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Brief communication

Impacts of maternal diet and exercise on offspring behavior and body weights☆

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ABSTRACT

Human and animal studies indicate that maternal obesity can negatively impact aspects of metabolism and neurodevelopment in the offspring. Not known, however, is whether maternal exercise can alter these adverse outcomes. In this study, Long-Evans female rats were provided a high fat (60%; HFD) or control diet (CD) 44 days before mating and throughout gestation and lactation. Running wheels were available to half of each diet group during the gestational period only, resulting in four conditions: CD diet with (CDRW) or without (sedentary; CDSED) exercise, and HFD with (HFRW) or without (HFSED) exercise. Only male offspring (one per litter) were available for this study: they were put on control diet two weeks after weaning and examined using behavioral evaluations up to four months of age. Before weaning, offspring of CDRW dams weighed less than offspring from CDSED or HFD dams. After weaning, the lower weight in CDRW offspring generally persisted. Adult offspring from HFSED dams performed worse than the HFRW group in a Morris water maze during initial spatial training as well as reversal learning; memory was not impacted. No differences between groups were seen in tests of novel object recognition, social approach, or chocolate milk preference. Thus, maternal diet and exercise produced differential effects on body weights and cognitive behaviors in the offspring, and the data demonstrate a positive impact of maternal exercise on the offspring in that it ameliorated some deleterious behavioral effects of a maternal high fat diet.

1. Introduction

The National Institutes of Health reports that more than two-thirds of American adults are overweight or obese, due to an energy imbalance from high-calorie intake and/or sedentary lifestyle (NIH, 2016). Overweight or obese adults have a greater risk of numerous health conditions (e.g., type 2 diabetes, metabolic syndrome), and in addition, mothers who were overweight or obese before becoming pregnant increase their chances of having pregnancy complications (e.g., gestational diabetes, preeclampsia) as well as offspring with physical or neurological defects (March of Dimes, 2016). Some studies have shown that maternal obesity and metabolic disorders are associated with greater risk of neurological syndromes such as autism spectrum disorders, developmental delays, and attention deficit disorders (e.g., Krakowiak et al., 2012; Van Lieshout et al., 2011).

In rodent models, there is a considerable literature showing that maternal obesity induced with high-fat or unbalanced diets produce metabolic disorders in offspring (Li et al., 2011). In addition, there is growing evidence of neurodevelopment changes in the offspring including spatial cognitive deficits, anxiety, and abnormal social behaviors, some of which have been associated with neural inflammation and altered development of neural pathways (e.g., Bilbo and Tsang, 2010; Can et al., 2012; Page et al., 2014; Sullivan et al., 2014; Tozuka et al., 2010; White et al., 2009). On the other hand, maternal exercise improves metabolic and cognitive function in offspring (e.g., Akhavan et al., 2008; Carter et al., 2013; Pampiansil et al., 2003; Robinson and Bucci, 2014; Stanford et al., 2015). Studies combining high fat diet and exercise with neurodevelopmental endpoints were not found.

In this study, female rats were provided a high fat (60%) or control diet before mating and throughout gestation and lactation. Running wheels were available to half of each diet group during the gestational period only. This paper presents exploratory data from male offspring

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that were placed on control diet after weaning and examined using several behavioral evaluations from 1 to 4 months after weaning.

2. Methods

2.1. Animals

2.1.1. Housing

The US Environmental Protection Agency (EPA) animal facility was fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) and studies were approved by the US EPA NHEERL Institutional Animal Care and Use Committee. All rats were housed in standard acrylic cages with dimensions of (h, l, w: 20, 42, 20 cm), with hardwood chip bedding (Beta-Chip®, Northeastern Products, Warrensburg, NY) and shredded paper (Enviro-Dri®, Shepherd Specialty Papers, Watertown, TN). Pregnant rats were singly housed, and after weaning, pups were housed two/cage until two months of age, when they were moved to single cages.

2.1.2. Maternal treatment

Initial dietary treatments and breeding were performed by Charles River Laboratories in their Raleigh NC facilities. Thirty day old female Long-Evans rats were started on either a control (TD.08806) or high fat (TD.06414) diet from Harlan Tecklad Diets (Madison, WI). The control diet (CD) had a caloric composition of 10.4% fat, 69.1% carbohydrate, and 20.5% protein, whereas the high fat diet (HFD) had a composition of 60.3% fat, 21.8% carbohydrate, and 18.4% protein. After 44 days on these diets, rats were bred over 3 consecutive days. Successfully mated rats (presence of sperm plug) were shipped to the US EPA animal facility on the same day of verification, which was designated as gestational day (GD) 1.

Upon receipt, the pregnant dams were assigned to either a sedentary (normal housing; SED) or active (placed in cages with running wheels; RW) group. Diet treatments were maintained through pregnancy, birth and lactation. Thus, the four maternal treatment groups were: control diet, sedentary (CDSED, $n = 13$); control diet, active (CDRW, $n = 14$); high fat diet, sedentary (HFSED, $n = 18$); and, high fat diet, active (HFRW, $n = 18$). The running wheels were removed on the day after birth to protect the pups from injury. For a more detailed explanation of maternal treatments, see Gordon et al. (2017).

2.1.3. Offspring

On postnatal day (PND) 6, litters were culled to 8 (4 males and 4 females where possible), and pups were weaned on PND21. Pups remained on the same diets as the dams until PND33 to allow time for planned physiological tests (reported elsewhere). At this age, the offspring used for behavioral testing were placed on Purina 5001 rat chow with a composition by calories of 13.4% fat, 56.7% carbohydrate, and 29.8% protein. One male per litter was randomly selected and assigned to the behavioral tests described here ($n = 11–14$/treatment). All other offspring were used for other studies. Dams and offspring were weighed approximately weekly.

2.2. Experimental procedures

2.2.1. Running wheels

The running wheel system was a stainless steel wire wheel (33 cm diameter; 1.02 m circumference; Starr Life Sciences, Oakmont, PA) placed in the home cage, and wheel revolutions were detected with a magnetic switch.

2.2.2. Novel object recognition

Rats were tested on PND54-57 for novel object recognition (Ennaceur and Delacour, 1988. Red acrylic test boxes were 60 × 50 × 36 cm, and the objects were travel mugs (filled with water) of similar size that differed in color, shape, and surface.

On the first day, rats were habituated to the empty box for 10 min. The next day, they were returned to the box with two different objects in adjacent corners, and allowed 5 min to interact with the objects. One hour later, one of the objects was replaced with a different, novel object, and the rat was allowed 2 min to explore these objects (counted when the rat's nose appeared to be less than about 2 cm from object). Between each rat, the box was cleaned with disinfectant.

The number of visits as well as time spent exploring each object were recorded in the training and test sessions by an observer who was unaware of the rat's treatment. Overall activity was the total time exploring and total number of visits to both objects. For the test session, preference for the novel object was calculated as the ratio of time spent or number of visits to the novel object compared to the total time or visits. Discrimination was measured by comparing each treatment group to a value of 0.5, which indicates equal exploration of both objects.

2.2.3. Social approach

Social approach testing (Varlinskaya and Spear, 2008) took place on PND63-64, using the novel object recognition box with clear acrylic dividers creating three compartments. The test rat was placed in the center compartment. A stimulus (stranger) rat, of the same age and gender as the test rat, was placed in one of the side compartments. Each rat was tested for 10 min, and the number of visits and time spent in close proximity (nose appearing to be less than 2 cm away) of either divider wall was recorded by an observer (unaware of the rat's treatment). The box was cleaned between each trial. Overall activity was calculated, and preference and discrimination were measured comparing exploration of the side with the stranger rat to the empty side.

2.2.4. Morris water maze

Rats were tested in the Morris water maze at age PND83-94. Test procedures have been previously described (Moser et al., 2001; Vorhees and Williams, 2006). Swimming was monitored by a video system (HVS Image, Hampton, UK), allowing analysis of latency, distance, location, and spatial patterns throughout the tank. For initial place training, rats were given two trials per day (approximately 5 min between trials) for 9 days, with a hidden platform in a fixed position. Starting positions varied in a pseudorandom order, and each rat was given 60 s to find the platform where it remained for 15 s. On the tenth day, a reference memory probe (spatial bias for the target quadrant) was conducted with no platform (60 s free swim). This was followed by a visual probe, in which a raised platform with a black band at the water's surface was located in a quadrant opposite that of the original target. For the next three days, using two trials per day, a reversal procedure was conducted with the submerged platform located in the opposite quadrant: all other aspects of training were the same as before.

Learning was evaluated as a change across days in latency, path length, and path ratio (ratio of path taken to the most direct path) to find the platform. On the no-platform probe, the time spent in the target quadrant was measured. For all procedures, percent time floating, active swim speed, and time in the thigmotaxis zone (within 7 cm of tank wall) and the middle annulus were recorded.

2.2.5. Chocolate milk preference

Rats were tested for chocolate milk preference on PND104–106. On the first day, rats were given a 1-h exposure to chocolate milk (1:3 dilution of 1% fat chocolate milk). The next day, water bottles were removed for 4 h, and then a bottle with tap water and one with chocolate milk were placed on each cage (milk bottle alternated sides across each group). After 2 h, bottles were removed and weighed to determine consumption. Preference was measured as the consumption of milk compared to total fluid intake. For additional details, see Slotkin et al. (1999) and Roegge et al. (2008).