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Female offspring born to obese and insulin resistant dams are not at increased risk for obesity and metabolic dysfunction during early development

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Abstract:

The percentage of women who are obese at the time of conception/pregnancy is increasing, with animal and human studies demonstrating that offspring born to obese dams/mothers are at increased risk for obesity and the metabolic syndrome. Our goal was to confirm in an experimental model of metabolic syndrome in the dam, whether the offspring would be at increased risk of obesity. Conversely, we observed that male offspring born to dams with metabolic syndrome had no alterations in their body weight profiles, whereas female offspring born to dams with metabolic syndrome were heavier at weaning, but exhibited no perturbations in energy metabolism. Moreover, they gained weight at a reduced rate versus female offspring born to healthy dams, and thus weighed less at study completion. Hence, our findings suggest that factors other than increased adiposity/insulin resistance during pregnancy are responsible for the increased risk of obesity in children born to obese mothers.

Key Words: Obesity, pregnancy, high fat diet, offspring, glucose homeostasis, metabolic syndrome

42 **Introduction**

43 Currently in Canada there are over 2 million children who are overweight/obese, and the majority
44 of these children will grow up to become adults who are also obese and at increased risk for
45 developing metabolic syndrome (Morrison et al. 2007). Of interest, the concept of “developmental
46 programming” suggests that an individual’s risk for developing disease throughout life is intricately
47 connected to the environment of the womb that individual was exposed to (Blackmore and Ozanne
48 2013). This idea was first put forward by David Barker and colleagues and termed the “fetal origins
49 hypothesis” to explain the inverse relationship between birth weight and mortality resulting from
50 ischemic heart disease (Barker et al. 1989). Over the years the “fetal origins hypothesis” has
51 continued to evolve, with a more recent evolution of this initial concept termed the “Developmental
52 Origins of Health and Disease (DOHaD)”. The DOHaD hypothesis postulates that the risk for disease
53 susceptibility in a developing individual is not simply confined to prenatal and early postnatal
54 development, but is actually influenced by a more broader developmental window that includes the
55 period from pre-conception to fertilization of the oocyte (Blackmore and Ozanne 2013; Pereira et al.
56 2015).

57 With regards to childhood obesity, as there are now an increasing percentage of pregnancies in
58 overweight/obese women at conception (Flegal et al. 2012), the DOHaD hypothesis would suggest
59 that this may be a significant factor accounting for the increased rates of obesity in our pediatric
60 population (Catalano 2003; Shankar et al. 2008). Indeed, a number of studies have demonstrated that
61 offspring born to obese dams are at increased risk for obesity and obesity-related metabolic
62 dysfunction, with many studies implicating a key role for epigenetic alterations in mediating this
63 phenomenon (Borengasser et al. 2013; Laker et al. 2014). For example, Howie and colleagues
64 demonstrated that offspring born to dams supplemented with a high-fat diet (HFD) prior to and during
65 pregnancy/lactation are more susceptible to obesity, regardless if they are weaned onto a low-fat diet
66 (LFD) or HFD (Howie et al. 2009). Moreover, it has been demonstrated that C57BL/6J mice fed a HFD
67 prior to and during pregnancy/lactation produce offspring at increased risk of glucose intolerance,

which was associated with reduced peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1 α) mRNA expression as a result of increased PGC1 α gene methylation (Laker et al. 2014). Nevertheless, there is inconsistency among studies exploring maternal obesity's actions on offspring development, as some studies have observed no impact of maternal obesity on offspring body weight (Buckley et al. 2005; Holemans et al. 2004).

Therefore, our objective was to develop an animal model of obesity and related metabolic dysfunction in the pregnant dam to study whether the DOHaD hypothesis would be relevant to and impact the developing offspring. If this proved to be correct, our secondary objective was to assess whether such findings may be due to altered expression of critical genes regulating energy metabolism.

Methods

Animal Care

All animals received care according to the Canadian Council on Animal Care and the University of Alberta Health Sciences Animal Welfare Committee. 8-week-old C57BL/6J (Jackson Laboratory) females were either fed a low-fat diet (LFD, 10% kcal from lard, Research Diets D12450J) or high-fat diet (HFD, 60% kcal from lard, Research Diets D12492) for 5 weeks. After 5 weeks of LFD/HFD supplementation, females were mated to a 12-week-old male C57BL/6J mouse supplemented with a LFD. Upon confirmation of a vaginal plug, the male breeder was removed and female dams remained on their respective diet throughout gestation. Following birth, pups born to the dam supplemented with a LFD remained with the dam throughout the 21-day nursing/lactation period, whereas offspring born to the dam supplemented with a HFD were cross-fostered to a surrogate dam supplemented with a LFD. This was carried out to ensure that potential body weight differences would not be due to offspring being nursed by an insulin resistant dam receiving their nutrition primarily from a HFD. A total of 4 dams were used for each experimental group (4 surrogate dams to foster pups from the 4 dams supplemented with a HFD, and 4 dams supplemented with a LFD). For each individual breeding, it

94 was ensured that litter sizes between the surrogate dam and the LFD dam were identical at 3 days
95 post-birth, with a maximum litter size of 5. Hence, if one dam was nursing 5 pups at 3 days post-birth
96 and another dam was nursing 4 pups, 1 pup was randomly euthanized from the dam nursing 5 pups,
97 such that both dams nursed an identical number of pups (4 pups) from 3 days post-birth until weaning.
98 At 21-days of age all offspring were weaned into separate cages and supplemented with a LFD until
99 euthanization at 14-weeks of age.

100

101 *Magnetic Resonance Imaging (MRI) Body Composition Analysis*

102 All female dams and offspring underwent assessment of body composition via quantitative nuclear
103 magnetic resonance relaxometry to quantify total lean/fat mass utilizing an EchoMRI-4in1/700 body
104 composition analyzer.

105

106 *Glycemic Assessments*

107 We assessed glucose homeostasis in female offspring as previously described (Ussher et al. 2014). In
108 brief, oral and intraperitoneal glucose tolerance were assessed either in female dams fed a LFD or
109 HFD for 5-weeks, or in female offspring following an overnight fast at 10- and 11-weeks of age,
110 respectively, using a glucose dose of 2 g/kg body weight. Intraperitoneal insulin tolerance was
111 assessed either in female dams fed a LFD or HFD for 5-weeks, or in female offspring following a 6 hr
112 fast at 12-weeks of age, using an insulin dose of 0.3 U/kg body weight (Novolin (biosynthetic human
113 insulin), Novo Nordisk).

114

115 *Indirect Calorimetry*

116 *In vivo* metabolic assessment via indirect calorimetry was performed in female offspring at 8-weeks of
117 age using an Oxymax comprehensive lab animal monitoring system (Columbus Instruments) as
118 previously described (Ussher et al. 2016).

119

Statistical Analysis

All values are presented as means $\pm$ standard error of the mean (SEM). Significant differences were determined by the use of an unpaired, two-tailed Student's *t*-test, or a two-way analysis of variance (ANOVA) followed by a Bonferroni post-hoc analysis.

Results

5-weeks of high fat feeding leads to a marked increase in adiposity and impairs glucose homeostasis in female dams.

8-week-old female C57BL/6J mice were fed either a LFD or HFD for 5 weeks prior to conception with a lean 12-week-old male C57BL/6J mice fed a LFD. 5-weeks of HFD supplementation lead to significant increases in body weight in female mice, and this was associated with a significant increase in total fat mass and percent body fat (Figure 1A-C). This increase in adiposity was associated with impaired glucose homeostasis in female mice fed the HFD, as they exhibited both worse glucose and insulin tolerance versus their female counterparts fed a LFD (Figure 1D/E).

Female offspring born to dams supplemented with a HFD are heavier at weaning but exhibit reductions in weight gain throughout early development.

Male offspring born to dams fed a HFD for 5 weeks prior to conception had similar body weights to their counterparts born to dams fed a LFD, while also gaining weight at a similar rate (Figure 2). Conversely, female offspring born to dams fed a HFD for 5 weeks prior to conception were significantly heavier at weaning than their counterparts born to dams fed a LFD (Figure 3A). This difference in body weight quickly dissipated by 6- to 8-weeks of age, as female offspring born to dams fed a LFD gained body weight at an accelerated pace (Figure 3B). At study completion when the female offspring were 14-weeks of age, those born to a dam fed a HFD actually weighed less than those born to a dam fed a LFD (Figure 3A). These differences in body weight at weaning and at study

completion in female offspring were not associated with changes in total fat mass and percent body fat (Figure 3C-E).

Glucose homeostasis and *in vivo* energy metabolism are normal in female offspring born to dams supplemented with a HFD.

Because the male offspring demonstrated similar body weight curves throughout their early development (Figure 2), we focused the rest of our studies on the female offspring. When all female offspring were 10- and 11-weeks of age, they underwent oral and intraperitoneal glucose tolerance tests, respectively, and we observed no differences in glucose tolerance between both groups of offspring (Figure 4A/B). In addition, the female offspring born to dams fed a HFD exhibited no abnormalities in insulin sensitivity at 12-weeks of age in response to an insulin tolerance test (Figure 4C). During assessment of indirect calorimetry, we observed no differences in whole body oxygen consumption rates normalized to lean body mass in female offspring born to dams fed a HFD (Figure 4D), while substrate preference also appeared similar as respiratory exchange ratios were comparable between groups (Figure 4E). The reduced body weight gain in female offspring born to dams fed a HFD is not due to changes in animal ambulatory activity (Figure 4F), but may involve changes in appetite, as 24-hr food intake demonstrated a trend to a mild reduction (Figure 4G).

Discussion

In this study we observed that female dams fed a HFD for 5 weeks prior to conception and during pregnancy, produced female offspring that exhibited no adverse alterations in glucose homeostasis throughout their juvenile and young adult development. In support of the DOHaD ideology, these female offspring were heavier at weaning, but unexpectedly gained less weight than their counterparts born to dams fed a LFD.

The principle concepts of DOHaD suggest that the risk for disease susceptibility in a developing individual is actually influenced by a more broader developmental window that includes the period

from pre-conception to oocyte fertilization, versus just the periods of prenatal and postnatal development (Blackmore and Ozanne 2013). As such, we hypothesized that dams fed a HFD prior to conception would produce offspring at increased risk for metabolic syndrome and early onset obesity. To our surprise, female offspring born to dams fed a HFD exhibited no abnormalities in glucose homeostasis. We initially surmised that reasons for this unexpected finding could be due to our experimental model of HFD supplementation in the dam not inducing any overt metabolic dysfunction or adiposity, but assessment of total adiposity in our dams fed a HFD prior to conception revealed robust increases in total fat mass, as well as significant impairments in glucose and insulin tolerance.

Taken together, it remains unclear why we did not observe changes in glucose homeostasis in our offspring born to dams fed a HFD prior to conception, and our findings are in contrast to previous studies indicating that offspring born to obese dams are at increased risk for both elevated weight gain and metabolic dysfunction. A study by Howie and colleagues demonstrated that both male and female rat offspring gained weight at increased rates regardless if they were supplemented with a standard chow diet or HFD at weaning if born to dams either fed a HFD (45% kcal from lard, Research Diets) throughout life, pregnancy, and lactation, or only throughout lactation and pregnancy (Howie et al. 2009). Conversely, studies from Laker and colleagues utilizing a pregnancy model in which female C57BL/6 dams were fed a HFD (60% kcal from lard, Research Diets) for 6 weeks prior to conception and during gestation, demonstrated no differences in offspring body weight over a 1-year period versus offspring born to dams fed a standard chow diet (Laker et al. 2014). Nevertheless, 9-month old offspring born to dams fed a HFD exhibited impairments in glucose and insulin tolerance versus offspring born to dams fed a LFD (Laker et al. 2014). The impairment in glucose homeostasis in the offspring born to dams fed a HFD was attributed to epigenetic changes in PGC1 α , a key regulator of mitochondrial function, as PGC1 α methylation was increased and subsequent PGC1 α mRNA expression decreased in skeletal muscles of these offspring. However, our measurements of soleus PGC1 α mRNA expression indicate similar levels at weaning in our female offspring born to dams fed a

196 HFD, and in 14-week-old female offspring born to dams fed a HFD, versus their female counterpart
197 offspring born to dams fed a LFD (Figure 4H), which may explain why glucose homeostasis was
198 unaltered in our studies. Conversely, it is possible that if we allowed our female offspring to age to 9-
199 months, we may have also observed impaired glucose homeostasis in the offspring born to dams fed
200 a HFD, supporting previous findings (Laker et al. 2014).

201 Of importance, a number of studies support the notion that epigenetic inheritance may be a
202 critical feature of the DOHaD ideology and the risk for disease susceptibility in a developing individual,
203 including pediatric obesity and metabolic syndrome. For example, dams made obese via liquid
204 enriched diet supplementation (40% excess calories) for 3 weeks led to robust alterations in offspring
205 DNA methylation of pro-adipogenic factors (e.g. CCAAT enhancer binding protein β) in white adipose
206 tissue stromal vascular cells (Borengasser et al. 2013). To ensure any potential epigenetic alterations
207 in this study were likely acquired via inheritance from the dam fed a HFD and exposure to an obesity-
208 like intrauterine environment, all offspring born to the dam fed a HFD were cross-fostered to a
209 surrogate lean dam. Conversely, the pregnancy models used by Howie and colleagues or Laker and
210 colleagues had the dams remain on a HFD during the nursing/lactation period. This differentiating
211 factor could influence epigenetic mechanisms controlling body weight gain in the offspring during
212 nursing, potentially explaining the early onset obesity in offspring weaned to a LFD and born to dams
213 fed a HFD observed by Howie and colleagues (Howie et al. 2009), or the impaired glucose tolerance
214 in offspring born to dams fed a HFD observed by Laker and colleagues (Laker et al. 2014). Hence, this
215 may explain why we did not see an increased risk for early onset obesity or metabolic syndrome in our
216 offspring born to dams fed a HFD for 5 weeks prior to conception, as we also cross-fostered our pups
217 to a surrogate dam fed a LFD immediately upon birth. Alternatively, our findings do have a significant
218 limitation in that we did not foster pups born to dams fed a LFD, and it is possible that our
219 observations in female offspring are the result of the stress of fostering, and not the offspring being
220 conceived by a dam fed a HFD and the subsequent intrauterine exposure to an obese metabolic
221 environment. Furthermore, it is possible that maternal care from a foster mother is not as strong as

that from the genetic mother, which could also contribute to our observations in the female offspring. However, we would thus expect to have seen a similar body weight pattern in our male offspring, but body weights in male offspring born to dams fed a HFD and fostered to a dam fed a LFD were identical at all time points when compared to male offspring born to dams fed a LFD without fostering. Nonetheless, this remains a very important lingering question that we plan to address in our future studies.

In spite of this limitation, our results are unique as our female offspring born to dams fed a HFD were actually heavier at weaning than their counterparts born to dams fed a LFD, but gained weight at a reduced pace that they actually weighed less once they reached 14-weeks of age. On the other hand, male offspring born to HFD supplemented dams in our study had normal body weights at weaning and gained weight at similar rates to their male offspring counterparts born to LFD supplemented dams. It remains unclear as to why we observed this unique sex-dependent body weight profile in our offspring versus those observed in previously aforementioned studies, though our results in male offspring are consistent with findings in male rat offspring born to HFD supplemented dams by Buckley and colleagues (Buckley et al. 2005). Of interest, our findings are consistent with the set-point theory of body weight regulation (Farias et al. 2011), and it is possible that the reduced rate of body weight gain in female offspring born to a dam supplemented with a HFD could be a centrally regulated defense mechanism resulting from an elevated weaning body weight and intrauterine exposure to an obese environment. As such, it would be interesting to have continually monitored glucose homeostasis in our offspring as they aged even further, and whether this could be negated by weaning the female offspring onto a HFD, though that is beyond the scope of this specific study. It is worth noting though if this set-point theory of body weight regulation is correct, it would have also been anticipated to occur in the male offspring, which we did not observe in our study.

Another potential factor is that we utilized a sucrose-matched low-fat control diet for our control dams, whereas most studies simply utilize standard rodent chow. One of the most common standard chow diets employed in animal facilities is the 2018S diet of Teklad, and the micronutrient

248 compositions of these standard chow diets can be vastly different from the micronutrient composition
249 of the Research Diets LFD/HFD utilized in our study. As such, we have observed differences with
250 regards to glucose tolerance and insulin tolerance when comparing 2018S versus Research Diets
251 LFD (*unpublished data*). This leads to the question of whether obesity itself, or the macronutrient
252 composition of the HFD, is responsible for the offspring phenotypes observed in previous studies, or is
253 it potential differences in micronutrient composition between the Research Diets HFD and the
254 standard chow diet the dam is exposed to that is contributing to the offspring phenotype.

255 In summary, our findings did not reproduce those of previously published papers supporting the
256 DOHaD ideology that obesity and metabolic dysfunction in the pregnant dam lead to increased risk for
257 early onset obesity and metabolic dysfunction in the developing offspring. Whether this is due to
258 differences in micronutrient composition in the diets we utilized versus previous studies, or because
259 we cross-fostered our pups during nursing/lactation to a lean dam fed a LFD, remains to be
260 determined. Future studies characterizing molecular factors that lead to increased body weight at
261 weaning, but reduce the rate of body weight gain in female offspring born to dams fed a HFD are
262 critical.

263

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268 Education.

269

270 **Disclosures**

271 The authors have no conflicts to disclose.

272

273

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320 **Figure Legends:**

321

322 **Figure 1.** *5-Weeks of HFD Supplementation Increases Adiposity and Precipitates Metabolic*
323 *Dysfunction in Female C57BL/6J Mice.* **A:** Body weights in female C57BL/6J mice fed a LFD or HFD
324 for 5 weeks (n = 6). **B:** Total fat mass in female C57BL/6J mice fed a LFD or HFD for 5 weeks (n = 6).
325 **C:** Fat mass as a percentage of total body weight in female C57BL/6J mice fed a LFD or HFD for 5
326 weeks (n = 6). **D:** Glucose tolerance in female C57BL/6J mice fed a LFD or HFD for 5 weeks (n = 6).
327 **E:** Insulin tolerance in female C57BL/6J mice fed a LFD or HFD for 5 weeks (n = 4). Values represent
328 mean $\pm$ SEM. Differences were determined by the use of an unpaired, two-tailed Student's *t*-test, or a
329 two-way ANOVA, followed by a Bonferroni post-hoc analysis. **P* < 0.05.

330

331 **Figure 2.** *Body Weights in Male Offspring.* Body weights from weaning until 14-weeks of age in male
332 offspring born to C57BL/6J dams fed a LFD or HFD for 5 weeks (n = 3, 4).

333

334 **Figure 3.** *Body Weights and Adiposity in Female Offspring.* **A:** Body weights from weaning until 14-
335 weeks of age in female offspring born to C57BL/6J dams fed a LFD or HFD for 5 weeks (n = 6). **B:**
336 Total body weight gain over an 11-week period in female offspring born to C57BL/6J dams fed a LFD
337 or HFD for 5 weeks (n = 6). **C:** Total fat mass and % fat mass in 4-week old female offspring born to
338 C57BL/6J dams fed a LFD or HFD for 5 weeks (n = 6). **D:** Total fat mass and % fat mass in 8-week
339 old female offspring born to C57BL/6J dams fed a LFD or HFD for 5 weeks (n = 6). **E:** Total fat mass
340 and % fat mass in 14-week old female offspring born to C57BL/6J dams fed a LFD or HFD for 5
341 weeks (n = 6). Values represent mean $\pm$ SEM. Differences were determined by the use of an
342 unpaired, two-tailed Student's *t*-test, or a two-way ANOVA, followed by a Bonferroni post-hoc analysis.
343 **P* < 0.05.

344

345 **Figure 4.** *Glucose Homeostasis and In Vivo Metabolism in Female Offspring.* **A:** Oral glucose
346 tolerance in 10-week-old female offspring born to C57BL/6J dams fed a LFD or HFD for 5 weeks (n =
347 5). **B:** Intraperitoneal glucose tolerance in 11-week-old female offspring born to C57BL/6J dams fed a
348 LFD or HFD for 5 weeks (n = 5, 6). **C:** Intraperitoneal insulin tolerance in 12-week-old female offspring
349 born to C57BL/6J dams fed a LFD or HFD for 5 weeks (n = 5, 6). **D:** Oxygen consumption in 8-week-
350 old female offspring born to C57BL/6J dams fed a LFD or HFD for 5 weeks (n = 3). **E:** Respiratory
351 exchange ratio in 8-week-old female offspring born to C57BL/6J dams fed a LFD or HFD for 5 weeks
352 (n = 3). **F:** Ambulatory activity in 8-week-old female offspring born to C57BL/6J dams fed a LFD or
353 HFD for 5 weeks (n = 3). **G:** 24-hr food intake in 8-week-old female offspring born to C57BL/6J dams
354 fed a LFD or HFD for 5 weeks (n = 3). **H:** *Ppargc1a* mRNA expression in gastrocnemius muscles from
355 3-week-old or 14-week-old female offspring born to C57BL/6J dams fed a LFD or HFD for 5 weeks (n
356 = 3, 4). Values represent mean $\pm$ SEM. Differences were determined by the use of an unpaired, two-
357 tailed Student's *t*-test, or a one-way ANOVA, followed by a Bonferroni post-hoc analysis. **P* < 0.05.
358
359

□ Female Offspring Born to a LFD Dam
 ■ Female Offspring Born to a HFD Dam