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Effects of paternal high-fat diet and rearing environment on maternal investment and development of defensive responses in the offspring

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ABSTRACT

Paternal preconception risk factors (e.g. stress, diet, drug use) correlate with metabolic dysfunction in offspring, which is often comorbid with depressive and anxiety-like phenotypes. Detection of these risk factors or deleterious phenotypes informs a female about prevailing ecological demands, in addition to potential adverse environment-induced phenotypes that may be disseminated to her offspring. We examined whether a F_0 male rat's prior exposure to an obesogenic high-fat diet (HFD) influences a female's attraction towards a male, subsequent mother-infant interactions and the development of defensive (emotional) responses in the F_1 offspring. Females displayed less interest in the HFD exposed F_0 males relative to control diet-exposed F_0 males. Dams that reared F_1 offspring in larger, semi-naturalistic housing provided more licking and grooming and active arched-back-nursing behavior. However, some of these effects interacted with paternal experience. F_0 HFD and maternal rearing environment revealed sex-dependent, between group differences in F_1 offspring wean weight, juvenile social interactions and anxiety-like behavior in adolescence. Our results show for the first time in mammals that male exposure to HFD may contribute to stable behavioral variation among females in courtship, maternal care, even when the females are not directly exposed to a HFD, and anxiety-like behavior in F_1 offspring. Furthermore, when offspring were exposed to a predatory threat, hypothalamic *Crf* gene regulation was influenced by early housing. These results, together with our previous findings, suggest that paternal experience and maternal rearing conditions can influence maternal behavior and development of defensive responses of offspring.

Significance statement

The differential allocation hypothesis implies that animals can detect qualities of a potential mate and then vary their own reproductive investment accordingly, yet little is known about the effects of paternal preconception diet in programming offspring development and anxiety behavior. The authors examine the effects of high-fat diet in male rats on female partner preference and maternal care, and show sex-specific changes in juvenile play and anxiety-related behavior. Epigenetic regulation of hypothalamic *Crf* in response to stress is also influenced by paternal high-fat diet, which is contextually-dependent on the rearing environment. This argues that preconception paternal high-fat diet and housing can influence maternal care and the development of defensive behaviors of offspring.

1. Introduction

Adverse intrauterine and early postnatal environments have lasting pathological consequences for hypothalamic-pituitary-adrenal (HPA) axis adaptation and metabolic programming, resulting in increased vulnerability for stress-related disorders in adolescence and adulthood (Sullivan et al., 2014; Lin et al., 2015). Clinical and epidemiological studies examining risk for common chronic health conditions, including obesity, hypertension, type 2 diabetes, cardiovascular disease and cancer have largely focused on maternal health before and during pregnancy, as well as perinatal factors and adult environmental exposures in the offspring (Vickers et al., 2007). Maternal exposure to high-fat diets (HFD) has been implicated in programming of offspring metabolic dysfunction and obesity risk (Chang et al., 2008) as well as anxiety-like behavior (Sasaki et al., 2014; Balsevich et al., 2015). However, it has become clear that paternal preconception risk factors, such as advanced age (Smith et al., 2013), smoking (Langley et al.,

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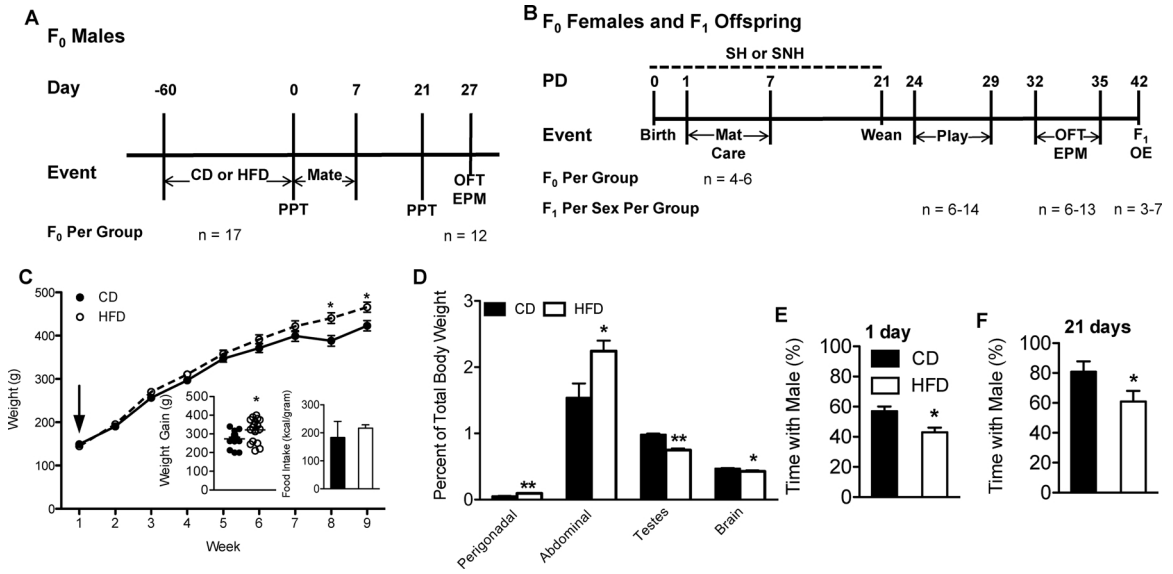

Fig. 1. Experimental timelines, weight gain, food intake, body weights in F_0 males following diet manipulation, and use of a partner preference test (PPT) to ascertain female preference for males previously exposed to high fat diet (HFD) relative to control diet (CD). (A) Timeline of treatment procedures involving F_0 males. Males were fed either control diet (CD) or high fat diet (HFD) for 60 days prior to a partner preference test (PPT) using sexually receptive virgin, naïve females. Within 12 h of the PPT, males were bred with *different* receptive naïve virgin females. Following confirmed mating, males were removed and females were left undisturbed until offspring were born. After mating, F_0 males were maintained on standard chow for 21 days before to a second PPT. One week after the second PPT, F_0 male anxiety behavior was assessed in the elevated plus maze (EPM) and open field test (OFT). (B) Timeline of treatment procedures for F_0 females and the F_1 offspring. Birth was considered postnatal day 0, and offspring were counted, sexed, and weighed before being transferred to either fresh standard housing (SH) or semi-naturalistic housing (SNH), with biological mothers, until weaning. Maternal behavior (Mat Care) was scored for 72 min, 5 times per day for 7 days. At PD 21, all offspring were weighed, weaned and placed in SH with a same-sex littermate. Play behavior was recorded in the home cage from PD 24–29, followed by exposure to the open-field test (OFT) and the elevated plus maze (EPM) on PD 32–35. F_1 OE took place on PD 42 with male and female offspring being exposed to either a control odor (CO) or predator odor (PO) for 30 min and then sacrificed. Sample sizes are provided for both F_0 and F_1 groups. (C) Weight gain F_0 male rats was significantly increased following HFD relative to CD, despite no difference in calorie intake during the final 5 days of diet manipulation (D) F_0 male rats exposed to HFD showed increases in perigonadal and abdominal fat pad mass and decreases in teste and brain weights 27 days after diet manipulation. (E–F) Female rats spent less time in the vicinity of HFD fed F_0 males relative to CD fed males during PPT, both 1 day and 21 days after the odor exposure had occurred in males. Percentage of time with males was calculated as the percentage of time spent with either a CD or HFD F_0 male per total time spent with both CD and HFD F_0 males. Data expressed as mean ± SEM; *HFD different from CD, $p \leq 0.05$, **HFD different from CD, $p \leq 0.005$.

2012), exposure to stress (Franklin et al., 2010; Dietz et al., 2011; Rodgers et al., 2015; Korgan et al., 2016), drug use (Vassoler et al., 2013) and being overweight (Fullston et al., 2015) impact offspring development. Futher, both stress and HFD correlate with metabolic dysfunction (Carone et al., 2010; Ng et al., 2010; Rando and Simmons, 2015) and occurrence of excess weight gain (Hoyer et al., 2013; Ost et al., 2014) in F_1 offspring, which is often comorbid with depressive and anxiety-like phenotypes (Joseph and Golden, 2016). Therefore, it is of great interest to identify the underlying mechanism(s) for the non-Mendelian inheritance of parental lifestyle and dietary risk factors that are critical for understanding the complex behavioral phenotypes in offspring, particularly with respect to metabolic programming and social-emotional development.

We previously demonstrated that maternal environment interacts with paternal stress (predator odor exposure), resulting in alterations to maternal behavior (Korgan et al., 2016). Increases in licking and grooming (LG) and high quality arch-back nursing (ABN) have also been observed following paternal enrichment (Mashoodh et al., 2012), indicating that females adjust investment in offspring based on perceived mate quality (Pryke and Griffith, 2009). Further, we have shown that these alterations in maternal behavior result in stable differences in offspring stress responsivity and anxiety-like behavior (Korgan et al., 2016). Mechanistically, both paternal exposure and maternal care affect offspring epigenetic programming of HPA and endocrine responses to stress. The effect of maternal behavior on shaping offspring programming of glucocorticoid receptor (GR) and anxiety-like behavior is well established (Weaver et al., 2004; Weaver et al., 2014). We have shown that maternal rearing in semi-naturalistic housing (SNH) increases dam LG-ABN behavior, and decreases offspring corticotrophin-releasing factor (CRF) immunoreactive neurons in the paraventricular nucleus of the hypothalamus (PVN) and H3K9ac at the *Crf* promoter (Korgan et al., 2015; Korgan et al., 2016). Contrarily, paternal predator odor stress increased H3K9ac at the *Crf* promoter region, providing further evidence that paternal stress contributes to changes in offspring HPA-axis functioning.

Recent research has identified mechanisms by which F_0 paternal metabolic experience can shape offspring gene regulation, reviewed in (Rando and Simmons, 2015). In *drosophila*, F_0 paternal sugar intake alters H3K9/K27me3 regulation of metabolic genes leading to excessive weight gain in F_1 offspring (Ost et al., 2014). In rodents, F_0 paternal obesity is linked to impaired glucose tolerance in offspring (Ng et al., 2010; Fullston et al., 2013; Wei et al., 2014). Specifically, F_0 paternal exposure to HFD alters global DNA methylation in both the testes and sperm, alters the sperm microRNA content, and increases F_1 offspring vulnerability to HFD feeding while decreasing reproductive success (Fullston et al., 2013). More recently, Govic et al. (2016) demonstrated that F_0 paternal exposure to caloric restriction (CR) decreased anxiety in F_1 offspring despite a corresponding decrease in maternal LG behavior, suggesting more complex variations in phenotypic alterations induced by manipulations to paternal diet.

To date, the role of F_0 paternal HFD induced obesity programming F_1 offspring anxiety-like behavior have been underreported. In the present study, we sought to identify differences in female preference for F_0 males exposed to either HFD or a control diet (CD). We then bred those males with females and measured effects on maternal care as a result of paternal diet and/or her exposure seminaturalistic housing (SNH) or to standard housing (SH). F_1 offspring play behavior, anxiety-like behavior, stress responsivity, and *Crf* expression and H3K9ac at the *Crf* promotor region were then measured to identify potential consequences of F_0 paternal HFD exposure and maternal environment.