No Significant Effect of Maternal Perception of the Food Environment on Reproductive Success or Pup Outcomes in C57BL/6J Mice

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Objective: Prior work concerning maternal perception of the food environment suggests that perceived disparities in food resources resulted in reduced pup mass and dam reproductive success. This study attempted to replicate this result with increased sample size and additional measures.

Methods: Female C57BL/6J mice ($n = 160$; 3 weeks old) were randomly assigned to either subject or peer and were pair housed in partitioned cages with olfactory and visual contact. After a 6-week maturation period on an energy-rich cafeteria diet, cages were randomly assigned to Control (subject and peer fed pelleted diet) or Treatment (subject fed pellets, peer fed cafeteria diet), and subjects were bred. After weaning, one pup from each sex per litter was reared to 5 months.

Results: Treatment did not affect the number of births, pup size at birth, or the proportion of pups surviving to weaning ($P > 0.09$). Treatment did not affect dam body or fat mass at parturition ($P > 0.22$), but these measures were higher in some Treatment dams at weaning ($P < 0.05$). Smaller female pups were weaned from Treatment dams pregnant on the first breeding attempt ($P = 0.01$), but no other pup effects were observed ($P > 0.07$).

Conclusions: Exposure to food-environment disparity in this study did not replicate previous findings or affect pup growth after weaning.

Introduction

The potential potency of transgenerational effects and the influences of the intrauterine and postnatal environments on long-term health and obesity are topics of active exploration. Indeed, at two ends of the developmental spectrum, in their strategic plans, the National Institute on Aging wrote that investigators should strive to “identify developmental, prenatal, early life, and environmental processes that affect individual differences in aging and risk of disease” (1), while the National Institute on Child Health and Human Development wrote, “Understanding the developmental origins of health and disease will benefit from interdisciplinary … studies … prioritizing research on today’s most common chronic conditions and diseases, such as obesity …” (2). Epidemiological studies and follow-up work in animal models suggest that alterations in maternal nutrition during pregnancy influence the long-term health of offspring, in some cases contributing to an obesogenic phenotype (3,4). Upon reviewing the state of research investigating developmental programming and its influence on obesity, researchers from a scientific symposium held at Pennington Biomedical Research Center in 2014 indicated a need for further research “identifying the mechanisms which cause or contribute to developmental programming” (5). The contribution of realized and perceived social disparity to...
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developmental effects remains poorly understood in part because of a lack of appropriate animal models of the condition. We propose such a model using C57BL/6J mice.

Previously we showed that a mother’s perception of the food environment might affect her offspring (6). Specifically, we tested the effects of maternal exposure to the sights and smells of conspecifics who were provided with an aromatic, varied, energy-dense food supply (i.e., a cafeteria diet) yet were themselves consuming an ordinary low-fat pelleted diet. In that study, we found a statistically significant effect of a perceived rich food environment that one cannot access, perhaps an experience of social disparity, on reducing pup mass and body fat at weaning and borderline results suggesting greater difficulty in achieving successful pregnancy for dams. We concluded, “Although limited in sample size and power, our results suggest that perceptions of the social energetic environment influence reproductive physiology and offspring body composition. This calls for additional experiments to replicate the findings and if confirmed, to test the generality across species, and the proposed hypotheses” (6). In the present report, we describe such an attempted replication.

Methods

Animals and general husbandry
All procedures were approved by and conducted in accordance with the guidelines of the University of Alabama at Birmingham Institutional Animal Care and Use Committee. Female (3 weeks old) C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, Maine) in August 2015. Upon arrival, females (n = 160) were housed in pairs (subject and peer) in the Optimice cage system (Animal Care Systems, Inc., Centennial, Colorado) at 22 ± 2°C on a 12-hour light/dark cycle (lights on at 4 AM). The clear polycarbonate cages were fitted with Unimice polycarbonate cage divider kits (Animal Care Systems, Inc.) that allowed visual, auditory, and olfactory (but no physical) contact between mice. Cages contained autoclaved hardwood chip bedding (NEPCO Bedding-Beta Chip, Warrensburg, New York), and each side of the cage contained a water bottle (autoclaved tap water), an isolated portion of the stainless steel food hopper, and Enviro-dri nesting material (Shepherd Specialty Paper, Milford, New Jersey).

Male (6 weeks old) C57BL/6J mice were purchased from Jackson Laboratory in September 2015. Upon arrival, males (n = 80) were group housed (two to three per cage) in polycarbonate cages containing hardwood chip bedding, Enviro-dri nesting material, and a Hydropac watering system (Lab Products, Inc., Seafood, Delaware). Males were given ad libitum access to a purified, pelleted, low-fat diet (10% kcal from fat) (D12450B, Research Diets, Inc., New Brunswick, New Jersey).

Phase I: maturation on cafeteria diet
Upon arrival, females (3 weeks old) were assigned to pairs (subject and peer) by using a random number generator, and each subject and peer were randomly assigned to either the right or left side of the partitioned cage (50/50 right/left). Females were fed a cafeteria diet daily for 6 weeks. Each day, females were proffered an item from each of three general categories (carbohydrate, fat/sugar, protein) (Table 1), and uneaten food was removed after 24 hours. Body mass was assessed weekly through 9 weeks of age to the nearest 0.01 g by using a precision balance. At 8 weeks old, one pellet of purified, low-fat diet (10% kcal from fat; D12450B, Research Diets, Inc.) was included in the food hopper with the cafeteria diet items to expose the mice to a pelleted diet for 1 week before the next phase of the experiment.

Phase II: breeding, gestation, and parturition
At 9 weeks of age, the body composition of females was assessed by quantitative magnetic resonance (QMR) (EchoMRI 3-in-1, software version 2013; EchoMRI LLC, Houston, Texas), as previously described and validated (7). Cages were then assigned to Treatment (n = 40) or Control (n = 40) by a random number generator. In the Treatment group, peers continued to receive the cafeteria diet feeding without low-fat diet pellets and subjects were switched from the cafeteria diet to the low-fat diet only. In the Control group, both peers and subjects were switched to the low-fat diet. Males (8 weeks old) were introduced to subjects for breeding, but peers were not bred. After 6 days, males were removed and returned to their original group-housed cage.

For 3 weeks following breeding, body mass and food intake (i.e., food proffered [g] – food remaining [g]) for subjects, both of which were on the low-fat diets, were assessed twice weekly. Frequent

| TABLE 1 Cafeteria food items |
|-------------------------------|
| **Carbohydrate** | **Fat/sugar** | **Protein** |
| Monday | Nacho cheese tortilla chips | Peanut butter candies | Hot dog |
| Tuesday | Rippled plain potato chips | Cinnamon raisin bagel | Sharp cheddar cheese |
| Wednesday | Raw macaroni pasta | Chocolate chips | Bologna |
| Thursday | Fruit cereal rings | Peanut butter cookie | Hot dog |
| Friday | Plain croutons | Vanilla cookie | Cocktail sausage |
| Saturday | Cheddar crackers | Chocolate rice crisp bar | Mozzarella cheese |
| Sunday | Chocolate puff cereal | Chocolate chips | Barbecue pork rinds |

During maturation period (age: 3 to 9 wk), all female mice proffered one item from each category each day. Only peer mouse in Treatment group remained on this diet throughout study. Uneaten food removed after 24 h.
measures of body mass for subjects assisted in monitoring body mass changes during gestation. As observed in the pilot study (6), steady increases in body mass beyond normal mass gain for virgin mice (approximately > 1.9 g/wk) indicated pregnancy. Rapid declines in the body mass of pregnant mice (approximately ≥ 1.9 g/wk) were indicative of apparent miscarriages. As subjects approached the third week of potential gestation, cages were monitored daily for signs of parturition. Within 24 hours of parturition, dams and their litters were weighed, the number of pups born was recorded, and the body composition of dams was assessed by QMR. Peers were removed and euthanized. Dams and litters were placed with home nesting material into a clean Optimice cage containing fresh bedding with the partition removed and were fed the low-fat diet ad libitum throughout lactation.

Four weeks after the first breeding attempt (i.e., Wave 1), subjects that did not become pregnant were paired for 6 days with males who had previously sired a litter. Subjects and peers were monitored for 3 weeks as described above, and the same procedures were followed upon parturition. Any subjects not producing a litter after the second breeding attempt (i.e., Wave 2) were euthanized along with their peers.

Phase III: lactation and weaning

During the 3-week lactation period, dams and litters were weighed every other day, food intake was measured, and the number of live pups was recorded. On day 21, pups were weaned and their body mass was measured. Body mass and body composition by QMR were assessed for dams. One female and one male pup with median body mass from each litter were selected and were haphazardly assigned (experimental group assignment blinded) to a same-sex pair cage with the cage divider kits allowing individuals to be observed longitudinally. Pups were given ad libitum access to the low-fat diet. Dams and the remaining pups were euthanized.

The body composition of euthanized pups was determined from carcasses by using chemical analysis. Briefly, carcasses were opened and dried at 65°C. The loss of mass during drying was body water. Fat mass for these pups was calculated as (fat-free dry mass – ash) + water mass.

Phase IV: pup growth and body composition

Pups were monitored daily through 21 weeks of age. Body mass and food intake for individual pups were assessed weekly. Body composition was measured by QMR every 4 weeks starting at 8 weeks old. Pups were euthanized at 21 weeks old.

Statistical analyses

Because of differences in the duration of exposure to Control or Treatment conditions between litter cohorts, data from dams pregnant on, and pups born from, the first breeding attempt (Wave 1) were analyzed separately from data collected from dams pregnant on, and pups born from, the second breeding attempt (Wave 2). The number of dams giving birth between the Control and Treatment groups was compared by using a χ² test, and the median numbers of pups born to either group were tested by using Wilcoxon score (rank sums) nonparametric tests. Dam body mass, composition, and total food intake during pregnancy were analyzed by using generalized linear models, and litter size was considered as a covariate but was removed from models when it lacked statistical significance. Pup size at birth, weaning, survival to weaning, and body composition at weaning were modeled by using mixed models with adjustments for relatedness by dam identification number as a random effect. Pup mass from ages 3 to 21 weeks (average mass and maximum mass) was analyzed by using a mixed linear model with dam identification set as a random effect and repeated measures modeled with an autoregressive moving average (1,1) covariance structure. An alpha level of 0.05 (two-tailed) was set as the significance level. All analyses were run using SAS 9.4 (SAS Institute, Inc., Cary, North Carolina).

Results

Maternal data: prebreeding, pregnancy, and lactation

By the end of the 6-week maturation period on the cafeteria diet, no significant differences were observed between groups for body (P > 0.631), fat (P > 0.744), or lean mass (P > 0.503; Table 2); however, dams that became pregnant on the first breeding attempt were larger (P = 0.011) and had more lean mass (P < 0.0001) (Table 2). No significant differences in total food intake during pregnancy were observed between groups for either Wave 1 or Wave 2 (P > 0.254; Table 3), and litter mass at birth was not a significant covariate for food intake during pregnancy (P > 0.105). When comparing prebreeding body mass to that at parturition, all dams gained body mass, and food intake was a significant covariate (P < 0.001); however, there were no significant group effects on the change in body mass (P > 0.207; Table 3). Analysis of body composition changes from prebreeding to parturition indicated a mean fat mass...
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TABLE 3 Total food intake during pregnancy and change in body mass during pregnancy, body composition changes from prebreeding to parturition, and body mass and composition changes from parturition to weaning, separated by group and wave

|                          | Control                  | Treatment                | P     |
|--------------------------|--------------------------|--------------------------|-------|
| Food intake (g)          | Wave 1: 39.9 ± 1.08 (22) | Wave 2: 40.7 ± 1.03 (24) | 0.580 |
|                          | Wave 2: 41.4 ± 1.26 (13) | Wave 2: 43.6 ± 1.42 (12) | 0.254 |
| Pregnancy                |                          |                          |       |
| Change in body mass (g)  | Wave 1: 3.19 ± 0.27 (22) | Wave 2: 2.77 ± 0.35 (24) | 0.166 |
|                          | Wave 2: 4.01 ± 0.37 (13) | Wave 2: 4.22 ± 0.41 (12) | 0.674 |
| Change in fat mass (g)   | Wave 1: −1.16 ± 0.24 (22) | Wave 2: −1.24 ± 0.27 (24) | 0.701 |
|                          | Wave 2: −1.36 ± 0.32 (13) | Wave 2: −1.32 ± 0.31 (12) | 0.833 |
| Change in lean mass (g)  | Wave 1: 3.51 ± 0.15 (22) | Wave 2: 3.25 ± 0.20 (24) | 0.198 |
|                          | Wave 2: 4.29 ± 0.26 (13) | Wave 2: 4.84 ± 0.39 (12) | 0.544 |
| Lactation                |                          |                          |       |
| Change in body mass (g)  | Wave 1: 1.25 ± 0.25 (20) | Wave 2: 1.24 ± 0.29 (17) | 0.985 |
|                          | Wave 2: 0.84 ± 0.45 (10) | Wave 2: 1.19 ± 0.39 (11) | 0.559 |
| Change in fat mass (g)   | Wave 1: 0.22 ± 0.11 (20) | Wave 2: 0.22 ± 0.13 (17) | 0.981 |
|                          | Wave 2: 0.48 ± 0.31 (10) | Wave 2: 0.48 ± 0.22 (11) | 0.992 |
| Change in lean mass (g)  | Wave 1: 1.49 ± 0.16 (20) | Wave 2: 1.57 ± 0.23 (17) | 0.368 |
|                          | Wave 2: 0.93 ± 0.30 (10) | Wave 2: 1.01 ± 0.26 (11) | 0.200 |

Values represent absolute means ± SE (n). Dams randomly assigned to equal food environment between subject and peer cage mates were classified as Control, and dams randomly assigned to disparate food environment between subject and peer cage mates were classified as Treatment. Subjects becoming pregnant on first breeding attempt were designated as Wave 1. Subjects becoming pregnant on second breeding attempt were designated as Wave 2. No covariates used in analysis.

loss of 1.27 ± 0.29 g SE and a mean lean mass gain of 3.97 ± 0.25 g SE across groups and waves, but there were no significant group effects (P > 0.166; Table 3) and litter mass was not a significant covariate (P > 0.05).

TABLE 4 Number of dams giving birth in Control and Treatment groups and number of dams not becoming pregnant after two breeding attempts (A group) and total number of pups born to each group (B group)

|                          | Control | Treatment | Total |
|--------------------------|---------|-----------|-------|
| A group                  |         |           |       |
| Wave                     | 1       | 2         |       |
| Control                  | 22      | 14        | 4     | 40   |
| Treatment                | 24      | 12        | 4     | 40   |
| Total                    | 46      | 26        | 8     | 80   |

Values represent absolute means ± SE (n). Dams experiencing equal food environment were classified as Control, and dams experiencing disparate food environment were classified as Treatment. Subjects becoming pregnant on first breeding attempt were designated as Wave 1. Subjects becoming pregnant on second breeding attempt were designated as Wave 2. (A: χ² = 0.241, df = 2, P = 0.887; B: Wilcoxon test P_{wave1} = 0.391, P_{wave2} = 0.206.)

No apparent miscarriages were observed for either group or wave of pregnancies. The number of dams giving birth per group did not differ significantly between the Control and Treatment groups (Table

TABLE 5 Dam body, fat, and lean mass at parturition and weaning

|                          | Control | Treatment | P     |
|--------------------------|---------|-----------|-------|
| Parturition              | Wave 1  | 23.1 ± 0.33 (22) | 22.6 ± 0.34 (24) | 0.219 |
|                          | Wave 2  | 22.7 ± 0.30 (14) | 23.3 ± 0.77 (12) | 0.734 |
| Fat mass (g)             | Wave 1  | 3.85 ± 0.10 (22) | 3.75 ± 0.08 (24) | 0.223 |
|                          | Wave 2  | 3.82 ± 0.17 (14) | 3.75 ± 0.22 (12) | 0.409 |
| Lean mass (g)            | Wave 1  | 17.9 ± 0.23 (22) | 17.5 ± 0.25 (24) | 0.300 |
|                          | Wave 2  | 17.4 ± 0.25 (14) | 18.2 ± 0.52 (12) | 0.164 |
| Weaning                  | Wave 1  | 24.7 ± 0.30 (20) | 24.6 ± 0.31 (17) | 0.715 |
|                          | Wave 2  | 23.6 ± 0.47 (10) | 25.3 ± 0.22 (11) | 0.026 |
| Fat mass (g)             | Wave 1  | 4.16 ± 0.09 (20) | 4.11 ± 0.09 (17) | 0.722 |
|                          | Wave 2  | 4.22 ± 0.26 (10) | 4.42 ± 0.25 (11) | 0.051 |
| Lean mass (g)            | Wave 1  | 19.5 ± 0.23 (20) | 19.5 ± 0.26 (17) | 0.837 |
|                          | Wave 2  | 18.4 ± 0.42 (10) | 19.7 ± 0.28 (11) | 0.017 |

Values represent absolute means ± SE (n). Dams experiencing equal food environment were classified as Control, and dams experiencing disparate food environment were classified as Treatment. Subjects becoming pregnant on first breeding attempt were designated as Wave 1. Subjects becoming pregnant on second breeding attempt were designated as Wave 2. Litter mass at birth was significant covariate for lean mass at parturition. No other covariates used.
The number of pups born per litter in the Control group ranged from five to nine for Wave 1 and one to eight in Wave 2. For both Wave 1 and 2, the number of pups born per litter in the Treatment group ranged from three to ten. No significant differences between groups were observed for the average number of pups per litter or the average pup size at birth ($P > 0.19$; Table 6). In Wave 1, a higher proportion of pups in the Control group survived to weaning ($P = 0.23$); however, in Wave 2, the trend reversed with a greater proportion of pups surviving in the Treatment group ($P = 0.09$; Table 6).

At weaning, female pups born to Treatment dams in Wave 1 were smaller than female pups born to Control dams ($P = 0.01$; Table 6), but male pups had similar body masses in each group ($P = 0.08$; Table 6). For Wave 2 pups, no significant differences in body mass were observed for female pups ($P = 0.48$; Table 6), with male pups showing marginally larger body mass in the Treatment group, $P = 0.05$; Table 6). The body composition of pups at weaning for both waves was not significantly different between groups ($P > 0.07$; Table 6). No significant differences in the number of female or male pups weaned were observed ($P > 0.632$; Supporting Information Table S1).

Pups followed to 21 weeks of age demonstrated no significant differences in average body mass ($P = 0.669$ for males and $P = 0.325$ for females) or maximum body mass ($P > 0.29$) (Figure 1). Monthly assessment of body composition by QMR revealed no significant group effects on body, fat, or lean mass (Table 7).

### Table 6 Litter size and body mass of pups at birth with body mass and composition data at weaning for pups

| Litter size at birth (#) | Control | Treatment | $P$ |
|-------------------------|---------|-----------|-----|
| Wave 1                  | 7.05 ± 0.22 (22) | 6.63 ± 0.32 (24) | 0.292 |
| Wave 2                  | 6.00 ± 0.66 (14) | 7.08 ± 0.53 (12) | 0.196 |

| Pup mass at birth (g)   | Control | Treatment | $P$ |
|-------------------------|---------|-----------|-----|
| Wave 1                  | 1.22 ± 0.01 (155) | 1.23 ± 0.01 (159) | 0.204 |
| Wave 2                  | 1.22 ± 0.03 (78)  | 1.24 ± 0.02 (85)  | 0.749 |

| Survival to weaning (%) | Control | Treatment | $P$ |
|-------------------------|---------|-----------|-----|
| Wave 1                  | 83.8 ± 3.64 (116) | 77.1 ± 3.95 (95) | 0.225 |
| Wave 2                  | 54.4 ± 9.51 (42)  | 78.7 ± 9.51 (64) | 0.082 |

Values represent absolute means ± SE (n). Pups born to dams experiencing equal food environment were classified as Control, and pups born to dams experiencing disparate food environment were classified as Treatment. Pups born from first breeding attempt were designated as Wave 1. Pups born from second breeding attempt were designated as Wave 2. Dam identification included as random effect.

4; $\chi^2 = 0.241$; $P = 0.887$). Dam body, fat, and lean mass at parturition did not differ significantly between groups for either Wave 1 or Wave 2 ($P > 0.16$; Table 5), and litter mass at birth was only a significant covariate for lean mass ($P < 0.02$). One dam in Wave 2 from the Control group cannibalized pups as they were born, so after QMR at parturition, no further measures were collected on the dam. During the lactation period (parturition to weaning), there were no significant group effects on changes in body mass ($P > 0.559$), fat ($P > 0.981$), or lean mass of dams ($P > 0.200$; Table 3), and litter mass at weaning was a significant covariate for change in lean mass. At weaning, no significant differences were observed between groups in Wave 1 for dam body, fat, or lean mass ($P > 0.53$; Table 5), even when adjusted for litter mass at weaning. In Wave 2, dams in the Treatment group had greater body mass ($P = 0.026$; Table 5) due to slightly higher fat mass ($P = 0.051$; Table 5) and lean mass ($P = 0.017$; Table 5).

**Figure 1** Growth curve of male and female pups isolated from Control and Treatment group litters. Pups born to dams experiencing equal food environment were classified as Control, and pups born to dams experiencing disparate food environment were classified as Treatment. Values represent mean body mass ± SE (n) = 9-29 for weekly body mass from 3 weeks of age (weaning) to 21 weeks of age. No significant differences in average body mass or maximum body mass were observed between groups or waves ($P > 0.238$).
Discussion

In the present study, we sought to replicate the original pilot experiment while doubling the sample size and incorporating additional measurements of ad libitum food intake during pregnancy, body mass, and body composition. The exposure to an apparent disparate food environment in this study did not significantly affect measured outcomes associated with dam physiology or reproduction. Similar to the original pilot study (6), dam body, fat, and lean mass were not differentially influenced by the perceived food environment. The additional measures in the present study of food intake during pregnancy, body mass, and composition changes from prebreeding to parturition and again after the lactation period did not reveal any significant group effects.

Unlike the original study (6), we did not observe any apparent miscarriages. In the present study, we measured pup outcomes from birth, through lactation, and at weaning and did not discern significant effects of dam perception of the food environment on the number or size of pups in litters. Survival of litters to weaning seemed to be reduced for dams in the disparate environment, but this trend was not significant and was reversed in the second set of pregnancies, also not a significant difference. Dam pregnancy only after a second breeding attempt may be related to moderately smaller body size prior to breeding, and this difference cannot be eliminated as a possible contribution to how successfully dams reared pups to weaning.

We did not observe significant effects of the dams’ perceived food environment on pup body mass or composition at weaning, which is in contrast to the original study in which pups born to dams in the disparate food environment had lower carcass mass and fat mass (6). To explore the potential long-term impacts of being born to dams of disparate food environments, we followed the food intake, body mass, and body composition of male and female pups from each group. We did not observe group differences in these outcomes for either sex by 20 weeks of age.

Methodological differences between the pilot study and the current study should be considered when interpreting the different outcomes. The breeding procedure used in the pilot study differed from that used in the present experiment. In the first study (6), 6-week-old males were housed with subject females (8 weeks old) for at least 2 weeks, possibly more, until females demonstrated mass gain indicative of pregnancy. During this time, females and males were proffered a double portion of 95% of the portion given to ad libitum-fed peer mice in the control group. The extended exposure to males made pinpointing the time frame of conception difficult in the pilot study. By condensing the breeding time to a 6-day period, we reduced exposure to the males in the present study, allowed ad libitum access to the diet during breeding, and were able to correlate measured values of dam body mass to the gestation period. Additionally, mice used in the current study were older at the time of breeding (females were at least 9 weeks old and males were at least 8 weeks old). Thus, the effects observed in the pilot study may be specific to the age of the females and/or additional potential stressors associated with longer housing with males.

We did not observe significant group effects on food intake for dams during pregnancy; however, the diet proffered was a low-fat formulation (10% kcal from fat, 20% kcal from protein, 70% kcal from carbohydrate). In human populations, diets consumed under disparate social conditions typically contain high proportions of fat and sugar (9). The diet used in the pilot study (6) was based on the NIH-31 open formula (7017, Teklad Diets; Envigo, Huntingdon, Cambridgeshire, UK) and contained slightly higher energy contributions from fat and protein (14% kcal from fat, 24% kcal from protein, 62% kcal from carbohydrate), and the diet from the present study contained a higher proportion of carbohydrates. A lower protein/carbohydrate ratio has been shown to improve metabolic outcomes in mice, in a manner similar to that of calorie restriction (10). Whether the slight differences in diet composition between the pilot study and the current work affected reproduction outcomes in dams is not known. Future work to develop animal models of social disparity should consider incorporating an element of choice among diets of varied nutritional composition (low fat, high fat, and/or high sugar). Differences in nutritional preferences under perceived disparate conditions may indicate behavioral influences on food intake and subsequent physiology.

Conclusions from the pilot study led to two proposed hypotheses to explain the mechanism by which reproductive physiology was apparently influenced by perceived disparity. The first hypothesis suggested the perception of an energy-rich environment without access to energy-rich food caused dams to initiate energy conservation that insufficiently supported gestation (6). We did not observe any physiological changes to suggest that dams had shifted from gestational

| TABLE 7 Body, fat, and lean mass for pups at 20 wk of age |
|----------------------------------------------------------|
| Control | Treatment | P         |
|---------|-----------|-----------|
| **Body mass (g)** | | |
| Wave 1  | Female | 22.4 ± 0.36 (20) | 22.9 ± 0.33 (17) | (0.30; 0.91) |
|         | Male    | 30.5 ± 0.69 (20) | 30.1 ± 0.84 (17) | (0.13; 0.54) |
| Wave 2  | Female | 22.3 ± 0.54 (9)  | 23.0 ± 0.57 (10) | (0.70; 0.92) |
|         | Male    | 29.5 ± 0.84 (9)  | 30.3 ± 0.85 (11) | (0.35; 0.54) |
| **Fat mass (g)** | | |
| Wave 1  | Female | 4.56 ± 0.24 (20) | 4.71 ± 0.25 (17) | (0.05; 0.59) |
|         | Male    | 8.16 ± 0.49 (20) | 8.09 ± 0.64 (17) | (0.90; 0.78) |
| Wave 2  | Female | 4.21 ± 0.24 (9)  | 4.66 ± 0.33 (10) | (0.35; 0.54) |
|         | Male    | 7.07 ± 0.58 (9)  | 7.81 ± 0.71 (11) | (0.35; 0.54) |
| **Lean mass (g)** | | |
| Wave 1  | Female | 16.9 ± 0.19 (20) | 17.3 ± 0.19 (17) | (0.05; 0.59) |
|         | Male    | 21.4 ± 0.22 (20) | 21.2 ± 0.24 (17) | (0.90; 0.78) |
| Wave 2  | Female | 16.9 ± 0.34 (9)  | 16.9 ± 0.30 (10) | (0.90; 0.78) |
|         | Male    | 21.0 ± 0.27 (9)  | 21.1 ± 0.30 (11) | (0.90; 0.78) |

Values represent absolute means ± SE (n). Pups born to dams experiencing equal food environment were classified as Control, and pups born to dams experiencing disparate food environment were classified as Treatment. Dam identification included as random effect in mixed model.
support to energy conservation for themselves. Perceptions of nutrient availability via sensory systems without realized differences in available nutrients have been shown to influence life-span in the fruit fly, *Drosophila melanogaster* (11), but to our knowledge, similar perception of energetic resources affecting reproductive success has not been reported in mammalian models. The second hypothesis suggested that the inability of Treatment dams to access energy-rich foods may have triggered a social disparity in which they experienced a lower position in the dominance hierarchy and potentially a perception of resource uncertainty (6). We did not evaluate dams for behavioral signs of anxiety or stress, and the physiologic metrics observed in the current study do not indicate signs of apparent social disparity.

Reproducibility among scientific studies aids in the advancement of hypothesis testing by directing future efforts either toward the paths highlighted from confirmatory results or toward revisiting the model of the phenomenon. Although the results of the present study did not replicate the earlier findings of the pilot study, we have learned that this particular model of perceived disparity may not appropriately illustrate the physiologic effects associated with this perception. Future studies can be designed to evaluate different potential triggers, and interactions among triggers, of perceived disparity and apparent transgenerational effects on metabolic health.

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References

1. National Institute on Aging. Aging well in the 21st century: strategic directions for research on aging. National Institute on Aging website. https://www.nia.nih.gov/about/aging-well:21st-century-strategic-directions-research-aging. Published 2016. Accessed May 18, 2017.
2. Eunice Kennedy Shriver National Institute of Child Health and Human Development. Scientific Vision: The Next Decade. Bethesda, MD: National Institutes of Health; 2012. https://www.nichd.nih.gov/publications/pubs/Documents/NICHD_scientific_vision120412.pdf. Accessed May 18, 2017.
3. Aiken CE, Ozanne SE. Transgenerational developmental programming. *Hum Reprod Update* 2014;20:63-75.
4. Vickers MI. Developmental programming and transgenerational transmission of obesity. *Ann Nutr Metab* 2014;64:26-34.
5. Sutton EF, Gilmore LA, Dunger DB, et al. Developmental programming: state-of-the-science and future directions - summary from a Pennington Biomedical symposium. *Obesity (Silver Spring)* 2016;24:1018-1026.
6. Schwartz TS, Gainer R, Dohn ED, Johnson MS, Wyss JM, Allison DB. Second-hand eating? Maternal perception of the food environment affects reproductive investment in mice. *Obesity (Silver Spring)* 2015;23:927-930.
7. Jones A, Johnson M, Nagy T. Validation of quantitative magnetic resonance for the determination of body composition of mice. *Int J Body Compos Res* 2009;7:67-72.
8. Debush G, Ankey C, Keremanz D. The effect of apparatus, extraction time, and solvent type on lipid extractions of snow geese. *Can J Zool* 1985;63:1917-1920.
9. Satia JA. Diet-related disparities: understanding the problem and accelerating solutions. *J Am Diet Assoc* 2009;109:610-615.
10. Solon-Burt SM, Mitchell SJ, Coogan SCP, et al. Dietary protein to carbohydrate ratio and caloric restriction: Comparing metabolic outcomes in mice. *Cell Rep* 2015;11:1527-1534.
11. Pletcher SD. The modulation of lifespan by perceptual systems. *Ann N Y Acad Sci* 2009;1170:693-697.