawal

Physical signs were compared across groups the day after the last IVSA session on Day 86. The rats were given systemic administration of the nicotinic receptor antagonist, mecamylamine to precipitate withdrawal (3.0 mg/kg, SC). A nicotine naïve control group of female (n = 4) and male (n = 5) rats were included that received mecamylamine alone. The occurrence of the following signs was recorded for 10 min: eye blinks, body shakes, gasps, writhes, headshakes, ptosis, and teeth clattering. Multiple successive counts of any sign required a distinct pause between episodes. If present continuously, ptosis was counted only once each min during the observation period. The observer was blind to the rats’ treatment condition.

2.7. Statistics

Glucose levels were analyzed using a 3-way mixed-model repeated measure analysis of variance (ANOVA) with diet (RD versus HFD) and sex (female versus male) as between subject factors and time (min) as a within subject factor. Food intake was adjusted to reflect differences in caloric value of the RD (g × 3.1) versus HFD (g × 5.1). Body weight was adjusted to reflect differences in percentage change (end value - initial value ÷ initial value × 100 percent) relative to the first day of nicotine IVSA. Nicotine, food, and water intake as well as weight change were analyzed in a similar manner but with nicotine dose as the within subject factor. Physical signs were analyzed using a 2-way ANOVA with diet and sex as between subject factors. In instances where interaction effects were observed, post hoc analyses compared between- and within-subject differences using protected Fisher’s least significant difference test (p < 0.05). All statistical analyses were performed on SPSS version 26, and all the graphs were generated using GraphPad Prism version 8.

3. Results

3.1. Glucose levels

Fig. 1 depicts glucose levels (mean ± SEM) expressed in mg/dL in female and male rats. The top panels reflect RD + Vehicle and HFD + Vehicle rats and the bottom panels (shaded in grey) reflect RD + STZ or HFD + STZ rats. Our analysis of the data in the top panels revealed that there was no interaction between diet, sex, and time [F(1,125)=1.23, p=0.29]. Also, there were no main effects of diet [F(1,25)=2.17, p=0.15] or sex [F(1,25)=2.44, p=0.13]. Our analysis of the data in the bottom panels revealed a significant interaction between diet, sex, and time [F(3,115)=2.48, p=0.03]. Our analysis also revealed significant main effects of diet [F(1,23)=19.16, p=0.0001] and sex [F(1,23)=15.57,
Glucose levels were higher at the 30- and 60-min time points for female HFD + STZ rats as compared to RD + STZ females (*p ≤ 0.05). Glucose levels were also higher for male HFD + STZ rats during baseline and the 15-, 30-, 60-, 120-, and 180-min time points as compared to RD + STZ males (*p ≤ 0.05). Regarding sex differences, glucose levels were higher during baseline and the 15-, 30-, 60-, and 180-min time points for male versus female HFD + STZ rats (*p ≤ 0.05).

### 3.2. Nicotine intake

Fig. 2 depicts active lever presses (mean ± SEM) in female and male rats. The top panels reflect RD + Vehicle and HFD + Vehicle rats and the bottom panels (shaded in grey) reflect RD + STZ or HFD + STZ rats. Our analysis of the data in the top panels revealed that there was no interaction between diet, sex, and nicotine dose \[F_{(2,50)}=1.18, p=0.31\]. Although there was no main effect of diet \[F_{(1,25)}=1.08, p=0.30\], we did observe a main effect of sex \[F_{(1,25)}=5.30, p=0.030\].

Overall, there was more active lever responding among female rats as compared to males (p ≤ 0.05). Our analysis of the data in the bottom panels revealed a significant interaction between diet, sex, and nicotine dose \[F_{(2,46)}=3.44, p=0.04\]. We observed a main effect of diet \[F_{(1,23)}=22.26, p=0.001\], but not sex \[F_{(1,23)}=3.65, p=0.06\].

Table 1

<table>
<thead>
<tr>
<th>Diet</th>
<th>Sex</th>
<th>Intake</th>
<th>Active</th>
<th>Inactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>RD + Vehicle</td>
<td>Female</td>
<td>1.34 ± 0.2</td>
<td>40.4 ± 6.4*</td>
<td>19.0 ± 5.8</td>
</tr>
<tr>
<td>HFD + Vehicle</td>
<td>Female</td>
<td>1.56 ± 0.1</td>
<td>50.0 ± 5.4*</td>
<td>16.1 ± 2.5</td>
</tr>
<tr>
<td>RD + Vehicle</td>
<td>Male</td>
<td>1.04 ± 0.1</td>
<td>31.6 ± 4.6*</td>
<td>15.2 ± 2.2</td>
</tr>
<tr>
<td>HFD + Vehicle</td>
<td>Male</td>
<td>1.19 ± 0.2</td>
<td>33.8 ± 5.4#</td>
<td>8.0 ± 1.5</td>
</tr>
<tr>
<td>RD + STZ</td>
<td>Female</td>
<td>1.46 ± 0.2</td>
<td>46.8 ± 6.2#</td>
<td>29.5 ± 6.7</td>
</tr>
<tr>
<td>HFD + STZ</td>
<td>Female</td>
<td>2.95 ± 0.4#</td>
<td>104.1 ± 13.0#</td>
<td>37.1 ± 6.3</td>
</tr>
<tr>
<td>RD + STZ</td>
<td>Male</td>
<td>1.40 ± 0.3</td>
<td>44.7 ± 9.6</td>
<td>28.8 ± 3.9</td>
</tr>
<tr>
<td>HFD + STZ</td>
<td>Male</td>
<td>1.99 ± 0.1</td>
<td>71.9 ± 7.5#</td>
<td>31.2 ± 5.5</td>
</tr>
</tbody>
</table>

*indicates different from controls (p ≤ 0.05).
|indicates different from male HFD + STZ rats (p ≤ 0.05).
#indicates different from inactive responses (p ≤ 0.05).

were more active lever responses among female HFD + STZ rats during IVSA of the 0.015 and 0.03 mg/kg dose of nicotine as compared to RD + STZ rats (*p ≤ 0.05). There were also more active lever responses among male HFD + STZ rats during IVSA of the 0.015 mg/kg dose of nicotine as compared to RD + STZ rats (*p ≤ 0.05). Regarding sex differences, there were more active lever responses made by female HFD + STZ rats during IVSA of the 0.015 and 0.03 mg/kg dose of nicotine as compared to male HFD + STZ rats (*p ≤ 0.05).

Table 1 includes our measures of total nicotine intake and active versus inactive lever responses across groups. The top rows include rats that received a RD or HFD regimen and the bottom rows (shaded in grey) display rats that also received STZ administration. Our analysis of total nicotine intake in the top rows revealed that there was no interaction between diet, sex, and nicotine dose \[F_{(2,50)}=0.95, p=0.39\]. There was also no main effect of diet \[F_{(1,25)}=0.78, p=0.38\] or sex \[F_{(1,25)}=2.95, p=0.10\]. Our analysis of the data in the bottom rows revealed that there was no interaction between diet, sex, and nicotine dose \[F_{(2,46)}=1.36, p=0.26\]. However, we did observe an interaction between diet and sex \[F_{(1,23)}=4.05, p=0.05\] and a main effect of diet \[F_{(1,23)}=10.31, p=0.01\] and sex \[F_{(1,23)}=5.24, p=0.03\]. Subsequent analyses revealed significantly higher levels of nicotine intake among female HFD + STZ rats as compared to RD + STZ rats (*p ≤ 0.05). Nicotine intake levels were also higher in female HFD + STZ rats as compared to their male counterparts (p ≤ 0.05). Our analysis of lever discrimination revealed that the rats displayed more active versus inactive presses across all groups, except for the male RD + STZ rats (paired sample t-test, *p ≤ 0.05).

### 3.3. Caloric intake

Fig. 3 depicts caloric intake (mean ± SEM) expressed in kcal/g in female and male rats. The top panels reflect RD + Vehicle and HFD + Vehicle rats and the bottom panels (shaded in grey) reflect RD + STZ or HFD + STZ rats. Our analysis of the data in the top panels revealed that there was no interaction between diet, sex, and nicotine dose \[F_{(2,50)}=0.07, p=0.92\]. There was a main effect of diet \[F_{(1,25)}=35.73, p=0.0001\] and sex \[F_{(1,25)}=55.90, p=0.0001\]. Specifically, caloric intake was greater for HFD + Vehicle versus RD + Vehicle rats (*p ≤
Also, females displayed lower caloric intake as compared to males ($p \leq 0.05$). Our analysis of the data in bottom panels revealed that there was no interaction between diet, sex, and dose ($F_{(2,46)}=1.04, p=0.36$). Although we did not observe a main effect of sex ($F_{(1,23)}=2.69, p=0.11$), there was a significant main effect of diet ($F_{(1,23)}=10.76, p=0.000$). Specifically, caloric intake was greater in HFD + STZ versus RD + STZ rats ($*p \leq 0.05$).

### 3.4. Water intake

Fig. 4 depicts water responses (mean ± SEM) in female and male rats. The top panels reflect RD + Vehicle and HFD + Vehicle rats and the bottom panels (shaded in grey) display rats that received a RD or a HFD in combination with STZ administration. The dagger denotes ($) a sex difference and the asterisk ($*$) denotes a difference from controls ($p \leq 0.05$).
Our analysis of the data in the bottom panels revealed a significant interaction between diet, sex, and dose \(F_{(2,46)}=4.52, p=0.01\). We also observed a main effect of sex \(F_{(1,23)}=22.97, p=0.0001\), but no main effect of diet \(F_{(1,23)}=3.98, p=0.058\). In females, there were no differences in water intake between HFD + STZ versus RD + STZ rats \(p \geq 0.05\). In males, water intake was higher in HFD + STZ rats during IVSA of the 0.03 and 0.06 mg/kg doses of nicotine as compared to RD + STZ rats \((p \leq 0.05)\). Regarding sex differences, water intake was higher in females during IVSA of the 0.03 mg/kg dose of nicotine as compared to RD + STZ rats \(p \leq 0.05\).
intake was greater in male HFD + STZ rats during IVSA of the 0.03 and 0.06 mg/kg doses of nicotine as compared to female HFD + STZ rats (*p ≤ 0.05).

3.5. Body weight

Fig. 5 depicts percent change in body weight (mean ± SEM) in female and male rats. The top panels reflect RD + Vehicle and HFD + Vehicle rats and the bottom panels (shaded in grey) reflect RD + STZ or HFD + STZ rats. Our analysis of the data in the top panels revealed that there was no interaction between diet, sex, and dose [F(2,50) = 1.93, p = 0.15]. There was no main effect of diet [F(1,25) = 0.17, p = 0.68] or sex [F(1,25) = 1.01, p = 0.32]. Our analysis of the data in the bottom panels revealed that there was no interaction between diet, sex, and dose [F(2,46) = 0.10, p = 0.90] and no main effect of sex [F(1,23) = 0.14, p = 0.70]. We did observe a significant interaction between diet and dose [F(2,46) = 15.91, p = 0.0001] and a main effect of diet [F(1,23) = 24.28, p = 0.001]. Female HFD + STZ rats weighed less during IVSA of the 0.03 and 0.06 mg/kg doses of nicotine as compared to female RD + STZ rats (*p ≤ 0.05). Male HFD + STZ rats weighed less during IVSA of the 0.03 and 0.06 mg/kg dose of nicotine as compared to male RD + STZ rats (*p ≤ 0.05).

3.6. Physical signs of withdrawal

Fig. 6 depicts total physical signs (mean ± SEM) in female and male rats. The top panels reflect RD + Vehicle and HFD + Vehicle rats and the bottom panels (shaded in grey) reflect RD + STZ or HFD + STZ rats. Our analysis of the data in the bottom panels revealed that there was no interaction between diet and sex [F(1,22) = 0.82, p = 0.37]. There were also no main effects of diet [F(1,22) = 1.87, p = 0.18] or sex [F(1,22) = 0.67, p = 0.42]. Our analysis also revealed that there was no interaction between diet and sex [F(1,23) = 3.99, p = 0.06]. There were also no main effects of diet [F(1,23) = 0.03, p = 0.86] or sex [F(1,23) = 0.17, p = 0.68]. To examine whether nicotine withdrawal was elicited, we compared physical signs between naïve rats and animals that received nicotine IVSA. Our results revealed that regardless of diet or sex, rats that received nicotine IVSA displayed greater physical signs of nicotine withdrawal as compared to naive controls (*p ≤ 0.05).

4. Discussion

In summary, the HFD regimen alone did not alter glucose levels, nicotine intake, physical signs, or weight change. Our findings with nicotine intake are consistent with a prior report showing that HFD-fed mice displayed similar nicotine CPP as compared to standard-chow controls (Blendy et al., 2005). In the present study, the combination of a chronic HFD with STZ produced an increase in food and water intake and a decrease in body weight. This regimen also produced insulin resistance that was greater in males versus females, consistent with prior reports (Alexander et al., 2007). Our major finding was that female HFD + STZ rats displayed greater nicotine intake than male rats. Interestingly, insulin resistance did not alter the physical signs of nicotine withdrawal in female or male rats in the present study. This finding contrasts a prior report from our laboratory showing that hypoinsulinemic male rats display greater physical signs of nicotine withdrawal versus healthy controls (Pipkin et al., 2017). The discrepancy in these reports may be due to a higher degree of hyperglycemia in the Pipkin et al. study that used a higher dose of STZ (45 mg/kg) than the present study (25 mg/kg).

A major finding of this study was that insulin resistance produced by HFD + STZ enhanced the reinforcing effects of nicotine. This is consistent with prior work in our laboratory demonstrating that a depletion of insulin via administration of a high dose of STZ (45 mg/kg) produced a dose-dependent increase in nicotine IVSA (O’Dell et al., 2014) and CPP produced by nicotine (Ibias et al., 2018; Pipkin et al., 2017). More recent studies have shown that the ability of STZ to increase nicotine IVSA is restored to healthy control levels in STZ-treated rats following insulin supplementation (Cruz et al., 2019). Together, these studies suggest that a disruption in insulin signaling alters biological/brain systems in a manner that enhances the reinforcing effects of nicotine.

With regard to sex differences, the present study revealed that nicotine intake was greater among females that received the HFD + STZ regimen as compared to males. This pattern of sex differences is consistent with a meta-analysis indicating that female rats generally self-administer more nicotine than males (see Flores et al., 2019). The latter effect may be modulated via estradiol (E2), as ovariectomized females display a reduction in nicotine IVSA that is returned to control levels following E2 supplementation (Flores et al., 2016). Thus, E2 may be a critical factor in mediating the strong reinforcing effects of nicotine observed in female versus male HFD + STZ rats. It is important to note that a previous report found that while nicotine CPP was increased in STZ-treated rats, this effect was greater in male versus female rats (Ibias et al., 2018). This contrasts our finding that HFD + STZ enhanced nicotine IVSA to a greater extent in female versus male rats. This discrepancy may be due to differences in the subjective effects of nicotine in behavioral procedures involving classical conditioning versus operant behavior and/or differences in the level of hyperglycemia. The possibility exists that the reinforcing effects of nicotine are more sensitive to insulin and glucose fluctuations in female versus male rats.

Recent work in our laboratory has revealed that insulin systems modulate the rewarding effects of nicotine via the mesolimbic dopamine pathway, which originates in the ventral tegmental area (VTA) and terminates in several forebrain structures including the nucleus accumbens (NAC; Koob, 1999; Mansvelder et al., 2003). Our prior work revealed that STZ administration reduced nicotine-induced dopamine release in the NAc as compared to healthy male rats. The latter effect was modulated via an increase in gamma-aminobutyric acid (GABA) inhibition and a decrease in glutamatergic excitation of dopamine cell bodies in the VTA (Cruz et al., 2020). The latter study also revealed that the neurochemical effects of STZ were reversed to control levels following insulin supplementation. These studies, along with the present findings, suggest that a disruption in insulin signaling increases nicotine reward processing, likely via control of dopamine release in the mesolimbic pathway. Indeed, it has been previously hypothesized that the high propensity of tobacco use among patients with diabetes is modulated via reduced dopamine transmission in the mesolimbic pathway (see O’Dell and Nazarian, 2016). Taken together, these findings suggest that the emergence of insulin resistance following a chronic HFD may disrupt dopamine systems to a greater extent in females versus males.

There are potential issues to be considered with the present work. The combination of STZ and HFD may have produced side effects that impacted operant responses in the present study. Indeed, one might wonder whether insulin resistant rats self-administer nicotine to alleviate neuropathic pain, particularly during withdrawal. However, we submit that the alleviation of pain does not entirely explain our results because nicotine-induced analgesia is observed following repeated administration of a bolus injection of nicotine (5 injections of 1 mg/kg; Cepeda-Benito et al., 2005). We recognize the possibility that hyperalgesia may have differed across our groups and our maximal level of nicotine intake in HFD + STZ rats (2.9 mg/kg; see Table 1) falls in an analgesic dose range. However, these values reflect intake over a 23-h period and our IVSA doses (0.015–0.06 mg/kg/0.1 mL infusion) are lower than those reported to induce analgesia in rats. Also, prior reports have shown that the side effects of STZ, such as neuropathic pain and catacaract formation, emerge 4 weeks after administration of higher STZ doses than were used here (45 versus 25 mg/kg; Morrow, 2004; Wei et al., 2003). Another consideration relates to group differences in nicotine metabolism, particularly given that persons with Type 2 diabetes metabolize nicotine faster (42% higher) than non-diabetic smokers (Keith et al., 2019). We have addressed this issue in prior studies that have shown that hypoinsulinemic rats that received a high dose of STZ...


