Maternal Obesity Alters Adiposity and Monoamine Function in Genetically Predisposed Offspring

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ABSTRACT

The impact of maternal obesity on brain monoamine function in adult offspring of dams selectively bred to express diet-induced obesity (DIO) or diet-resistance (DR) was assessed by making dams obese or lean during gestation and lactation. After 12wk on chow and 4wk on a 31% fat diet, offspring hypothalamic nucleus size and binding of $^3$H nisoxetine binding to norepinephrine transporters (NET) and $^3$H paroxetine binding to serotonin transporters (SET) was measured. Offspring of obese DIO dams became more obese than all other groups but maternal obesity did not alter weight gain in DR offspring (25). Maternal obesity was associated with 10-17% enlargement of ventromedial (VMN) and dorsomedial nuclei in both DIO and DR offspring. Offspring of obese DIO dams had 25-88% lower NET binding in the paraventricular (PVN), arcuate, VMN and the central amygdalar nuclei, while offspring of obese DR dams had 43-67% higher PVN and 90% lower VMN NET binding and a generalized increase in SET binding across all hypothalamic areas compared to other groups. Thus, maternal obesity was associated with alterations in offspring brain monoamine metabolism which varied as a function of genotype and the development of offspring obesity.

Key Words: diet-induced obesity, norepinephrine, serotonin, hypothalamus, amygdala, neural plasticity, insulin, leptin
INTRODUCTION

An increasing number of studies in humans and rodents suggest that the state of maternal nutrition (14;36;39;42) and the presence of obesity (25) or diabetes (30-32) can all have long-lasting and important effects on the development of obesity and diabetes in offspring. All of these predisposing conditions can alter central nervous system function by either changing the neuroanatomy (30) or neuronal function in select brain areas (15;16;30;32;34). Because neural function is difficult to assess in living humans, such studies have only been carried out in animal models.

The impact of maternal obesity on subsequent weight gain and neural development in the offspring has not been clearly elucidated. To study this issue, we developed a rat model of diet-induced obesity (DIO) in which outbred Sprague-Dawley rats were selectively bred over several generations for their propensity for high (DIO) versus low (diet resistant [DR]) weight gain on a diet of relatively high caloric and fat content (high energy [HE] diet; 31% fat) (24). This generated two substrains which appear to inherit their weight gain characteristics as a polygenic trait, similar to the inheritance pattern of most human obesity (4). Because these substrains breed true to their weight gain phenotype, we were able to manipulate the maternal diet to make DIO and DR dams either relatively lean or obese during gestation and weaning (25). That study showed that offspring of DIO dams always became more obese than those of DR dams regardless of the state of maternal adiposity. However, when both DIO and DR dams were made obese during gestation and lactation, the offspring of obese DIO, but not obese DR dams, developed increased carcass adiposity, hyperleptinemia and hyperinsulinemia as adults. These differences were accentuated when offspring were
placed on HE diet for 4wk after having been fed a low fat chow diet from weaning to 16wk of age (25). The morphometric and metabolic results of that study have been previously reported (25). Since norepinephrine (NE) injections into the medial hypothalamus stimulate (17), and serotonin (5HT) injections inhibit food intake (19), we measured binding to the NE transporter (NET) and serotonin transporter (SET) to assess the effect of maternal obesity and genotype on NE and 5HT function in the brains of the adult offspring reported in the previous study (25). These transporters function as a reuptake mechanism to regulate the amount of NE and 5HT available for synaptic transmission. Here we report that maternal obesity was associated with a genotype-specific reduction of NET binding in select hypothalamic and amygdalar nuclei and with a larger size of some hypothalamic nuclei in offspring of obese DIO dams.

**METHODS**

**Animals and experimental protocol**

*Dams and breeding.* Use of animals was in accordance with the guidelines set up by the animal use committee of the E. Orange VA Medical Center. All studies were carried out in keeping with the guiding principals for research involving animals and human beings of the American Physiological Society (1). The breeding pairs for this experiment were derived from our two colonies of rats bred selectively for their propensity to develop DIO or DR, respectively (24). These colonies were originally derived from outbred Sprague-Dawley rats (Charles River Labs). Rats were kept at 23-24°C on a 12:12 h light: dark cycle. One month prior to breeding, 18 DIO and 27 DR females were divided into one of five groups of 9 dams each: 1) *DR Chow* dams were continued on Purina rat chow which
contains 3.30 kcal/g with 23.4% as protein, 4.5% as fat and 72.1% as carbohydrate which is primarily in the form of complex polysaccharide (26). They remained lean throughout pregnancy and lactation on this diet (25); 2) DR HE diet dams were fed a high energy diet composed of 8% corn oil, 44% sweetened condensed milk and 48% Purina rat chow (Research Diets). This diet contains 4.47 kcal/g with 21% of the metabolizable energy content as protein, 31% as fat and 48% as carbohydrate, 50% of which is sucrose (26). They remained lean throughout pregnancy and lactation on this diet (25); 3) DR Ensure dams were given HE diet ad libitum and, in addition, were given access to Ensure (Ross Products) which is a liquid diet containing 1.06 kcal/ml with 14% of the metabolizable energy content as protein, 22% as fat and 64% as carbohydrate. These dams overate and became obese during pregnancy and lactation on this diet (25); 4) DIO Chow dams were fed chow. They remained lean (although more obese than DR chow-fed dams) throughout pregnancy and lactation on this diet (25); 5) DIO HE diet rats were fed the HE diet. They became obese on this diet (25). Dams were kept on their respective diets through gestation and lactation. At two weeks of gestation all dams underwent tail bleeding for plasma glucose, insulin and leptin levels and were then mated with males of the same genotype. At birth, litter sizes ranged from 4-15 pups with means in the five groups ranging from 10-14 without any significant differences in litter sizes among the groups. All litters were culled to 4-10 pups, i.e. random pups from litters larger than 10 were discarded.

Pups and diet manipulations. After weaning, all male pups were separated from their dams and fed chow for 16wk followed by an additional 4wk on HE diet. Rats studied for brain NET and SET binding (n=6 rats/ group) were killed by decapitation between 0800
and 1100h and the brains were removed and rapidly frozen in powdered dry ice. Brains were stored at -80°C until they were assayed for ³H nisoxetine and ³H paroxetine binding. In addition, their retroperitoneal, perirenal and epididymal adipose depots were removed and weighed and their trunk blood was collected for plasma glucose, insulin and leptin levels as reported previously (25).

**Autoradiographic NET and SET binding assays.** Frozen brains were cut in 20µm serial sections through the forebrain and the sections were thaw-mounted on gel-coated slides, vacuum desiccated overnight and stored at -80°C until assayed. NET binding was assessed using ³H nisoxetine ([N-methyl-³H] nisoxetine; 3.01 TBq/mmol; Amersham). Sections were incubated for 2h at 4°C for 30min with 1nM ³H nisoxetine in Tris buffer (Tris 50mM; NaCl 300mM; KCl 5mM, pH=7.4). Additional sections were incubated with both ³H nisoxetine and 1.0µM mazindol to define non-specific binding (<15% of total binding). Slides were then washed twice with incubation buffer at 4°C and the sections were dried on a warming plate. SET binding was carried out using 1nM ³H paroxetine ([phenyl-6'-³H] paroxetine; 1.03 TBq/mmol; New England Nuclear). Sections were incubated for 1h at 4°C in 50mM Tris buffer, pH 7.4 (see above), washed twice in ice-cold Tris buffer and dried. Non-specific binding was assessed in the presence of 30µM fluoxetine (<30% of total binding). Slides from both assays were apposed Hyperfilm (Amersham) and kept at 4°C for 6-8wk. The brain sections used to generate the autoradiograms were stained with cresyl violet and these were used to define the anatomical limits of the various nuclei. Digitized images of the autoradiograms were overlaid on digitized images of the stained sections and binding to various forebrain areas involved in energy homeostasis regulation was determined using computer-assisted
densitometry (Drexel University). Four to eight bilateral density readings from each area were averaged and converted to binding as previously described (20). Brain areas read included the hypothalamic arcuate (ARC), dorsomedial (DMN), ventromedial (VMN) and paraventricular (PVN) nuclei and the central amygdalar nucleus (ACN). Areal measures were also made in 2-4 cresyl violet stained sections per hypothalamic nucleus for determination of nuclear size (mm$^2$). Areal measures of the PVN were taken at the point of largest diameter. Measures of the ARC, VMN and DMN were made at the midpoint of the ARC in which neuropeptide Y neurons involved in energy homeostasis are found in both the ARC and DMN (22).

**Assays of glucose, insulin and leptin.** Samples of trunk blood were collected into heparinized tubes and the plasma removed for assay. Glucose was assayed by automated glucose oxidase method (Beckman) and both insulin and leptin were analyzed by radioimmunoassays (Linco) using antibodies to authentic rat insulin and leptin, respectively as reported previously (25).

**Statistics.** One way analysis of variance (ANOVA) was used for single point measures of total food intake, terminal body and fat depot weights, plasma glucose, insulin and leptin levels. Binding of $^3$H nisoxetine and $^3$H paroxetine and areal measures were first compared across brain areas by two-way ANOVA (genotype x phenotype). Additional comparisons were made by one-way ANOVA by experimental group. When significant intergroup differences were found by ANOVA ($P \leq 0.05$), post-hoc comparisons were carried out using Scheffe analysis.
RESULTS

Summary of morphometric and metabolic data. The data in Table 1 describe the status of the dams during the second week of pregnancy and of their offspring who were fed chow from weaning to 16wk of age and then HE diet for an additional 4wk as previously reported (25). At 2wk of gestation, DIO Ensure dams were the heaviest and had higher plasma insulin levels than all other groups. They had a tendency towards higher leptin levels but these did not differ significantly from DIO chow or DR Ensure dams. The DIO chow and DR Ensure dams had comparable body weights with intermediate plasma insulin and leptin levels. DR chow and DR HE dams had comparable body weights and plasma leptin levels although the DR HE dams had slightly higher insulin levels than DR chow dams.

Collectively, offspring of all DIO dams were 30% heavier and had 47% higher plasma insulin levels than all offspring of DR dams after 16wk on chow, regardless of maternal diet. At this time, there were no differences in body weight between offspring of DIO chow (lean) and DIO HE dams (obese). But offspring of obese DIO dams had heavier retroperitoneal fat pad weights than all other groups (25). Following an additional 4wk on HE diet, a second group of DIO offspring collectively gained 50-100% more weight than offspring of DR dams. Offspring of obese DIO dams gained the most weight and had the heaviest total fat pad mass and highest plasma leptin levels of all other groups. Interestingly, there was an increase in the ratio of the weight of 4 fat pads to final body weights across the groups as a function of maternal genotype, phenotype during pregnancy and maternal diet. Thus, this ratio increased progressively from offspring of
DR chow (lean) to DR HE (lean) to DR Ensure (obese) to DIO chow (lean) to DIO HE (obese; Table 1). Obese DR offspring had a 12% significantly higher ratio of fat pads to body weight and a non-significant, 20% higher leptin level than DR chow offspring. Similarly, offspring of obese DIO dams had a 30% higher fat pad to body weight ratio and 14% higher leptin level than offspring of lean DIO dams.

Effect of maternal genotype and phenotype on the areal size of hypothalamic nuclei. When the areas of the four hypothalamic brain areas were considered together, there were no significant differences in size across the four brain areas that were dependent upon maternal genotype (DIO vs. DR; Table 2). However, offspring of obese dams (DR Ensure, DIO HE), regardless of their genotype, had larger hypothalamic areas overall than those from lean dams (DR chow, DR HE, DIO chow; F[1,99]=5.61; P=0.020). When considered by individual brain areas, offspring of obese dams had 17% larger DMN (F[1,23]=6.58; P=0.018) and 10% larger VMN areas (F[1,24]=5.02; P=0.035.

Forebrain norepinephrine and serotonin transporter binding. There were significant differences in \(^3\)H nisoxetine binding which were dependent upon the combination of maternal diet and genotype in four of five areas assessed (Figure 1). In the PVN, the diet by genotype interaction (F[4, 25]=12.31; P=0.001) showed that offspring of obese DIO dams had 34-41% lower binding while offspring of obese DR dams had 43-67% higher binding than the offspring of lean DR and DIO dams. In the ARC (F[4,25]=3.78; P=0.015) and ACN (F[4,25]=4.63; P=0.001), offspring of obese DIO dams had reduced binding of 25-55% and 72-88%, respectively, compared to all other groups. In the VMN,
obesity in the dam was associated with markedly lower $^3$H nisoxetine binding (89-94%) in offspring compared to offspring of lean dams regardless of maternal genotype ($F[4,25]=2.78; P=0.05$). There were no intergroup differences in DMN $^3$H nisoxetine binding.

Across all four hypothalamic nuclei, offspring of obese DR and DIO dams had higher $^3$H paroxetine binding than those from lean DR and DIO dams ($F[1.97]=4.98; P=0.028$). However, this was primarily due to the fact that offspring of obese DR dams had the highest binding across all areas overall (Figure 2). When analyzed by individual hypothalamic nuclei, offspring of obese DR dams had significantly higher binding than all other groups in the VMN ($F[4,20]=13.63; P=0.001$) and ARC ($F[4,20]=8.43; P=0.001$). In the PVN ($F[4,20]=16.31; P=0.001$) and DMN ($F[4,20]=13.94; P=0.001$), offspring of both lean DIO and obese DR dams had higher binding than offspring of obese DIO and lean DR dams. Thus, the non-obese offspring of obese DR dams had an overall increase in hypothalamic $^3$H paroxetine while offspring of lean DIO dams had reduced $^3$H paroxetine binding in select hypothalamic nuclei.

**DISCUSSION**

This study emphasizes the importance of the interaction between environment and genetic background and its influence upon the development of the brain in DIO. Our original report of the metabolic characteristics of the rats described here (25) showed that the offspring of obese DIO dams (DIO HE) had heavier fat pads at 16wk of age on chow. They also gained the most weight after 4wk of HE diet, accompanied by greater adiposity
and higher leptin and insulin levels than all other groups. As expected (23;24), offspring of lean DIO dams (DIO chow) also gained more weight and were more obese on both chow and HE diet than all three groups of DR offspring. Maternal obesity in DR rats (DR Ensure) was also associated with a higher ratio of the weight of 4 fat pad depots to total body weight (not reported in the original study), although there were no significant differences in insulin and leptin levels among the DR offspring groups after 4wk on HE diet. Thus, maternal obesity enhanced the development of obesity in offspring who were already genetically predisposed to become obese and might also have increased the tendency to become obese in obesity resistant rats.

The current studies further characterize these rats with regard to the effect of perinatal and postnatal manipulations on the development of the nervous system, with emphasis on forebrain monoaminergic systems. In general, offspring of obese DIO dams had reduced NET binding across most brain areas assessed when compared to offspring of all other groups. Somewhat paradoxically (see below), hypothalamic SET binding was also increased in offspring of lean DIO dams. Genotype played a critical role in the outcome of these studies since offspring of obese DR dams had increased NET binding selectively in the PVN and a generalized increase in hypothalamic SET binding. Finally, maternal obesity in both DIO and DR offspring was associated with increased VMN and DMN areal sizes and reduced VMN NET binding. Since offspring were studied well into adulthood, after exposure to two different diets, it is uncertain whether these altered neural parameters were the cause or the effect of the resultant metabolic picture seen in the various groups. However, it seems unlikely that adult onset obesity alone caused the selective reduction in NET binding in offspring of obese DIO dams since reduced
binding was not present in the offspring of lean DIO dams, even though they were also more obese than offspring of lean DR dams. Similarly, the alterations in NET and SET binding seen in offspring of obese DR dams is unlikely to have been primarily a function of adult onset obesity since they were only marginally more obese and had similar leptin and insulin levels compared to offspring of lean DR dams. The larger areal size of the VMN and DMN in DIO offspring might be due to difference in brain size between DIO and DR rats. However, since neither brain size nor an exhaustive volumetric measure of the entire size of the nuclei was made, these data must be interpreted with caution. Finally, dietary content alone could not explain any of these findings since all groups of offspring were exposed to the same set of dietary manipulations. This makes the interaction of maternal environment with genetic background the most likely critical variable in explaining the intergroup differences.

There are several possible explanations for the alterations in NET and SET binding. Binding to transporters has been used as an index of both dendritic (10) and presynaptic axonal (27) density. However, the expression and function of transporters can also be altered by the metabolic state of the animal. Insulin reduces the expression of NET mRNA (8). Leptin decreases SET binding (5) even though brain SET binding is increased when outbred rats develop DIO (29). Thus, reduced NET binding in offspring of obese DIO dams could represent either a reduced number of noradrenergic terminals or a normal number of noradrenergic terminals with a decreased complement of NET’s per terminal. Since NET’s are responsible for removal of NE after its release, synaptic NE would be increased if NET number were decreased in the latter circumstance. This situation occurs in offspring of dams treated with insulin or in offspring of dams
undernourished during the third trimester. These offspring become obese as adults and have increased ventromedial hypothalamic extracellular NE (16). Acutely, injection of NE into the PVN increases food intake (17) and chronic PVN NE infusion produces hyperphagia and obesity (18). Thus, the reduced NET’s in the PVN of offspring of obese DIO dams could have contributed to their greater obesity as adults. By the same line of reasoning, the increased PVN NET binding in the offspring of obese DR dams would have reduced synaptic PVN NE availability and opposed any tendency they might have had to become obese. However, since both leptin and insulin can alter the number of NET’s and SET’s, the greater obesity and higher leptin levels in offspring of obese DIO dams might have contributed to their altered transporter binding without necessarily affecting the overall NE or 5HT innervation of the areas examined.

A similar problem arises in the interpretation of the SET binding data. If increased SET binding in offspring of obese DR dams reflects an increased number of transporters per terminal, rather than a change in the absolute number of 5HT terminals, then their synaptic 5HT would be reduced. This could contribute to their propensity to become more obese than the offspring of lean DR dams since hypothalamic 5HT normally inhibits ingestive behavior. Central 5HT injections inhibit (6;19) and 5HT antagonists stimulate food intake (7), while SET blockers reduce food intake and body weight (9;38), presumably by increasing synaptic 5HT. Unfortunately, this argument does not explain how increased hypothalamic SET binding in offspring of lean DIO dams contributed to their reduced adiposity compared to the offspring of obese DIO dams. In fact, any chronic effect of either NE or 5HT on energy intake can be largely discounted as a primary cause of increased adiposity since there were no significant differences in intake
among individuals within each genotype group. Interpretations of the data are further complicated because of the complex interaction between NE and 5HT transmitter release (41) and receptor function (3). Thus, without actual measures of NE or 5HT release and/or turnover, no definitive role for the observed alterations of NET or SET binding can be assigned to explain the differences in energy homeostasis among the groups.

Given this caveat, the main importance of these studies is that they emphasize the critical interaction between genotype and the environment in determining the development of brain monoamine systems. Alterations in the maternal milieu and the perinatal metabolic environment have been well documented to alter adiposity, glucose metabolism and neural development (14-16;25;30-32;34;36;39;42). In addition, genotype and diet interact to alter both noradrenergic (21) and serotonergic (11;29) function in adult DIO and DR rats. DIO rats develop hyperinsulinemia and hyperleptinemia and both insulin (12;13;15;16;28;33;35;37) and leptin (2;40) can alter neural plasticity in the developing and adult brain. But insulin and leptin cannot be the only determinants. Offspring of obese DIO and DR dams were both exposed to comparable levels of both, yet the offspring of obese DIO dams had a generalized decrease in NET binding, while the offspring of obese DR dams had a generalized increase in hypothalamic SET binding. Thus, our data suggest that if maternal hyperinsulinemia or hyperleptinemia account for the current findings, they must do so in a genotype-specific way. Neither can the findings be easily explained by dietary perturbations during the post-weaning period, since all groups were exposed to exactly the same diet manipulations during this period. Thus, the current descriptive studies support the general concept that genetic background provides the template upon which environmental factors act to determine the neural and metabolic
future of the individual. Further, they emphasize the importance of the maternal environment in these genotype-specific outcomes. Given the design of the current studies, we cannot assign relative roles of pre- vs. postnatal environmental factors to the observed outcomes. Clearly, further studies are required to identify the specific environmental variables that determine the propensity of an individual to develop DIO.

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REFERENCES


**FIGURES**

**Figure 1:** DR and DIO dams were made obese or remained lean during gestation and lactation by manipulating their diets. Lean DR dams received either chow (DR Chow [Lean]) or HE diet (DR HE [Lean]) and obese DR dams received Ensure (DR Ensure [Obese]). Lean DIO dams received chow (DIO Chow [Lean]) and obese DIO dams received HE diet (DIO HE [Lean]). Male offspring (n=6/group) were fed chow from weaning until 16wk of age and then HE diet for an additional 4wk. Binding to the norepinephrine transporter was assessed by receptor binding autoradiography with $^3$H nisoxetine in the hypothalamic paraventricular (PVN), ventromedial (VMN), dorsomedial (DMN) and arcuate (ARC) nuclei and in the central nucleus of the amygdala (ACN). Bars with differing superscripts were significantly different from each other by post hoc test at the P=0.05 level or less after significant intergroup differences for each brain area were found by one way ANOVA. Bars are mean ± SEM.

**Figure 2:** Binding to the serotonin transporter was assessed by $^3$H paroxetine binding in the groups described in the legend of Figure 1.
Table 1. Effect of genotype, maternal and postnatal environment on metabolic status. Body and adipose depot weights, plasma insulin, leptin and glucose levels in dams at the second week of gestation and their offspring after being fed chow from weaning to 16wk of age and then HE diet for 4wk. DR dams were fed either chow (n=7), HE diet (n=6) or HE diet + Ensure (n=6) while DIO dams were fed chow (n=3) or HE diet (n=3) for 1mo prior to conception and then throughout gestation and weaning. Body Wtₚ = Body weight at 16wk of age on chow; Offspring= 6 rats/ group. Body Wtₚ = Body weight after 4wk on HE diet; Body Wtₕ = body weight gain after 4wk on HE diet. Data are mean ± SEM taken from (25). Fat pad weight is the total weight of the retroperitoneal, perirenal and epididymal fat pads. Parameters with differing superscript letters differ from each other by P < 0.05 by post hoc t-test after intergroup differences were found by ANOVA.
Table 2. Effect of genotype, maternal and postnatal environment on hypothalamic nuclear sizes. Cresyl violet stained sections through the hypothalamic paraventricular (PVN), ventromedial (VMN), dorsomedial (DMN) and arcuate (ARC) nuclei were assessed for nuclear area in offspring of lean and obese DR and DIO dams on various diets. Across all brain areas, offspring of obese dams, regardless of their genotype, had larger hypothalamic areas than those from lean dams (F[1,99]=5.61; P=0.020). Post-hoc one way ANOVA by individual brain areas showed significantly larger DMN (F[1,23]=6.58; P=0.018) and VMN areas (F[1,24]=5.02; P=0.035) in offspring of obese vs. lean dams. N = 6 rats / group. Data are mean ± SEM areas (mm$^2$).
FIGURE 1

$^3$H Nisoxetine (fmol/mg protein)
FIGURE 2

$^3$H Paroxetine (fmol/mg protein)