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Early life exposure to a high fat diet promotes long-term changes in dietary preferences and central reward signaling

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Abstract

Overweight and obesity in the United States continues to grow at epidemic rates in large part due to the over consumption of calorically-dense palatable foods. Identification of factors influencing long-term macronutrient preferences may elucidate points of prevention and behavioral modification. In our current study, we examined the adult macronutrient preferences of mice acutely exposed to a high fat diet during the third postnatal week. We hypothesized that the consumption of a high fat diet during early life would alter the programming of central pathways important in adult dietary preferences. As adults, the early exposed mice displayed a significant preference for a diet high in fat compared to controls. This effect was not due to diet familiarity as mice exposed to a novel high carbohydrate diet during this same early period failed to show differences in macronutrient preferences as adults. The increased intake of high fat diet in early exposed mice was specific to dietary preferences as no changes were detected for total caloric intake or caloric efficiency. Mechanistically, mice exposed to a high fat diet during early life exhibited significant alterations in biochemical markers of dopamine signaling in the nucleus accumbens, including changes in levels of phospho-DARPP-32 Thr-75, Δ FosB, and Cdk5. These results support our hypothesis that even brief early life exposure to calorically-dense palatable diets alters long-term programming of central mechanisms important in dietary preferences and reward. These changes may underlie the passive overconsumption of high fat foods contributing to the increasing body mass in the Western world.

Keywords

dopamine; striatum; macronutrient; development

The obesity epidemic in the United States continues to grow, with recent statistics indicating that over 60% of American adults are currently overweight or obese (Ogden et al. 2006). Another, equally important trend is the increasing rate of childhood obesity (Ogden et al. 2002). Children in Western societies, in addition to an increased sedentary lifestyle, are exposed to a wide variety of foods high in fat and calories that contribute to the development of obesity. Obese children are more likely to become obese adults, perhaps in part because of the persistence of habits and programming of dietary preferences developed during childhood (Serdula et al. 1993).

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Studies have shown that exposure to certain taste stimuli during infancy and early childhood can alter dietary preferences in children years later (Johnson et al. 1991; Kern et al. 1993; Liem and Mennella 2002; Mennella and Beauchamp 2002). However, the mechanisms whereby such long-term effects occur have not been elucidated. Therefore, we examined the effects of early life exposure to a high fat diet on adult macronutrient preferences in mice. Mice were exposed to a high fat diet for one week, from postnatal days 21-28 (P21-28), the time during which they begin to consume solid food and are no longer dependent on the dam for nutrition. At weaning, mice were returned to standard house chow and examined for macronutrient choice preference and caloric intake on a chronic high fat diet as adults. Based on previous studies showing an effect of palatable diets on brain reward centers and changes in dopamine signaling (Teegarden and Bale 2007; Teegarden et al. 2008), we also examined biochemical markers in the ventral striatum of these mice. We hypothesized that exposure to and withdrawal from a high fat diet during early life would lead to an increased preference for diets high in fat in adulthood via changes in reward circuitry that promote intake of energy-dense, palatable food.

Experimental Procedures

Animals and Early Diet Exposure

Mice were generated on a mixed C57Bl/6:129 background as part of our in-house breeding colony. These mice have been on a mixed background population for more than ten years (Bale et al. 2000), with introduction of a new gene pool every two years by breeding with an F1 C57Bl/6:129 cross. At 3 wks of age, litters were exposed to the high fat diet (Research Diets, New Brunswick, NJ) for one week. The high fat diet contained 4.73 kcal/g and consisted of 44.9% fat, 35.1% carbohydrate, and 20% protein. Control litters remained on standard house chow (Purina Lab Diet, St. Louis, MO). House chow contained 4.00 kcal/g and consisted of 12% fat, 60% carbohydrate, and 28% protein. This time period for diet exposure was selected as by 3 wks of age, offspring are consuming solid food and are not dependent on the mother for nutrition. After weaning, all mice (n = 16 control, 14 early high fat exposed) were maintained on house chow until 3 months of age. All studies were conducted according to protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee, and all procedures were performed in accordance with institutional guidelines.

Macronutrient Choice Preference

In order to examine how early exposure to a macronutrient-enriched diet would affect adult food preferences, 3 month-old mice were examined for macronutrient choice preference over 10 days. Mice were allowed to habituate to individual housing for 1 wk prior to choice preference. Pre-weighed pellets of high fat, high carbohydrate, and high protein diets (Research Diets) were placed on the floor of the cage. Mice and food pellets were weighed daily. High carbohydrate diet contained 3.85 kcal/g consisting of 10% fat, 70% carbohydrate and 20% protein. High protein diet contained 4.29 kcal/g and consisted of 29.5% fat, 30.5% carbohydrate, and 40% protein. The high fat diet used was identical to that used for the early exposure.

In order to control for the effects of diet familiarity on macronutrient preferences, we also examined separate litters exposed to the high carbohydrate diet (Research Diets, as described above), again from 3-4 wks of age and tested for macronutrient choice preference as adults (n = 6).

Adult chronic high fat diet exposure

Following macronutrient choice preference, a subset of mice (n = 7 control, 9 early high fat exposure) were exposed to the high fat diet alone for 15 wks in order to examine the consumption and effects of chronic high fat diet and the possible development of obesity in

mice that had been exposed to this diet during early life. Mice were weighed weekly during this period, and 24-hr food intake was measured during a one week period following 6 wks of chronic exposure. At the end of the chronic high fat diet period, mice were sacrificed by decapitation following brief isoflurane anesthesia, and adipose tissue, plasma, and brains were collected for analysis.

Adiposity and plasma leptin

At sacrifice, mice were weighed and brown adipose tissue and reproductive and renal white adipose tissue depots were removed and also weighed. Trunk blood was collected in tubes containing 50 mM EDTA and centrifuged for 10 min at 5000 rpm and 4°C to separate plasma. Plasma was stored at -80°C until assayed. Leptin levels were determined by radioimmune assay (Linco Research, St. Charles, MO). Fifty microliters of plasma were used per sample, and all samples were run in duplicate. The sensitivity of the assay was 0.2 ng/ml, and the intra- and interassay coefficients of variance were 7.2% and 7.9% respectively.

Biochemical analyses

At sacrifice, the brain was rapidly removed, the ventral striatum (approximately 0.5 - 1.75 mm from bregma, at a depth of 3.5 - 5.5 mm) was dissected (Teegarden and Bale 2007), and the tissue immediately frozen in liquid nitrogen. Western blots (n = 4 control, n = 5 early high fat exposure) were performed as previously described using a phosphatase inhibitor cocktail (P2850 Sigma, St. Louis, MO) to preserve phosphorylation state (Bale et al. 2003; Teegarden and Bale 2007). Antibodies used were FosB (1:200; Santa Cruz Biotechnology, Santa Cruz, CA), Cdk5 (1:500; Santa Cruz Biotechnology), phospho-DARPP-32 Thr 75 (1:200; Cell Signaling Technology, Danvers, MA), phospho-DARPP-32 Thr 34 (1:500; PhosphoSolutions, Aurora, CO), total DARPP-32 (1:500; R&D Systems, Minneapolis, MN), and mu opioid receptor (1:500; Abcam, Cambridge, MA). ΔFosB was distinguished from full length FosB by weight (Nestler et al. 2001). All blots were stripped and reprobed for β-actin for normalization (1:1000; Sigma, St. Louis, MO). Blots were analyzed using IPLab software (Teegarden and Bale 2007). Optical density values for target proteins were divided by values for β-actin within each sample to correct for loading error.

Statistics

All data were analyzed using a student's t-test with early diet treatment as the independent variable. All data are presented as the mean ± SEM.

Results

Macronutrient Choice Preference

In order to determine how early diet exposure affected adult dietary preferences, mice exposed to a high fat diet from 3-4 wks of age were examined for macronutrient choice preference for 10 days beginning at 3 months of age. Preference for the high fat diet (reported as the percent of total calories consumed as high fat diet; Fig. 1A) was significantly greater in mice that had been exposed to the high fat diet during early life ($P < 0.05$). Preference for the high protein diet was not significantly altered by early diet exposure ($P = 0.17$). Mice previously exposed to the high fat diet consumed significantly less of the high carbohydrate diet than controls ($P < 0.05$). Average daily caloric intake between control and early high fat exposed mice was not different (Fig. 1B). When daily intake was expressed as grams of food consumed, there were again no significant differences between groups (control = 3.29 ± 0.13 g/day, early high fat exposed = 3.15 ± 0.14 g/day).

Average body weights were not significantly different between treatment groups before or after macronutrient choice preference (Fig. 1C). Caloric efficiency was calculated as weight gained (g) / calories consumed (kcal) over the course of the experiment. There was no difference in caloric efficiency between groups while on macronutrient choice preference (Fig. 1D). This suggests that while early exposure to a high fat diet increases adult preference for a high fat diet, it does not lead to changes in overall caloric intake or efficiency.

In order to control for effects of diet familiarity on long-term diet preference, a separate cohort of mice received the high carbohydrate diet from 3-4 wks of age. These mice showed no changes in macronutrient preferences for high carbohydrate or high fat diets relative to controls (Fig. 1E), supporting the powerful effect specific to a high fat diet on brain systems governing food preferences.

Chronic High Fat Diet

Mice were exposed to a chronic high fat diet and food intake, body weight, adiposity and plasma leptin levels were measured. There were no significant differences in average daily food intake, final body weight, or caloric efficiency during high fat diet exposure (Fig. 2A-C). There were no differences in the relative amounts of body fat between groups after 3 months on high fat diet (Fig. 2D). Further, there were no differences between groups in plasma leptin levels following chronic high fat diet (Fig. 2E).

Biochemistry in the Ventral Striatum

Following chronic high fat diet exposure, biochemical markers of reward signaling were analyzed in these mice. Mice exposed to the high fat diet during early life displayed significantly elevated levels of the transcription factor Δ FosB ($P < 0.05$; Fig. 3A). Δ FosB has been shown to induce expression of cyclin-dependent kinase 5 (Cdk5) (Bibb et al. 2001). In keeping with this model, early high fat diet exposed mice also exhibited elevated levels of Cdk5 in the striatum ($P < 0.05$; Fig. 3B). Cdk5 phosphorylates the protein dopamine and cAMP-regulated phosphoprotein, molecular weight 32 kDa (DARPP-32) at threonine 75 (Bibb et al. 1999). Mice exposed to a high fat diet during early life also showed significantly higher levels of phospho-DARPP 32 Thr 75 ($P < 0.05$; Fig. 3C). These mice also showed a non-significant trend for a corresponding reduction in phosphorylation of DARPP-32 at Thr 34 ($P < 0.10$; Fig. 3D). Levels of total DARPP-32 protein in the striatum were not altered by early diet treatment ($P = 0.78$; Fig. 3E). Activation of the opioid system in the striatum is also associated with increased consumption of palatable foods. In particular, the mu opioid receptor has been closely linked with increased consumption of preferred diets. Therefore, we investigated levels of the mu receptor in this area (Zhang et al. 1998). Levels were not different between control and early high fat diet exposed mice ($P = 0.90$; Fig. 3F).

Discussion

Studies of food preferences in infants and children have shown that early exposure to different flavors can lead to increased acceptance of and preferences for these flavors in later life (Liem and Mennella 2002; Mennella and Beauchamp 2002). As children are increasingly exposed to foods high in fat during early life, it is important to determine how exposure to certain diets during this time may affect food preferences during adulthood and be a possible contributing factor to the increased intake of energy-dense palatable foods. In the current study, we examined how exposure to a high fat diet during the periweaning period (3-4 wks of age), when mice are consuming solid food and are no longer dependent on the dam for nutrition, would affect adult macronutrient preferences, food intake, and weight gain.

In a 10-day macronutrient choice preference test, high fat diet early-exposed mice showed a significantly greater preference for a high fat diet as adults, measured as the proportion of total daily caloric intake. As a control for diet familiarity, mice exposed to the high carbohydrate diet during early life showed no differences in adult macronutrient preferences, suggesting that changes in adult preference are not simply a result of prior experience with the diet. Changes in the maternal diet have been associated with altered preferences for macronutrients, with both low protein and high fat diets increasing preference for high fat diet at early ages, although these differences lessen with age (Bellinger et al. 2004; Kozak et al. 2005). However, these manipulations occur during gestation and lactation when the brain is still developing and thus are unlikely to be responsible for the effects observed here. Interestingly, exposure to a novel sweet treat (Froot Loops cereal) from P22-27 has been shown to increase consumption of this item in adulthood (Silveira et al. 2008). However, conclusions from this work further suggested that the changes in consumption were due more to the limited access provided and the novel environment in which the food was presented than to any change in the rats' inherent preference for it. By using a nutritionally complete, macronutrient-rich diet presented ad libitum in the home cage environment, we were able to assess changes in global dietary preferences. Because the timing of the diet presentation occurred to late in development, it is less likely that changes in neural wiring in feeding and reward circuits are responsible for the observed changes in behavior, and that other mechanisms, such as epigenetic changes, may be present.

Despite the increased proportional intake of the high fat diet observed in the early-exposed mice, there were no differences in total daily caloric intake or weight gain during the macronutrient choice preference period. Mice consuming more of the high fat diet compensated for the excess calories by reducing their intake of the other macronutrient-enriched diets, particularly the high carbohydrate diet. Overall, these results suggest that the impact of the early exposure is on preference alone, and not overall food intake or metabolism. It is possible that had the length of the macronutrient choice preference test been increased, differences in body weight and caloric efficiency would have emerged due to the more prolonged increase in intake of dietary fat. However, during the chronic high fat diet exposure, we did not observe differences between groups in intake, weight gain, or adiposity, further supporting an effect of early life exposure specific to dietary preference.

Mechanistically, we investigated the possible contributing factors for the increased dietary fat preference. The timing of the diet exposure in the current study made it unlikely that direct effects on the hypothalamus were responsible for the phenotype. The circuitry of the arcuate nucleus, the primary center governing food intake, is formed largely during the second week of life, with the connections resembling that of the adult animal by P18 (Bouret et al. 2004). Expression of the main orexigenic and anorexigenic peptides, neuropeptide Y (NPY) and pro-opiomelanocortin (POMC), also change over the course of early postnatal development, reaching adult levels around the third week of life (Ahima and Hileman 2000; Grove et al. 2003; Leibowitz et al. 2005). Arcuate neurons become responsive to leptin and ghrelin between two and four weeks after birth (Mistry et al. 1999; Proulx et al. 2002). Most studies on the effects of early nutrition in rodents involve dietary manipulations during gestation and/or lactation, in order to capitalize on this period of plasticity in the rodent hypothalamus. By the fourth week of life, when our high fat diet exposure was initiated, hypothalamic development is largely complete. However, there is some evidence for limited plasticity in the adult hypothalamus (Horvath 2005; Kokoeva et al. 2005). We cannot rule out the possible contribution of such changes to our end phenotype.

Preferences for palatable diets have been closely linked with reward systems, with intake of preferred foods having profound effects on dopamine (DA) release in the nucleus accumbens, and alterations in DA function leading to changes in feeding behavior (Blum et al. 2000; Colantuoni et al. 2001; Colantuoni et al. 2002; Cagniard et al. 2006). In addition, early

nutritional manipulations or exposure to rewarding stimuli in rodents have been shown to affect the long-term functioning of the DA system (Sato et al. 1991; Zippel et al. 2003; Kelley and Rowan 2004). We have previously reported that withdrawal from a high fat diet can have profound and long-lasting effects on the DA system (Teegarden and Bale 2007; Teegarden et al. 2008). Thus, in the current study we hypothesized that reward signaling might be altered in mice exposed to high fat diet during early life. To test this hypothesis, mice were sacrificed following chronic high fat diet exposure and markers of reward signaling in the ventral striatum were examined. We found that mice exposed to high fat diet during early life had significantly higher levels of the transcription factor Δ FosB in the ventral striatum following chronic high fat diet exposure in adulthood. Δ FosB is induced in the nucleus accumbens following chronic exposure to drugs of abuse and natural rewards (Nestler et al. 2001; Teegarden and Bale 2007; Wallace et al. 2008). Mice overexpressing Δ FosB in dynorphin-positive accumbal medium spiny neurons show an increased motivation to obtain a food reward due to a basal dysregulation of DA signaling (Olausson et al. 2006; Teegarden et al. 2008). Our own work has shown that these mice are more vulnerable to high fat diet withdrawal and show dramatic changes in markers of DA signaling following high fat diet exposure (Teegarden et al. 2008). We also observed a significant increase in cyclin-dependent kinase 5 (Cdk5) and dopamine and cAMP-regulated phosphoprotein, molecular weight 32 kDa (DARPP-32) phosphorylated at threonine 75, as well as a trend for a corresponding reduction of pDARPP-32 Thr 34. In the progression of signaling following reward experience and elevation of Δ FosB, levels of Cdk5 begin to rise (Bibb et al. 2001). As a negative regulator of DA neurotransmission and neuronal excitability (Chergui et al. 2004; Benavides et al. 2007), Cdk5 phosphorylates DARPP-32 at threonine 75 (Bibb et al. 1999). Interestingly, phosphorylation of DARPP-32 at this site attenuates D1 DA receptor activity via direct inhibition of protein kinase A and inhibits phosphorylation at Thr 34 (Benavides and Bibb 2004). Overall, these biochemical measures are highly suggestive of a reduction in DA signal transduction in the striatum during high fat diet exposure in mice previously exposed to and then withdrawn from a high fat diet during early life. We hypothesize that the reduced DA signaling observed during high fat diet exposure likely contributes to the increased preference for high fat diet during macronutrient choice preference. During chronic high fat diet exposure, it is likely that intake is limited by total caloric consumption, and thus no behavioral differences were observed. Our data is in line with clinical reports that suggest reduced DA signaling in obese patients (Wang et al. 2001). The increase in preference for high fat diet in adulthood may be a compensatory response by the organism to normalize dopaminergic tone (Blum et al. 2000; Wang et al. 2004; Teegarden et al. 2008).

The mechanism behind these changes in dopamine signaling remains to be elucidated. It is important to note that changes in opioid signaling in the ventral striatum have also been closely linked to changes in palatable feeding and dopaminergic signaling. In particular, stimulation of the mu opioid receptor leads to a robust increase in intake of a diet high in fat (Zhang et al. 1998), and exposure to a high fat diet can alter opioid signaling (Blendy et al. 2005; Jain et al. 2004). However, we observed no differences in levels of the mu opioid receptor in the striatum between control and early high fat diet exposed mice. While this does not rule out a role for mu receptor signaling or other opiodergic factors, our data indicate that the change in dietary preference is due to changes in dopamine signaling that are unrelated to changes in mu opioid receptor levels.

In the rat, dopamine neurons are born around embryonic day 12 (E12) and begin to extend processes at E13. Innervation of the striatum extends into the first postnatal week, and reorganization continues at least until the third postnatal week (Van den Heuvel and Pasterkamp 2008). Thus, the dietary manipulation paradigm in the current study is not likely to alter the initial formation of the mesolimbic dopamine system. Changes in fatty acid levels during development and later life can also affect DA and DA receptor levels in the frontal

cortex of adult rats (Delion et al. 1994; Delion et al. 1996; Zimmer et al. 1998), and maternal consumption of high fat diet can alter the functioning of the DA system in adult offspring, possibly leading to desensitization of dopamine receptors (Naef et al. 2008). Although the diets used in our present study contained a balanced variety of fatty acids, the possibility remains that subtle variations in dietary fat content may alter long-term DA signaling. In addition, direct developmental effects that may be observed in models of maternal diet manipulation are unlikely to be responsible for the current results due to the late timing of the diet exposure, suggesting that epigenetic mechanisms may play a role. Plasticity in the nucleus accumbens is also observed following treatment with drugs of abuse. Cocaine, nicotine and amphetamine increase spine density in this area (Robinson and Kolb 2004). These changes last for months after the last drug exposure, and can be induced by only a single experience (Kolb et al. 2003). We have previously shown that withdrawal from a high fat diet in adults produces changes in stress and reward pathways in mice (Teegarden and Bale 2007). Therefore, it is possible that the brief exposure and withdrawal of this diet during early life produces similar effects that reprogram these circuits. Finally, another candidate for mediating long-term changes in gene expression is epigenetic regulation. Dietary manipulation could also lead to long-term programming of gene expression via changes in DNA methylation or histone acetylation. Changes in methylation of genes in the DA system have been linked to psychiatric and mood disorders as well as addiction (Abdolmaleky et al. 2008; Hillemecher et al. 2008). While these studies do not directly address the effects of a high fat diet on DA system plasticity, they raise the intriguing possibility that the functioning of this system may be altered long-term by a natural reward during early life. These mechanisms may be further investigated in future studies.

In conclusion, the present study demonstrates that a brief exposure to a palatable, high fat diet during early life programs an increased preference for this diet during adulthood that is not based on diet familiarity. Mechanistically, reduced DA signal transmission in the ventral striatum in these mice may result in an increased preference for the high fat diet in an attempt to normalize DA levels. The data then suggest that exposure to a palatable, high fat diet during early life may lead to long-term reprogramming of the reward system, leaving the organism at risk not just for maladaptive eating habits but perhaps also to other disorders of the reward system.

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List of Abbreviations

P, postnatal day; Cdk5, cyclin-dependent kinase 5; DARPP-32, dopamine and cyclic adenosine monophosphate regulated phosphoprotein, molecular weight 32 kDa; Thr, threonine; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; DA, dopamine; E, embryonic day.

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Figure 1.

Brief early life exposure to a high fat diet results in an increased preference for fat during adulthood. (A) Mice exposed to a high fat diet immediately prior to weaning (Early HF) consumed a significantly greater proportion of their calories in the form of high fat diet (HF) during adulthood ($P < 0.05$), and significantly less high carbohydrate diet (HC) than controls (Ctrl; $P < 0.05$). Preference for the high protein diet (HP) was not significantly different between groups. * $P < 0.05$ HF and HC consumption compared to control group. (B) Total 24-hr caloric intake was not different between groups. (C) Body weights were not affected by early diet exposure at age 6 wks (2 wks post-weaning), 12 wks (immediately prior to macronutrient choice preference), or 13.5 weeks (at the end of macronutrient choice preference). (D) Caloric

efficiency during macronutrient choice preference was not different between groups. (E) As a control for diet familiarity, macronutrient preferences in mice exposed to the HC diet from 3-4 wks of age (Early HC) did not differ from controls. All data are represented as the mean $\pm$ SEM.

Figure 2.

No differences were observed between groups for food intake and body weight during 3-month chronic high fat diet exposure. (A) Daily caloric intake was not different between control (Ctrl) and early high fat exposed (Early HF) mice when the mice were consuming the high fat diet chronically. (B) Average body weight at the end of the diet period was not affected by early diet exposure. (C) Mice exposed to the high fat diet just prior to weaning showed no difference in caloric efficiency during chronic high fat diet exposure in adulthood. (D) Weights of brown, reproductive and renal white adipose tissue depots, normalized to body weight, were not different between groups. (E) Circulating leptin levels following 3 months of high fat diet

exposure were not affected by early high fat diet treatment. All data are represented as the mean $\pm$ SEM.

Figure 3.

Markers of dopamine signaling in the ventral striatum were altered in mice briefly exposed to a high fat diet in early life (Early HF). (A) Levels of the transcription factor Δ FosB were significantly elevated in the ventral striatum of adult mice exposed to a high fat diet from 3-4 weeks of age compared to controls (Ctrl). (B) Levels of cyclin-dependent kinase 5 (Cdk5) were also significantly elevated in early high fat exposed mice. (C) Phospho-DARPP-32 Thr 75 was significantly elevated in early high fat exposed mice. *P < 0.05. (D) Phospho-DARPP-32 Thr 34 levels in mice exposed to high fat diet during early life showed a non-significant trend to be reduced (P < 0.10). (E) Levels of total DARPP-32 protein were not different between groups. (EF) No differences in mu opioid receptor levels were observed between groups. For each blot,

representative bands are shown for control (C) and early high fat exposed (HF) groups. All data are represented as the mean $\pm$ SEM.