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Paternal line multigenerational passage of altered risk assessment behavior in female but not male rat offspring of mothers fed a low protein diet

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HIGHLIGHTS

- Multigenerational behavior effects by paternal line
- Maternal protein restriction causes multigenerational effects on corticosterone.
- Multigenerational sex behavioral difference by paternal line

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ABSTRACT

Maternal low protein (MLP) diets in pregnancy and lactation impair offspring brain development and modify offspring behavior. We hypothesized multigenerational passage of altered behavioral outcomes as has been demonstrated following other developmental programming challenges. We investigated potential multigenerational effects of MLP in rat pregnancy and/or lactation on offspring risk assessment behavior. Founder generation mothers (F_0) ate 20% casein (C) or restricted (R) 10% casein diet, providing four groups: CC, RR, CR, and RC (first letter pregnancy, second letter lactation diet) to evaluate offspring (F_1) effects influenced by MLP in F_0. On postnatal day (PND 250), F_1 males were mated to non-colony siblings producing F_2. On PND 90, F_2 females (in diestrous) and F_2 males were tested in the elevated plus maze (EPM) and open field. Corticosterone was measured at PND 110. Female but not male CR and RC F_2 made more entries and spent more time in EPM open arms than CC females. Overall activity was unchanged as observed in male F_1 fathers. There were no open field differences in F_2 of either sex, indicating that multigenerational MLP effects are due to altered risk assessment, not locomotion. MLP in pregnancy reduced F_1 male and F_2 female corticosterone. We conclude that MLP in pregnancy and/or lactation increases the innate tendency to explore novel environments in F_2 females via the paternal linage, suggesting lower levels of caution and/or higher impulsiveness to explore unknown spaces. Further studies will be necessary to identify the epigenetic modifications in the germ line through the paternal linage.

1. Introduction

Developmental programming of offspring as a result of a suboptimal intra-uterine environment predisposes to adverse health outcomes in offspring throughout life [1–4]. Epigenetic factors may also play a role in the long-term effects on progeny through multiple generations [4–7]. Recently studies show that germ line epigenetic changes can influence offspring brain and behavior development [8]. The impetus to such multigenerational effects can result from chemical environmental exposure or sub-optimal maternal diet.

Female F_2 rats conceived by F_1 intra-uterine growth restricted mothers and then transferred as embryos to control rats show hyperglycemia, hyperinsulinemia, increased hepatic weight and unsuppressed hepatic glucose production in F_2 female offspring [9] while perinatal protein restriction results in increased body mass in F_2 male rats [10]. Glucose metabolism is also adversely affected in the F_3 generation of F_0 female rats fed with a low protein diet during early development [11].

F_0 maternal undernutrition during pregnancy programs reduced birth weight, glucose intolerance and obesity in both the F_1 and F_2.

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Sex-specific transmission of phenotypes could implicate several mechanisms including alterations in metabolic environment (transmaternal inheritance of obesity), gene expression, mediated by developmental and epigenetic pathways (transpaternal inheritance of low body weight), or both (impaired glucose tolerance) [12]. In addition multigenerational effects are often offspring sex dependent. For example, male and female F_3 progeny exhibit opposite anxiety-like behavior following F_0 exposure to vinclozolin, a fungicide with antiandrogenic activity [13].

We have shown that the timing of protein restriction during development mediates sex-dependent inheritance in the F_2, as male rats developed insulin resistance in response to post-natal protein restriction while females developed sensitivity following prenatal protein restriction [14]. In addition to multigenerational effects of restricted maternal diet, maternal high fat diet during pregnancy and lactation produces increased body length, reduced insulin sensitivity, and reduced leptin levels over two generations [15]. Only females displayed the increased body size phenotype in the F_3 generation and this was via the paternal lineage [16].

To date no study to our knowledge has investigated multigenerational behavioral effects following perinatal protein restriction by paternal line. We hypothesized that perinatal protein restriction in F_0 dams results in altered behavioral outcomes in F_2 progeny via the paternal linage.

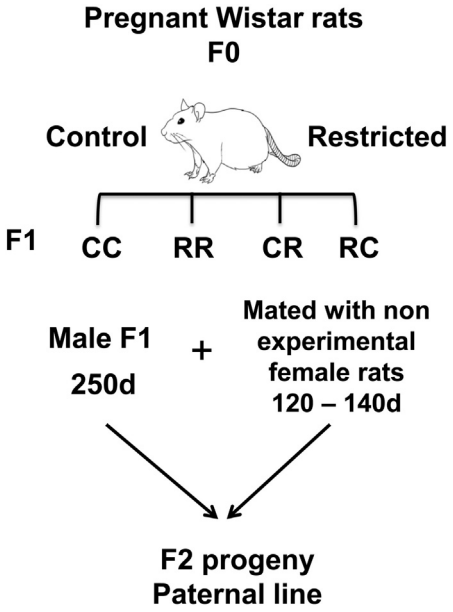

Fig. 1. Schematic of the study design: first letter defines the diet during pregnancy and second letter means diet during lactation. Control (C) and restricted (R).

2. Methods

2.1. Care and maintenance of animals

2.1.1. Breeding and maintenance of F_0 female rats

All procedures were approved by the animal Experimentation Ethics of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City. Fifty virgin female albino Wistar rats aged 16–18 weeks, weighing 220 ± 20 g were obtained from the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (Mexico City, Mexico). Rats were maintained under controlled lighting (lights from 07.00 to 19.00 h) at 22–23 °C. Prior to breeding male and female subjects were maintained on Zeigler, RQ 22-5 rodent diet. Female rats were mated overnight with male breeders and the day on which spermatozoa were present in a vaginal smear was designated as day of conception (day 0). Only rats that were pregnant within 5 days were retained in the study. Pregnant rats were transferred to individual cages and allocated at random to one of two groups to be fed either 20% casein (control diet) or 10% casein isocaloric diet (restricted). Food and water were available ad libitum for all animals.

All F_0 rats delivered the F_1 offspring by spontaneous vaginal delivery. Timing of delivery of the F_1 and F_2 pups and morphometric measurements was recorded at birth. Ano-genital distance was measured with calipers to determine offspring sex [17]. To ensure homogeneity of study subjects, litters of over 14, or less than 10, pups were excluded from the study. Litters were adjusted to 10 pups for each dam while maintaining as close to a 1:1 sex ratio as possible.

Four groups were established. C represents control and R the restricted diet. The first letter defines the diet mothers received during pregnancy and the second the diet in lactation: CC control during pregnancy and lactation, RR restricted during pregnancy and lactation, CR restricted only during lactation and RC restricted only during pregnancy. After weaning all pups were maintained on Zeigler RQ 22-5 rodent diet.

2.2. Breeding of F_1 males

To produce the F_2, F_1 males aged 250 days were bred with non-experimental female Wistar rats aged 16–18 weeks with regular estrous cycles. During pregnancy and for the rest of the study, all rats were fed with control diet. All F_1 females underwent spontaneous vaginal delivery (Fig. 1).

2.3. Elevated plus maze (EPM)

At PND 75, two weeks prior to all behavioral testing, a reverse light cycle was implemented (lights off at 07.00 h, and on at 19.00 h) so that testing could be conducted in the animals' dark phase corresponding to the investigators' day time. For females behavioral assessments were performed during the diestrous cycle. Body weight was recorded at the beginning of the behavioral test. Subjects were assessed at the same time of the dark cycle between 08.00 h and 14.00 h. The EPM was constructed of dark gray plastic and was situated 64 cm above the floor. It consisted of two unprotected arms (open arms, 45 cm × 10 cm each) facing each other and two arms protected by high gray walls at 90° from the open arms (closed arms, 45 cm × 10 cm each), all extended from a common central platform (10 cm × 10 cm). The light level was 30 lx in the open arms and 6 lx in the closed arms. The rat's position on the maze was recorded via a video camera mounted on the ceiling above the center of the maze. The camera was connected to a video tracking motion analysis system (Ethovision, Noldus Information Technology by Wageningen, The Netherlands) running on a personal computer. To start the session each rat was placed individually at the center of the maze facing an open arm. After 10 min of EPM exploration, the rat was returned to its home cage and the EPM was cleaned with 70% ethanol. An experimenter blind to the subject's treatment group manually scored the number of entries into the predefined zones of the open and closed arms, while the Ethovision system measured the distance traveled and the time spent in the different areas. An arm entry was scored only if the rat's center of gravity entered into the arm. All subjects were tested in a randomized sequence.

2.4. Open field

The day following EPM testing, the same subjects were evaluated in a 60-minute open field test. The open field, made of dark gray Plexiglas, consisted of a square arena (101 cm × 101 cm and 34 cm high) which was located in an experimental room illuminated by low light (12 lx).