Published in final edited form as:

Neuroscience. 2016 June 21; 326: 170–179. doi:10.1016/j.neuroscience.2016.04.002.

Removal of high fat diet after chronic exposure drives binge behavior and dopaminergic dysregulation in female mice

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Abstract

A significant contributor to the obesity epidemic is the overconsumption of highly palatable, energy dense foods. Chronic intake of palatable foods is associated with neuroadaptations within the mesocorticolimbic dopamine system adaptations which may lead to behavioral changes, such as overconsumption or bingeing. We examined behavioral and molecular outcomes in mice that were given chronic exposure to a high fat diet (HFD; 12 weeks), with the onset of the diet either in adolescence or adulthood. To examine whether observed effects could be reversed upon removal of the HFD, animals were also studied 4 weeks after a return to chow feeding. Most notably, female mice, particularly those exposed to HFD starting in adolescence, demonstrated the emergence of binge-like behavior when given restricted access to a palatable food. Further, changes in dopamine-related gene expression and dopamine content in the prefrontal cortex were observed. Some of these HFD-drive phenotypes reversed upon removal of the diet, whereas others were initiated by removal of the diet. These findings have implications for obesity management and interventions, as both pharmacological and behavioral therapies are often combined with dietary interventions (e.g., reduction in calorie dense foods).

Keywords

dopamine; high fat diet; sex difference; reward; binge; adolescence

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1. Introduction

Multiple factors interact to drive obesity risk, including physical activity, genetics, stress and food consumption, specifically the overconsumption of readily available, energy dense, palatable foods (Marcus & Wildes, 2014). Palatable foods are rewarding and acute consumption leads to the release of the neurotransmitter dopamine (DA) (Geiger et al., 2009). Animal models of diet-induced obesity (DIO) involving the chronic consumption of palatable foods have documented DA adaptations in response to chronic consumption of a high fat diet (HFD). Unlimited access to HFD causes lower basal extracellular dopamine levels in the nucleus accumbens (NAc) and the ventral tegmental area (VTA) (Geiger et al., 2007; Geiger et al., 2009; Cone et al., 2010; Rada et al., 2010). DIO models also demonstrate lower DA turnover (Davis et al., 2008), decreased DA release (York et al., 2010), and reduced DA clearance (Speed et al., 2011) in the NAc. Dopamine-related molecules, including tyrosine hydroxylase, the DA transporter, and the DA receptors DR1 and DR2, are all decreased in DIO animal models at the level of mRNA (Alsiö et al., 2010, Huang et al., 2005, Carlin et al., 2013) and protein (Johnson & Kenny, 2010, Huang et al., 2006, South & Huang, 2008). However, nearly all of the published rodent studies have been conducted exclusively in adult male rodents, leaving open the question of whether these effects extend more broadly to females and/or other developmental ages. This is significant as women exhibit higher rates of obesity, including as adolescents (Wang & Beydoun, 2007), highlighting the importance of studying females' DA response to palatable foods.

Important developmental differences in response to HFD consumption have also been observed. The majority of DIO rodent studies begin exposure to the diet in adulthood (Baladi & France., 2009, Kanoski & Davidson, 2010; Lassiter et al., 2010), and exposures during earlier developmental times are relatively understudied. During critical periods of development, such as lactation through adolescence, the brain is particularly sensitive to environmental changes (King, 2006). For example, high fat feeding was sufficient to disrupt prepulse inhibition (PPI) when given during the peripubertal period, yet high fat feeding during adulthood failed to affect PPI (Labouesse et al., 2013). Similarly, HFD consumption during adolescence, but not in adulthood, disrupts hippocampal neurogenesis and memory (Boitard et al., 2012), a pattern which is also observed in response to high fructose corn syrup consumption (e.g., memory impairment and hippocampal inflammation with adolescent exposure, but not adult exposure, (Hsu et al., 2014)). However, the impact of adolescent HFD exposure on the DA system is unknown.

Reduced levels of DA in the synapse or reduced DA signaling, as suggested by the current literature, could subsequently reduce the sensitivity of the animal to natural rewards. Alterations in dopaminergic circuitry after high fat intake have been associated with decreases in sucrose preference (Carlin et al., 2013, Vucetic et al., 2012), a decrease in operant responding for a sucrose pellet (Davis et al., 2008), and a decrease response to food reward (Corwin et al., 2011, Cottone et al., 2008, Johnson and Kenny, 2010). These adaptations can lead to an increase in compulsive behaviors, including the overconsumption of food or drugs of abuse (Verbeken et al., 2012, Volkow & Li, 2004). Binge eating involves uncontrolled food intake, typically of highly palatable food, and is a component of eating disorders such as bulimia nervosa and binge eating disorder. Binge eating affects both sexes

but is more common in women (Hudson et al., 2007), a finding replicated in animal models, with binge eating behavior seen more frequently in female rats than in males across a number of different experimental paradigms (Klump et al., 2013). Consistent with its role in hedonic feeding, dopamine dysregulation has been linked to binge eating. For example, dopamine neurons in the VTA were shown to be activated by binge-like intake of high fat diet in mice (Valdivia et al., 2015), while in human patients with binge-eating disorder (BED), food image-evoked dopamine levels in the caudate were shown to be increased only in BED patients, and correlated with binge eating scores (Wang et al., 2011). Additional studies in both rodents and humans have been reviewed (Broft et al., 2011, Naef et al., 2015, van Gestel et al., 2014). Therefore, we examined whether the age of onset of the HFD consumption or sex could influence the development of binge behavior.

A final important question is whether these dopamine-related neuroadaptations persist upon the removal of the HFD. Diet reversal studies involve a return to normal chow after an extended period of HFD consumption. Following relatively short periods of diet reversal (1–2 weeks), reports have found a persistence of HFD-driven neuroadaptations (Alsio et al., 2010, Johnson & Kenny 2010, South & Huang, 2008). However, a number of recent reports have found a normalization of diverse HFD-driven phenotypes, including hypothalamic inflammation (Berkseth et al., 2014), memory impairments and IL-1 β levels in hippocampus (Sobesky et al., 2014), and dopamine-related changes (Carlin et al., 2013) using a longer reversal period (4 weeks). The following experiments were designed to test the hypothesis that beginning a high fat diet earlier in life would result in greater and more lasting changes in the dopamine reward system. Understanding which neurobiological changes reverse or persist after HFD-removal may inform the high failure rates of human dieting and relapse to unhealthy eating habits.

2. Materials and Methods

2.1 Animals and experimental model

C57BL/6J females were bred with DBA/2J males (The Jackson Laboratory, Bar Harbor, ME) and were fed control diet (Test Diet, Richmond, IN #5755; 18.5% protein, 12% fat, 69.5% carbohydrate) throughout pregnancy and lactation. Offspring were weaned at 3wks of age and a third of the animals were placed on high fat diet (Test Diet, #58G9; 18% protein, 60% fat, and 20.5% carbohydrate). Another third of the animals were placed on high fat diet at 6wks of age. The final third were kept on standard chow. Animals remained on the high fat diet for 12 weeks. In an additional cohort of animals, after 12 weeks *ad lib* access to the high fat diet, the diet was returned to control chow for 4 weeks. Separate cohorts of animals were used for the behavioral studies and the molecular studies, and animals were group housed (5 mice/cage), except where noted for behavioral experiments. Body weights were recorded weekly, and both male (n=5–10/experiment) and female (n=5–10/experiment) mice were used for the behavioral experiments. Given that binge behavior was only observed in females, experiments to measure dopamine and dopamine-related gene expression changes were conducted in female mice. The Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania approved all procedures.

2.2 Fat pad Weights

Animals (n=5–10/group) were euthanized with an overdose of carbon dioxide, followed by cervical dislocation; a method recommended by the Panel on Euthanasia of the American Veterinary Medical Association. Body weights were taken and abdominal, gonadal, and inguinal fat pads were removed and weighed separately. Fat pad mass was normalized to body weight.

2.3 Sucrose Preference

After 12 week exposure to HFD or HFD + 4 weeks chow, mice were individually housed in standard cages for 3 days with *ad lib* access to food. In a two bottle choice task, one bottle was filled with 200 ml of 4% sucrose solution (w/v), another with 200 ml of tap water. Sucrose (ml), water (ml), and food consumption (g), were measured daily. Preference was calculated using the measurements from the last 2 days only as follows: preference % = [(sucrose consumption/sucrose + water consumption) × 100] (n=8–10/group).

2.4 One-Hour Palatable Food Intake

After 12–14 weeks on the HFD or control diet, mice were individually housed (n=11–12/group) in standard cages for eight days and maintained on their respective *ad libitum* diets (chow for all groups except the HFD-fed animals). Each day, home cage food intake and body weight were measured. Animals were given access to a bowl with either palatable high fat/high sugar food (Reese's Peanut Butter chips) or control diet for one hour. Daily caloric intake of *ad libitum* chow, palatable food, and percent calories/day were calculated and averaged across the final 6 days of the test, to account for acclimation during the first 2 days. Because animals consuming the HFD may respond differently to the palatable food presentation (solely because of the presence of the calorie-dense HFD), a separate cohort of animals was acutely placed on *ad libitum* control chow during the experiment. This group is labeled HFD + acute chow and compared to HFD and HFD + 4 week chow groups.

2.5 Total RNA isolation from the brain

Animals (n=5/group) were euthanized and brains were rapidly removed and placed in RNAlater (Ambion, Austin, TX) for 4–6 hours before dissection. Brain dissections to isolate the prefrontal cortex, the nucleus accumbens and the ventral tegmental area were performed as previously described (Reyes et al. 2003, Vucetic et al. 2010). Total RNA was isolated using AllPrep DNA/RNA Mini Kit (Qiagen).

2.6 Gene expression analysis by quantitative Real-Time PCR

For each individual sample, 500ng of total RNA was used in reverse transcription using High Capacity Reverse Transcription Kit (ABI, Foster City, CA). Expression of target genes was determined by quantitative RT-PCR using gene specific Taqman Probes with Taqman gene expression Master Mix (ABI) on the ABI7900HT Real-Time PCR Cycler. Gene probes are listed in supplemental material. Relative amount of each transcript was determined using delta CT values as previously described in (Pfaffl, 2001). Changes in gene expression were calculated against an unchanged GAPDH standard.

2.7 Ex vivo Dopamine Measurement by HPLC

Tissue samples were homogenized in 0.1 N perchloric acid with 100 μ M EDTA (15 μ l/mg of tissue) using a handheld homogenizer. Samples were centrifuged at 15,000 rpm for 15 min at 2–8 °C. The supernatant were analyzed the Bioanalytical Systems HPLC (West Lafayette, IN, USA) with a PM-80 pump, a Sample Sentinel autosampler, and an LC-4C electrochemical detector. Samples (12 μ l) were run through a reverse phase microbore column at a flow rate of 0.6 ml/min and electro detection at +0.6 V. Separation for dopamine was accomplished by a mobile phase consisting of 90mM sodium acetate, 35mM citric acid, 0.34mM ethylenediamine tetraacetic acid, 1.2mM sodium octyl sulfate, and 15% methanol v/v at a pH of 4.2 (Mayorga et al., 2001; Vucetic et al., 2010). High performance liquid chromatography was used to measure dopamine content in the PFC and NAc (n=8–12). Brains were collected from animals and cut into right and left hemispheres. NAc and PFC were dissected and quickly frozen by dry ice and stored at –80°C. Samples were analyzed by the Bioanalytical Systems HPLC (West Lafayette, IN, USA) with a PM-80 pump, a Sample Sentinel autosampler, and an LC-4C electrochemical detector. Samples (12 μ l) were run through a reverse phase microbore column at a flow rate of 0.6 ml/min and electro detection at +0.6 V. Separation for dopamine and dopamine metabolites was accomplished by a mobile phase consisting of 90mM sodium acetate, 35mM citric acid, 0.34mM ethylenediamine tetraacetic acid, 1.2mM sodium octyl sulfate, and 15% methanol v/v at a pH of 4.2 (Mayorga et al., 2001; Vucetic et al., 2010).

2.8 Statistical Analysis

Because the gene expression data, body weights, and fat pad weights were collected at different ages for the different groups (e.g., adolescent onset HFD: 3 wks age + 12 wks HFD = 15 wks of age; adolescent onset HFD + 4 wk chow: 3 wks age + 12 wks HFD + 4 wks chow = 19 weeks of age), these data were analyzed using Student's t-test comparing each experimental group to aged matched controls. The alpha level was adjusted for the multiple brain regions surveyed using a Bonferroni correction (e.g. significance of a gene used in one brain region was $p=.05$; for two regions, $p=0.025$, for 3 brain regions $p=.016$). Sucrose preference, palatable food intake, HPLC measurements, food intake, were analyzed using oneway ANOVA to compare control, HFD, and HFD + chow groups. Bonferroni–corrected posthoc comparisons were used and significance was set at an alpha level of $p=.05$. All data were normally distributed, as confirmed by Shapiro-Wilk normality test (when $n>6$) or KS normality test (when $n \leq 6$) completed using Prism 6.0.

3. Results

3.1 Body and Fat pad weights

Animals that started the high fat diet at weaning are labeled “Adolescent-onset HFD”, while animals that started the high fat diet at 6 weeks of age are labeled “Adult-onset HFD.” Within 6 weeks on HF diet, animals reached significantly higher body weight than controls and remained significantly heavier than controls after a 4-week chow period in both Adolescent-onset HFD (Figure 1A, $F(18,100)=110.4$, $p<0.0001$) and Adult-onset HFD males (Fig 1A $F(20,108)=97.60$, $p<0.0001$). Adolescent-onset HFD females gained weight at a faster rate than Adult-onset HF females but both Adolescent-onset HFD and Adult-onset

HFD females were significantly heavier than their controls after 12 weeks on high fat diet (Figure 1B $F(17,95)=56.57$, $p<0.0001$; $F(20,115)=34.89$, $p<0.0001$). As in males, 4-weeks on standard chow was not sufficient to fully normalize body weights in either HFD-exposed female group. Figures 2A, 2B show the terminal body weights of HFD-fed animals, along with the direct age-matched controls. HFD-fed animals, with or without the 4 week chow exposure, weighed more than their age-matched controls in all groups; male Adolescent-onset HFD (Figure 2A (left), $t_{(13)}=7.464$, $p<0.0001$; $t_{(17)}=4.581$, $p=.0003$), male Adult-onset HFD (Figure 2A (right), $t_{(14)}=8.131$, $p<0.0001$, $t_{(14)}=3.234$, $p<0.006$), female Adolescent-onset HFD (Figure 2B (left), $t_{(27)}=8.235$, $p<0.0001$; $t_{(28)}=8.267$, $p<0.0001$) and female Adult-onset HFD (Figure 2B (right), $t_{(27)}=4.598$, $p<0.0001$; $t_{(27)}=4.748$, $p<0.0001$). Differences in abdominal fat pad mass followed a pattern similar to body weight (no differences were observed between groups for gonadal or inguinal fat pad weight, data not shown). Fat pad mass was significantly greater in the HFD-fed animals compared to their age-matched controls; males (Figure 2C, $t_{(20)}=17.19$, $p<0.0001$; $t_{(17)}=15.83$, $p<0.0001$) and females (Figure 2D, $t_{(17)}=10.26$, $p<0.0001$; $t_{(15)}=9.416$, $p<0.0001$). Four weeks after removal of the HFD, fat pad mass decreased, but remained significantly greater than that of controls in all groups; male Adolescent-onset HFD and Adult-onset HFD groups (Figure 2C, $t_{(15)}=2.263$, $p<0.0389$; $t_{(11)}=5.609$, $p=0.0002$) and female Adolescent-onset HFD and Adult-onset HFD groups (Figure 2D, $t_{(14)}=3.171$, $p<0.0068$; $t_{(17)}=3.460$, $p=0.003$).

3.2 Sucrose Preference

Sucrose preference was used to assess the animals' response to a natural and nutritive rewarding stimulus. All animals preferred the 4% sucrose solution to water (preference > 50%, Fig 3A, 3B). In males, sucrose preference was significantly reduced after 12 weeks HFD diet in the Adolescent-onset HFD animals (Figure 3A, $F(2,17)=17.58$, $p<0.0001$), yet unchanged in Adult-onset HFD animals. Sucrose preference was decreased in both female groups (Figure 3B, Adolescent-onset HFD: $F(2,16)=12.07$, $p<0.0006$) and adult: ($F(2,15)=7.497$, $p<0.0055$) after 12 wks high fat diet. Preference in all groups where sucrose preference was decreased returned to control levels after a 4 week return to standard chow feeding.

3.3 One-Hour Palatable Food Intake

For eight days, animals were given one-hour access to high fat/high sugar food (peanut butter chips) to assess their response to restricted access to palatable food. In Adolescent-onset HFD males, one-way ANOVA revealed a significant difference across groups (Figure 4A, $F(3,31)=15.94$, $p<0.0001$), such that chronic HFD reduced the intake of palatable food, and removal of the HFD, either during the 8 days of testing or for 4 weeks, normalized this intake to control levels. Adult-onset HFD males showed an identical pattern (Figure 4A, $F(3,28)=12.52$, $p<0.0001$), with a significant reduction in 1hr palatable food intake in animals consuming the HFD, which normalized with removal of the HFD. Intake of the animals' *ad libitum* diet was largely similar to the intake patterns of the PB chips (Figure 4C). Males on the HFD had a decrease in 24-hr caloric intake, that largely normalized with removal of the HFD (adolescent onset: $F(3,32)=5.41$, $p<0.005$; adult onset: $F(3, 32)=10.82$, $p<0.0001$).

Palatable food intake also differed across the female groups. In the adult-onset HFD females, again, palatable food intake was decreased while animals were on the HFD, and this normalized in the 4 week HFD removal groups (Figure 4B, $F(3,31)=8.68$, $p<0.0005$). However, a different pattern was observed in the adolescent-onset female group (Figure 4B, $F(3,32)=12.06$, $p<0.001$). These animals tended to reduce their 1hr palatable food intake while on HF diet, although this difference was not statistically reliable given the greater variance in the control group. However, after removal of the HFD for 4 weeks, adolescent-onset HFD females consumed significantly *greater* amounts of the palatable food as compared to controls. Interestingly, in females, the intake pattern of the *ad lib* diet was somewhat different than what was observed in males (Fig 4D). In females, intake of the *ad lib* diet significantly decreased when HFD was acutely removed (as opposed to males when intake was decreased *on* the HFD), and returned to control levels of intake after 4 weeks on chow (adolescent onset: $F(3,32)=15.11$, $p<0.0001$; adult onset: $F(3, 31)=4.72$, $p<.01$).

A binge episode can be operationally defined as the consumption of greater than 25% of daily calories within the binge period (Halpern et al., 2013). Therefore, the amount of peanut butter chips eaten in 1hr as a percent of total daily calories was calculated. Data were analyzed using a t-test against a theoretical mean of 25. For males, none of the animals reached binge-levels of intake within the 1hr period of testing, regardless of experimental group (Fig 4E, all means were significantly $<25\%$). However, a markedly different pattern was observed in the females, with HFD removal emerging as a powerful stimulus to drive binge behavior. Adolescent-onset HFD females reached binge level intake (Fig 4F; 25% daily intake) after both acute ($t(8)=2.7$, $p=.03$) and chronic ($t(8)=1.23$, n.s. (not different from 25%)) removal of the HFD. For females in the adult-onset HFD exposure group, palatable food intake reached binge levels after chronic removal of the HFD ($t(8)=0.54$, n.s. (not different from 25%)).

3.4 Gene Expression

Given that only females showed binge behavior, mechanistic follow-up studies were next conducted in females. In mice naïve to behavioral testing, dopamine-related gene expression was examined within the reward circuitry. Table 1 summarizes in detail the statistical analysis and gene expression results in the VTA, NAc, and PFC of both female diet groups compared to their aged matched controls ($n=5/\text{group}$). Gene expression data is presented as fold change from the appropriate aged-matched controls for either the HFD or HFD+ 4 week chow cohorts. Only the adolescent-onset control group is shown in Fig 5 for clarity in presentation (as both control groups are set to 1). Key findings will be highlighted here. Significance was determined at a corrected alpha level of $p<(0.05/\# \text{ brain regions assayed})$.

In the adolescent exposed group, DAT expression was increased, while TH expression was decreased, and neither of these changes normalized after 4 weeks on standard chow. Adult-onset exposure was less potent as compared to adolescent-onset, as the increased DAT expression did not normalize after HF diet removal, and TH expression was not affected by diet (Fig 5A, 5B).

In the NAc (Fig 5C, 5D), the regulation of DRD1 and DRD2 are similar across the two age groups; DRD1 expression is decreased, but then rebounds/normalizes, while D2R expression

is also decreased, but does not normalize with removal of the HFD. Gene expression analysis in the PFC revealed further differences between the two age groups (Fig 5E, 5F). In general, adolescent exposure to the HFD decreased expression of D1R and D2R, however this response was absent in the adult exposed animals. In the adolescent onset group, the decreased expression of D1R and D2R normalized after removal of the HFD.

3.5 Dopamine levels

Given the changes in gene expression for dopamine (DA) regulating genes in the VTA, DA was quantified in regions that receive projections from the VTA: NAc and PFC (n=7–10/group). Figure 6 shows DA levels in the NAc and PFC of female mice. Dopamine levels were unchanged by diet in the NAc in either age group (Figure 6A). In the PFC, female animals exposed to HFD as adolescents or adults had increased DA levels that normalized after a 4wk on chow (Figure 6B, $F(2,26)=3.845$, $p < 0.05$, $F(2,23)=6.839$, $P < 0.01$).

4. Discussion

Three key findings emerge from the present work; (1) females exposed to HF diet are more likely to develop binge behavior than males, (2) dopamine dysregulation is apparent in the reward-circuitry of these females, and (3) removal of the HF diet and a return to chow can normalize some endpoints (sucrose preference, some gene expression, and dopamine levels in the PFC), while driving others (binge behavior and rebound mRNA levels of DRD1).

4.1 Sucrose preference

Sucrose preference is a broad measure of natural reward intake, and a decrease in sucrose consumption is considered an animal model of anhedonia, which is consistent with the hypo-reward responses often associated with obesity (e.g., obese patients show decreased activity in reward related regions when ingesting a palatable solution (Stice et al., 2010)). We found a decrease in sucrose preference in all females exposed to HFD and male mice exposed to HFD as adolescents, indicating that female sex and adolescent exposure to HFD are variables that can increase susceptibility to adverse effects of HFD on reward-related endpoints. We also observed normalization of sucrose preference after 4 weeks on standard chow, demonstrating that the decrease in sucrose preference was related to the presence of the HF diet and not obesity per se, as the behavior normalized with removal of the HFD when animals were still significantly heavier. Presence of the calorically-dense HFD may also have contributed to the reduced intake of sucrose, as HFD can alter satiety responses (e.g., by altering GLP-1 responses (Duca et al., 2015) or CART (Bharne et al., 2015)). Our data are consistent with findings in the OLETF CCK-1 knockout rats. These animals become obese on control chow and do not have a decreased preference for sucrose (Marco et al., 2012), providing additional support that a decrease in sucrose preference is linked to diet, rather than obesity.

Sex has previously been shown to be important in self-administration of other rewarding substances such as saccharin, cocaine and morphine (Carroll et al., 2002, Dess et al., 1998; Dess, 2000;) with females having a more rapid rate of acquisition and an increase in administration. The decrease in the value of natural rewards could lead to overconsumption

of rewarding food in order to increase total reward levels, and these data suggest that adult females, as compared to males, would be more susceptible to the effects of chronic high fat diet consumption. This conclusion is supported by the results from the palatable food intake experiments.

4.2 Binge behavior

To examine the risk of overconsumption of palatable food, mice were given daily 1-hour access to high fat/high sugar food for eight days. Intermittent access has been previously used to elicit binge eating in *ad lib* fed rodents (Corwin et al., 2004), which typically takes >4 weeks to develop. Therefore, the protocol used here can be considered sub-threshold, and allowed for the identification of the unique vulnerability of adolescent-onset females. In this group, removal of the HFD was a potent trigger for binge eating, greatly *increasing* the palatable food intake, such that binge levels of consumption were reached. In adult-onset females, a similar pattern was observed, with binge levels of consumption occurring after 4-weeks off the HFD. In males, the intake of both the palatable PB chips and the *ad lib* diet decreased in the presence of HFD, and largely returned to normal after removal of the diet, and in no condition did males reach binge levels of intake. Indeed, binge eating is more prevalent in females than in males (Kessler et al., 2013). Estrogen may play a role in this difference, as the estrogen metabolite 2-hydroxyestradiol has been found to increase binge eating (in rats) (Babbs et al., 2013). This study identifies the removal of the HFD as a potent trigger for overconsumption, particularly in females. Interestingly, the expression pattern for DRD1 in the PFC matched this pattern of binge behavior, with a significant increase in expression after removal of the HFD in both groups of females.

4.3 Dopaminergic gene expression

Additional support for the increased vulnerability of the adolescent exposed group is found in the gene expression results. For the VTA and the PFC, adolescent-onset exposure to high fat diet was more impactful than adult-onset exposure. In the adolescence-exposed group, HFD increased DAT and decreased TH in the VTA, and these changes persisted after removal of the HFD. In adults, only DAT was increased (no change in TH), and this normalized after removal of the HFD. Further, in the PFC, DRD1 and DRD2 mRNA was decreased in the adolescence- exposed animals, yet none of these genes were altered when the diet was started in adulthood.

The response pattern of the dopamine receptors in the NAc and PFC may be informative in understanding the observed binge behavior; (1) decreased DRD2 expression in the NAc and (2) increased DRD1 expression in PFC. In both age groups, exposure to HFD decreased DRD2 expression in the NAc, and this decrease did not normalize after 4 weeks on chow. Low dopamine receptor D2 levels are implicated in obesity, drug addiction and impulsive behaviors (Wang et al., 2001), and decreased DRD2 in reward regions has been shown to directly predict reward dysfunction and binge-like behavior (Johnson & Kenny, 2010). In the PFC, dopamine content was significantly increased while animals were on the HFD, and these levels normalized once animals were returned to chow feeding. However, after 4 weeks of chow feeding, DRD1 mRNA was significantly elevated in the PFC, to levels above the baseline controls. This “overshoot” of DRD1 mirrors the binge behavior seen in the females

(binge behavior emerging with the removal of the HFD) and may be in response to the decrease / normalization of dopamine levels in the PFC, however, this remains speculative until a causative relationship between dopamine levels and DRD1 levels can be directly tested. A better understanding of how the dopamine system responds to the *removal* of a highly palatable diet is clearly needed, particularly in an effort to better understand how repeated cycling between food restriction (dieting) and excessive intake of palatable food interact to affect dopaminergic activity in the brain. In future studies, it would be valuable to extend analyses to later time points, to examine whether behavioral and molecular changes that do not normalize with the removal of the high fat diet, may normalize once body weight and/or adiposity normalize. Extending the dopamine analyses to male animals is an important future direction as well.

4.5 Conclusions

In summary, we have shown that chronic HF diet leads to changes in palatable food intake, sucrose preference, and dopamine circuitry. It is possible to reverse some of these changes before body weight normalization with a 4 week standard chow replacement. Importantly, the age of onset of the HFD was a critical variable that affected both the response to the HFD as well as the reversal. In general, adolescent exposure led to more significant changes in response to the diet, and these changes were less readily normalized. It is possible that in these groups, reversal would occur at a later time point and that remains to be tested. Women, and adults who were obese as children, generally find it more difficult to lose weight as compared to the rest of the population. Changes in dopaminergic gene expression and/or function that persist during “dieting” may contribute to high failure rates of dieting seen in these populations. Understanding how age of onset and sex interact to affect dopamine function in response to HFD is an important step in better understanding the neural adaptations that impede or promote the development of healthy patterns of food intake.

Acknowledgments

Funding and Disclosure:

Work was supported by NIH grants TL1TR000138 (JLC), T32-GM008076 (JLC), and MH087978 (TMR). The authors have no financial interests or conflicts of interest to disclose. Experiments were designed and analyzed by JLC, IL and TMR; experiments were performed by JLC, SEM, THS, NMG, and RG, and the manuscript was written by JLC and TMR, and edited by SEM, NMG and IL.

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Highlights

- Removal of high fat diet after 12-week exposure drives binge behavior in female but not male mice
- Dopamine receptor type 1 (DRD1) expression levels in the prefrontal cortex may be related to binge-like behavior
- Some, but not all, behavioral and molecular responses to high fat diet exposure are reversed after high fat diet removal
- Adolescent versus adult onset of high fat diet consumption leads to more persistent behavioral and molecular changes

Figure 1. 12 week high fat diet increases body weight.

Body weights (in grams; $n=10/\text{group}$) were measured weekly in Adolescent-onset HFD (black circles) and Adult-onset HFD animals (gray circles) in males (A) and females (B). Dashed line depicts time on HFD diet; solid line depicts return to chow feeding. Control animals (black squares) remained on standard chow throughout the experiment.

Figure 2. 12 week high fat diet increases terminal body weight and fat pad weight.

Bar graph of terminal body weights of each HF group compared to their age-matched control; control (white bar), HFD (black bar), control + 4 weeks (light gray) and HF+ 4 week chow (dark gray) males (A) and females (B). Normalized Abdominal Fat Pad weights in males (C) and females (D) shown as percentage of final body weight, each group compared to their age-matched controls. Post hoc comparisons shown at alpha levels *p<.05, **p<.01, ***p<.001, ****p<.0001 significantly different from aged matched control group.

Figure 3. Chronic HFD affects sucrose preference.

Percent preference for 4% sucrose was evaluated in control (white bars) HFD (black bars) and HFD + 4 week chow (gray bars). Adolescent-onset HFD and Adult-onset HFD age groups ($n=8/\text{group}$), males (A) and females (B). Post hoc comparisons are shown at alpha levels * $p<.05$, ** $p<.01$, *** $p<.001$ significantly different from own control group.

Figure 4. Chronic HFD affects palatable food intake.

Average intake (g/BW) of PB chips during one-hour palatable food access was measured in control (white bars) HFD (black bars) and HFD + acute chow (1 week; light gray bars) and HFD + 4 week chow (dark gray bars) for Adolescent-onset HFD and Adult-onset HFD males (A) and females (B). Average 24-hr intake (kcal/BW) of *ad libitum* diet during palatable food access was also measured in males (C) and females (D). Percent of daily calories eaten during the 1 hr palatable food access was calculated for both male (E) and female (F) mice. Dotted line depicts 25%, the level at or above which is considered binge levels of intake. Post hoc planned comparisons shown at alpha levels * $p < .05$, ** $p < .01$, *** $p < .001$ significantly different from own control group. For E and F, * indicates $>25\%$, \$ indicates = 25%.

Figure 5. Chronic high-fat diet (HFD) alters dopamine-related gene expression in females. Gene expression was measured in the ventral tegmental area (VTA; A, B), nucleus accumbens (NAc; C, D), and prefrontal cortex (PFC; E, F) of control (set to 1; white bars), HFD (solid bars) and HFD + 4 week chow (gray bars) Adolescent-onset HF and Adult-onset HFD female age groups (n=5/group). HFD and HFD + 4 week chow groups are shown as fold change from their own aged matched controls. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to own aged matched control group.

Figure 6. Dopamine levels are increased after chronic HFD.

Dopamine (DA) was measured in the NAc (A) and PFC (B) of control (white bars), HFD (solid bars) and HFD + 4 week chow (gray bars) Adolescent-onset HFD and Adult-onset HFD age groups (n=8–10/group). *p<0.05, significantly different from control group, #p<0.01, significantly different from HFD group.

Table 1.
Gene Expression Summary and Statistics.

Gene expression results for female ventral tegmental area (VTA), nucleus accumbens (NAC), and prefrontal cortex (PFC) in both Adolescent-onset HFD and Adult-onset HFD females. Summary of fold change, significance and p-values are presented for HFD and HFD + 4 week chow groups as compared to their age matched control.

	Adolescent Onset		Adolescent Onset + 4 week chow		Adult Onset		Adult Onset + 4 week chow	
GENE	Fold change	P value	Fold change	P value	Fold change	P value	Fold change	P value
VTA								
DAT	1.76	0.0304	1.46	0.0104	1.28	0.0546	0.692	0.0219
TH	0.558	0.0157	0.446	0.0004	0.937	0.3179	0.907	0.2155
NAC								
DRD1	0.256	0.0030	2.95	<0.0001	0.374	0.0023	0.833	0.0355
DRD2	0.483	0.0042	0.346	0.0004	0.178	<0.0001	0.353	0.0019
PFC								
DRD1	0.338	<0.0001	2.01	0.0108	1.129	0.5851	3.08	<0.0001
DRD2	0.609	0.0195	2.43	0.0722	2.49	0.0897	1.32	0.4875