The Effect of Maternal Diet on Fetal Development

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1. INTRODUCTION

During fetal development, the child’s growth and maturation within the womb depends largely on the food and nutrients provided to the infant through the placenta (Liang, 2009). Recent studies suggest strong connections between environmental conditions during fetal growth and development and lifelong effects on growth, maturation, and long-term disease risk (Prater, 2008). The developmental origins of health and disease, or “DOHaD” hypothesis states that the fetal environment will lay the groundwork for many health risks later in life (Hales, 2001). There is evidence that chronic adult diseases such as obesity, osteoporosis, cardiovascular disease, renal disease, and diabetes can trace their origins back to the gestational environment of a child. While genetics will influence the mature phenotype and influence the body’s sensitivity to environmental factors later in life, early developmental environment plays a significant role (Lanham 2009). Neonatal size is determined primarily by the intrauterine environment (62%); parental genetic determinants are secondary (20% maternal, 18% paternal) (Davies 2005, Penrose 1952).

Lack of proper nutrients stemming from an unhealthy diet of the mother during critical periods of fetal development may limit growth and maturation (Sirinivasan 2006). It’s the offspring’s metabolic and physiologic functions may be “programmed” based on exposure to nutrients, toxins, and hormones in utero. The fetus essentially assumes that the in utero environment is predictive of the extra-utero or post-natal environment. Adaptations to a stressful or
compromised fetal environment lead to negative programming of both physiology and metabolism that can persist throughout life (Barker, 1995).

One critical environmental determinant is maternal diet. Placental and fetal stress may be induced by consumption of a high fat diet (HFD) or high sugar diet (HSD). Diets high in saturated fat – including heavy reliance on fast-food items – lead to excess adiposity (body fat) that exceeds the body’s energy requirements. Increased fat consumption is a common feature of the modern “western diet”. Western diet foods are often heavily processed and chemically modified, which has led to increased daily caloric in-take. Portion sizes in fast food restaurants have grown 2-5 times in size compared to 20 years ago (Ledikwe, 2005). These larger portion sizes in restaurants have been positively linked to elevated caloric intake and increased overall body mass (Ledikwe, 2005). Increased body mass of the population extends to include more women being overweight or obese at the time of conception and pregnancy.

1.1 OBESITY

Obesity is a problem that is reaching epidemic proportions worldwide, particularly in highly technologically developed countries. Obesity is generally defined a person’s body mass exceeds the “healthy” range by 16-20%. It was reported by the Center for Disease Control (CDC) that in 2007-2008 there were 72.5 million obese adults in the United States (www.cdc.gov). Diseases associated with obesity have caused a 27% increase in healthcare costs, which amounts to approximately $147 billion dollars annually (www.cdc.gov). This
trend affects not only adults, but children as well. In 1980 it was reported that
6.5% of children under 19 years of age were obese. By 2002 that number had
increased to 15.8%; the national trend is still rising today (www.cdc.gov).

Infants born to obese mothers are often in the 90th percentile or greater
for gestational growth during the third trimester (Heerwagen, 2010). This rapid
late-term growth is associated with a tendency for increased adipose (fat) tissue
at birth, which in turn has been linked to obesity and metabolic syndromes in
adulthood (Heerwagen, 2010). Increased neonatal adiposity is explained in part
by altered lipid metabolism in obese pregnant mothers. During early pregnancy,
lean women demonstrate a net increase in lipogenesis (conversion of simple
sugars to fatty acids) during the first trimester. In the third trimester, a switch to
lipolysis occurs, where triglycerides become broken down to simply fatty acids
(Catalano, 1998). Conversely, in obese women lipogenesis stops significantly
earlier in the pregnancy and lipolysis is much more prominent during the second
and third trimesters (Catalano, 1998). The increase in lipolysis increases
placental lipid levels – an early warning sign of insulin resistance (Catalano,
1998). Studies have also demonstrated when an obese mother begins a healthy
diet at the time of conception, continuing through breastfeeding, offspring are
spared from adverse intrauterine programming effects (Gallou-Kabini, 2006).

Altered birth outcomes between lean and obese mothers are also
manifested through gene expression. DNA encodes every gene in the human
body; variations in genetic sequences and gene expression create phenotypic variations among humans. Gene expression is altered in placentas of obese women who have greater than 16% body fat. Numerous genetic pathways are subject to modification, in obese women compared to lean women (< 8% body fat), including: energy storage, cell growth, cell death, and the phospholipase A2 (PLA2) genes (Heerwagen, 2010). The PLA2 genes are intimately involved in development of atherosclerosis, and accumulation of fat in the heart valves (www.cdc.gov).

Maternal health during gestation also alters post-natal feeding habits of offspring. These habits have been shown to be impaired; children have been found to have a larger caloric intake if the mother was overweight at the time of pregnancy (Ledikwe, 2005). A correlation has been found between maternal prenatal diet and offspring diet. Maternal consumption of a HFD during pregnancy, in mice, is associated with have increased caloric intake by offspring post-natally and through adulthood (Heerwagen, 2010). This may be a result of neurologic programming. Idiopathic obesity can be linked to dysfunctional perception of hunger and satiety by the brain. These hunger signals are regulated through hormonal mechanisms including leptin resistance and ghrelin production, leading to an increased calorie intake (Heerwagen, 2010). In some studies; however, female mouse offspring have been found to be resistant to the maternal HFD-induced post-natal hyperphagia during childhood (Gallou-Kabani, 2010). Those studies showed that some of the female offspring maintained
similar eating habits to mice whose mothers consumed a normal control diet
during pregnancy (Gallou-Kabani, 2010).

1.2 OSTEOPOROSIS

Osteoporosis is a skeletal disorder defined by low bone mass and
deterioration of the micro-architecture of bone tissue, leading to bone fragility
(Goodfellow 2010). Osteoporosis disproportionately affects women, generally
beginning after menopause when estrogen production begins to decline
(www.cdc.gov). Decreased bone density can lead to osteoporotic fracture –
often a result of injury (falls, automobile accidents) combined with poor bone
strength (Cooper, 2006).

Skeletal health is determined beginning with in utero development and
continuing through infancy, childhood, and adulthood. Nutrition, environment,
and activity during these periods are critical to determining bone mineral density
later in life (Harvey, 2010). Bone development begins in the womb with
formation of a cartilage “template”, the precursor to bone formation, at about
week 5 in the standard gestation period. By the second trimester, these bones
begin to mineralize and lengthen (Goodfellow, 2010). Cell division is especially
sensitive to environmental influences during the second trimester when the
appendicular skeleton is forming (Prater, 2008). Over 80% of neonatal bone
volume is accumulated at this time, when bone growth is at its peak (Harvey,
2010). Growth of long bones is particularly important, as these bones are
susceptible to fracture by injury or fatigue later in life. Neonatal accumulation of
Bone mass is affected by both environmental and genetic factors (Lanham, 2009). Studies have demonstrated a relationship between a girl’s weight at one year of age and her adult bone mineral content (BMC) at regions prone to osteoporotic fracture -- namely the lumbar spine and femoral neck (Goodfellow 2010).

Twin studies can highlight the critical role of intra-uterine development and the spine. Nearly 66% of monozygotic (identical) twins share a placenta, meaning that the babies are competing for resources. This often leads to one twin being significantly smaller than the other at birth. It is possible to see the trend between birth weight and bone density later, even after the twins reach adulthood (Antoniades, 2003). Low adult bone density in the smaller, less well-nourished baby illustrates how the unequal sharing of placental resources impacts the skeletal health in adulthood.

Bone density can be increased through the mechanical loading, which stimulates anabolic bone formation and maintenance bone cell health (Erlich, 2002). Obesity studies in animals show that high fat diet (HFD) negatively impacts the structure of cortical and trabecular bone, despite the increased bone mass and body weight forces (Cao, 2009). These structural defects can include: shorter long bones, skeletal malformations, and disorganized trabecular architecture (Liang, 2009). Bone strength is also impacted by a lack of nutrients, notably vitamin D and calcium that tend to be missing in a HFD (Yin, 2010). Mice
on a HFD demonstrate a decreased potential for osteoblastic differentiation of cells (Lanham, 2010). This causes problems in overall bone growth and remodeling.

1.3 DIABETES

Another impact of increased body mass is the increased risk for type II diabetes. More than 80% of people with type II diabetes are overweight (Sirinivasan, 2006). Offspring exposed in utero to a HFD have been shown to suffer increased risk for hyperglycemia, insulin resistance, and type II diabetes in adulthood (Liang, 2009). Generational type II diabetes is preventable if the mother changes to a healthy diet at the time of conception through the time they are breastfeeding their offspring (Gallou-Kabani, 2010).

There is significant evidence that demonstrating a high prevalence of glucose intolerance and increased serum triacylglycerol concentration (insulin resistance syndrome) in malnourished pregnant women (Barker, 1993). Offspring of mothers suffering from obesity have been shown to have significant glucose intolerance in adulthood (Han, 2005). Specifically, these female offspring have been found to have reduced serum insulin values, suggesting that pancreatic beta cells were damaged during growth and development (Han, 2005). Lack of growth and development normally stems from the suppression of beta cell transcription factor, pancreatid duodenal homeobox-1 (Pdx-1) (Heerwagen, 2010). This is often an adaptive response of the fetus to a high stress
environment. Rodent models have shown a decrease in renal output during adulthood even with a healthy diet after birth (Lanham, 2010). Further, animal models have also shown up two times plasma insulin level in offspring of HFD mothers who also consumed a similar diet (Merrill, 2010). The same study also showed that even rats whose mothers consumed a HFD and then switched to a standard healthy diet still had higher plasma insulin values (Sirinivasan, 2006).

High insulin values have been demonstrated in animal models and human populations. The Pima Indians were studied and a relationship was found between babies exposed to diabetes in utero and babies who were not. Offspring of mothers with diabetes were 10x more likely to develop diabetes in early adulthood than infants whose mothers were not diabetic (Heerwagen, 2010).
2. MATERIALS and METHODS

The principles of animal laboratory care under the guidelines of “SUNY Upstate Committee for the Humane use of Animals”, protocol 216 were followed completely, in accordance with AAALAC guidelines (Association for Assessment and Accreditation of Laboratory Animal Care). Male and female C57BL/6J mice at 4 weeks of age were obtained from Jackson Laboratories (Bar Harbor, MI). Mice were group housed, 5 to a cage, on a 12 hour light/dark schedule at 22° +/- 1°C with 40-60% humidity. Upon arrival female mice were divided into five groups (A-D, AA) and fed a high fat diet (HFD), high sugar diet (HSD) and a control diet (CD). All males were fed the control diet. The HFD was Teklad Diet TD.06414, composed of 18% kcal protein, 21.3% kcal carbohydrates and 60.3% kcal fat [Harkland Teklad Custom Research Diet]. The HSD was Teklad Diet TD.86489, composed of 20% protein, 67.3% carbohydrate, and 12.8% fat [Harkland Teklad Custom Research Diet]. The carbohydrate portion of the diet was equal parts (wt/wt) of sucrose and cornstarch. The CD diet was Formulab Diet 5008, composed of 26.849% protein, 16.710% fat, 56.441% carbohydrates [Formulab Diets] (Table 1). Mice were given food and water ad libitum.

<table>
<thead>
<tr>
<th></th>
<th>HFD (5.1 Kcal/g)</th>
<th>HSD (3.7 Kcal/g)</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein (kcal)</strong></td>
<td>18.4 %</td>
<td>20.0 %</td>
<td>26.8 %</td>
</tr>
<tr>
<td><strong>Carbohydrate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(sucrose/cornstarch)</td>
<td>21.3 %</td>
<td>67.3 % (53%/47%)</td>
<td>56.4 %</td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Saturated/Unsaturated)</td>
<td>60.3 % (37%/63%)</td>
<td>12.8 %</td>
<td>16.7 %</td>
</tr>
</tbody>
</table>

Table 1: Description of dietary constituents.
After four weeks on their assigned diet, males and females were bred overnight at a 1:2 (male:female) ratio. Mating was deemed successful by the presence of a vaginal plug (Figure 1). The presence of a vaginal plug was considered gestation day 0.

Figure 1: Image of vaginal plug (www.thefunmouse.com)

Offspring were collected at PND 0 (Post Natal Day), PND 7, and PND 14 for euthanasia and processed following the model found in “Micro CT Evaluation of Murine Fetal Skeletal Development” (Oest, 2008) briefly summarized as follows: Pups were preserved in ethanol until they could be scanned using a high-speed in vivo micro-computed tomography (micro-CT) system [Scanco Medical, Bassersdorf, Switzerland]. Still-born pups of all groups and incidental perinatal deaths from all groups were scanned. Reconstructed 3-D grayscale images of the pups were generated using Scanco’s imaging software, then converted to the Digital Imaging and Communication in Medicine format
(DICOM). The DICOMs were exported to a local computer, MacBook Pro [Apple Computer in Cupertino, CA].

Limb length measurements of each fetus were then collected using OsiriX. OsiriX is a DICOM-compatible medical imaging freeware program. Each DICOM file was imported into the program and a 3-D volume rendering of the micro-CT image was done. Image sets could be individually rotated 360° so each limb could be measured in its plane of reference, obtaining a maximum limb length. A sample of a PND 0 pup DICOM can be seen in Figure 3.

![Figure 3: Image of PND 0 pup (HSD) as viewed in OsiriX.](image)

The limb lengths were measured using the OsiriX length measurement tool, calibrated to the micro-CT voxel size. Limb lengths for both the right and left side of the fetus were taken for the mandible, humerus, radius, ulna, femur, tibia and fibula. The reason for measuring both right and left side limbs was to account for human error. This allowed measurement variability for each mouse
to be averaged together which removes some of the operator-associated measurement variability. Right and left side data for each mouse was averaged together to generate an average limb length for each mouse. The same limb length procedure was utilized for all samples.

Once limb lengths were recorded they were sorted by group and litter. Data from each fetus of a litter was averaged together and then litter data was averaged to other litters whose mothers ate the same diet. These limb length averages were taken for each group, HFD, HSD and CD. These averages for limb length were then compared to one another.

In addition to limb length Bone Volume (BV) and Bone Mineral Density (BMD) were taken from each mouse. These numbers were generated using the micro-CT image analysis software. Both BV and BMD were taken for each mouse in a litter and then averaged together. These averages were then averaged with other litters of the same group and then compared with the other two groups.

Finally, data was run through statistical software, StatView Software [SAS Institute, Inc]. Statistics were run using Analysis of Variance (ANOVA) analysis with Fisher’s pairwise-comparisons with significance determined using a p-value of 0.05. ANOVA uses observed variance of a specific variable for example limb length, bone volume, or bone density. ANOVA utilizes the Students T-test for multiple groups and determines whether the means of several different groups are equal to each other.
3. RESULTS

Utilizing data from the micro-CT and measurements from OsiriX, this study focused primarily on analyzing and comparing limb lengths, bone volume and bone density of mouse pups whose mothers had consumed a HFD, HSD or CD prior to conception and continuing throughout gestation and lactation.

The first time point came from groups harvested at PND 0. These pups were harvested within 12 hours of birth. Figure 4 shows representative samples of images collected for pups at PND 0 for the HFD, HSD and CD.

![Figure 4: Images of PND 0 pups.](image)

Visible differences can be noted from these images; the HFD pups have underdeveloped skull bones. Conversely, it appears that the pups in HSD have over-ossified skull bones compared to the pups in the CD group. Additionally, HSD pups have more prevalent spinal curvature defects. These pups exhibit excessive lordosis, (inward curvature) of the cervical and lumbar regions of the spine. There also appears to be some evidence of excessive kyphosis (outward curvature) in the cervical and thoracic spinal regions.
In Figure 5 data from limb length bone measurements of PND 0 pups is presented. There were 41 pups at this time point; 14 pups on the CD, 13 pups on the HFD and 14 pups on the HSD. Long bones measurements included the humerus, radius, ulna, femur, tibia and fibula. The flat mandible bone was also measured.

![Limb Lengths at PND 0](image)

**Figure 5**: PND 0 limb lengths.
* denotes p < 0.05, + denotes p = .0623, n = 41.

As can be seen above, the pups whose mothers consumed the control diet during gestation have longer long bones than pups whose mothers consumed the HFD and HSD. For all measurements except the HFD mandible, HFD and HSD limb lengths were significantly shorter than those of CD offspring (p < 0.05). Comparison the HFD mandible to CD mandible (p = .0643) showed a trend, not a significant difference.
In Figure 6 data of bone volume measurements of PND 0 pups can be found. This data was generated from the micro-CT.

**Figure 6**: PND 0 bone volume measurements. *denotes p < 0.05, n = 41.

Bone volume was found to be significantly decreased in pups born to mothers consuming both the HFD and HSD compared to controls. There was no significant difference between pups in the HFD and HSD groups.

Figure 7 shows data of bone mineral density measurements of PND 0 pups was found. This data was also generated by the micro-CT.
Bone volume was found to be significantly less for the HSD compared to the CD and the HFD. However, bone mineral density for pups in the HFD group compared to the CD demonstrated a trend with a p-value of 0.0608. With PND 0 density a significant difference was also found between the HFD and the HSD.

The second time point came from pups harvested at PND 7. These pups were euthanized one week after birth, while still being nursed by their mothers. While a subset of the mothers were switched to the control diet after consuming a HFD or HSD during gestation, altering post-natal maternal diet did not significantly affect offspring development (data not shown). Therefore, all pups

Figure 7: Post-natal day 0 bone mineral densities. * denotes $p < 0.05$, + denotes $p = 0.0608$, $n = 41$. 
were analyzed together based on peri-natal diet. Figure 8 shows representative samples of images collected for pups at PND 7 for the HFD, HSD and CD.

![Images of PND 7 pups](image1)

**Figure 8**: Images of PND 7 pups.

Visible observations show that in general, the pups on the HFD caught up to the pups on the CD for long bone growth and development. There are still qualitatively noticeable delays in skull bone development. The pups on the HSD continued to show excessive spinal curvature and some rib malformations, including fused ribs, were visible.

Limb length bone measurements of PND 7 pups are shown in Figure 9. There were 22 pups at this time point, with 7 pups on the CD, 8 pups on the HFD and 7 pups on the HSD. The same bones were analyzed as with the previous time point.
As pups began to grow and develop there were less noticeable differences in limb lengths. A significant difference was noticed between two shortest long bones, tibia and fibula, where limb length was significantly shorter in HFD and HSD pups than offspring on the control diet. Another interesting finding demonstrated that the HSD pups had significantly longer radii and not a significant difference in length with the ulna; however a trend was seen with a p value of .0682.

In Figure 10 shows the data of bone volume measurements of the PND 7 pups. This data was calculated during the micro-CT scan.
There was no significant difference in bone volume measurements for the three groups. However, a trend was seen between the HFD and the HSD.

Figure 10: PND 7 bone volume measurements. * denotes $p = .0578$. $n = 22$.

Micro-CT generated data of bone mineral density measurements of PND 7 can be found in Figure 11.
Bone mineral density was found to be significantly less in the HFD compared to the CD and a trend was noticed between the CD and the HSD with a p value of .0593.

The final time point came from pups harvested at PND 14. These pups were collected two weeks after birth, prior to being weaned. Again, there were no significant effects resulting from switching mothers to the control diet after consuming a HFD or HSD during gestation (data not shown). Therefore, pups were grouped for analysis based on gestational diet. Figure 12 shows representative samples of images collected for pups at PND 14 for the HFD, HSD and CD. At this time point all of the pups seem visually similar.
In Figure 13 data from limb length bone measurements of PND 14 pups.

There were 25 pups at this time point, with 4 pups on the CD, 10 pups on the HFD and 11 pups on the HSD. The same bones were analyzed as with the previous time points.
As growth and development continues there is almost no noticeable difference in limb lengths; although in some cases the HSD pups have longer limb lengths than both the pups on the HFD and the CD.

The data from the micro-CT scan of PND 14 pups in Figure 14, show bone volume measurements.

![Bone Volumes at PND 14](image)

**Figure 14**: PND 14 bone volumes.

* denotes p = .0578, n = 25.

There was no significant difference in bone volume measurements for the three groups.
Bone density measurements, generated by micro-CT analysis of PND 14 pups are illustrated in Figure 15. There was again no significant difference in bone density between treatment groups.

**Figure 15:** PND 14 bone mineral density measurements. * denotes p < 0.05, + denotes p = .0593, n = 25.
4. DISCUSSION

It has been well documented that a fetus developing in a hostile intrauterine environment suffers negative effects on the growth and development. The Barker Hypothesis, or “developmental origins of disease” hypothesis (Barker, 2003) has been evaluated using many models. Continuing research in this area includes both human epidemiological studies and animal model studies, and routinely shows a negative relationship between low birth weight and adult-onset diseases including diabetes, obesity, cardiovascular disease, and osteoporosis (Liang et al, 2009). This study endeavored to quantify maternal dietary effects on early postnatal development by studying the limb lengths, bone volume and bone density of mouse pups. Body weight, though commonly used in clinical settings, is not an ideal indicator of postnatal growth. By studying bone growth and development through birth and early stages of weaning in an animal model, an attempt was made to correlate maternal diet to alterations in bone development (Liang, 2009). This work extended previous research studying HFD and pre-natal development by following offspring through early post-natal growth, and addition of a HSD treatment group.

Basic visual observations show that at birth there are noticeable differences in skeletal development. Effects of poor diet manifest through delayed skeletal formation in the HFD group. Conversely, in the HSD experimental group there seems to be excessive, premature mineralization of the skull bones. This may suggest the mechanisms by which HFD and HSD modify
fetal development are different, as long bones and flat bones mineralize through different cellular mechanisms. Also, pups in the HSD show excessive spinal curvature in the form of lordosis in the cervical and lumbar portion of the spine. As the pups age, this may affect the heath and degeneration of intervertebral disks in the spine, contributing to back pain and loss of function. Finally, the over-ossification seen in HSD offspring skulls is also accompanied by rib cage malformations. Many of the specimens were found to have fused ribs.

When studying the limb lengths of pups harvested at the PND 0 time point, some definite trends were observed. It was clear that pups that had developed with mothers consuming either the HFD or HSD had shorter long bones. It was also found that there was no significant statistical difference between the HFD and HSD experimental groups. This suggests that the changes in maternal diet can affect both intramembranous and endochondral ossification processes (Liang, 2009). Related studies illustrate that mice on a HFD diet suffer impaired osteoblastic differentiation, contributing to delayed mineralization (Lanham, 2010). A trend was seen when looking at the mandible, another flat bone; while the HSD showed a significant difference in bone length for the CD with the mice in the HFD, the p-value was only equivalent to 0.0623. This suggested that there may be a different signaling or growth mechanism involved with long bone formation as opposed to flat bone formation.
Bone formation is regulated through the combined interactions of osteoblasts, osteoclasts, and osteocytes. First, osteoblasts deposit an unmineralized bone matrix called an osteoid. The osteoid is mainly type I collagen containing low mineral density. The osteoid then begins to accumulate mineral and reorganize the extracellular matrix as entrapped osteoblasts become osteocytes (Zhou, 2010). Osteoclasts aid in bone remodeling by demineralizing osteocytes in their vicinity so that osteoblasts can follow to secrete collagen and mineral. Osteocytes, osteoblasts and osteocytes work together to form and remodel bone (Zaidi, 2010). This balance of bone formation and resorption may change because of changes in gene pathways from a hostile intra-uterine environment, causing deterioration of bone micro-architecture (Zaidi, 2010). When studying bone health and strength, the rate of bone remodeling can be a predictor of fracture risk (Ammann, 2003).

Data from BV and BMD studies aid in understanding bone homeostasis. There was a significant difference in BV for both the HFD and HSD compared to the CD in our study. This observation must also take into account that the bones in the HFD and HSD were found to be shorter, inherently decreasing the skeletal volume. Analysis from BMD also shows a significant decrease from the CD and both experimental groups. This decrease in overall BMD suggests that by changing the diet in the two experimental groups, ossification was delayed in utero compared to the CD. However this study showed that there were not only differences found between the CD and experimental groups, but also between
the HFD and HSD. Pups consuming the HSD also had a significantly higher BD than pups in the HFD diet. This observation suggests that HFD and HSD affect pup growth and development differently.

Further body developmental differences were observed with pups harvested at Day 7. It appeared that the HFD pups had caught up to the CD pups in long bone growth, although the skull bones had qualitatively noticeable delays in development. In rats and humans, fetal growth impairment followed by catch-up growth is associated with reduced lifespan (Singhal, 2004; Nuyt, 2009). The HSD pups showed the same accentuated spinal curvature that was present at birth as well as some altered rib development still present.

Analysis of limb length growth at this stage showed mixed results. Measurements of the shorter long bones, tibia and fibula still showed the HFD and HSD significantly shorter than the CD pups. This was similar to what was observed birth. However, measurements of the femur and ulna showed no significant difference or trend between any of the three experimental groups and when examining the radius and ulna there was a significant increase in height between the HSD and the CD. This suggests that the HFD caused pups to begin to play catch up after birth. While the HSD caused pups to catch-up at an accelerated rate and in some cases bones over developed compared to pups on the CD. Studies have found that this “catch-up” growth has many negative long-term effects. During skeletal growth, there is net bone formation, at peak bone
mass the amount of osteoclastic bone resorption matched by the amount of osteoblastic new bone formation (Davies, 2004). If this process is compromised and is happening at an accelerated rate, new bone formation will be unable to develop to the optimal size and material properties for a particular set of normal or daily physiological conditions (Ionova-Martin, 2010). A third observation when examining the mandible shows that there is no longer a significant difference between pups in CD, HFD or HSD again suggesting that the signaling mechanism may be different between flat bones and long bones.

By looking at BV between the groups at Day 7 it is clear that the pups consuming a HSD and HFD have succeeded in closing the significant gap between the pups on the CD. Although, the pups on the HFD still have less BV the difference is no longer significant. However, it can also be observed that the pups on the HSD have a statistically higher BV then the pups consuming the HFD; they have a higher BV than the CD but it is not enough to be significant. This adds further evidence that both experimental groups have begun to “catch-up” in BV to the pups on the CD, in only one week of post-natal growth. This supports findings from other studies that, bone grew faster in the HFD. It has been hypothesized that the bones will accelerate growth to accommodate for the additional weight of animals on a HFD (Ionova Martin, 2010). This causes bone mass to accumulate faster than it can be remodeled, resulting in dense but poor quality bone (Ionova Martin, 2010).
The trend seen with BV is not continued when examining BMD. The pups on the HSD have significantly less BMD than the pups on the CD, and the pups on the HFD show a trend with less BMD, the p-value is 0.0593. Bone is made up of mostly collagen and mineral the content and organization of which regulate its mechanical properties (Xiao, 2010). By noting that the BMD is decreased with both experimental groups it is possible to draw the conclusion that mineral content and therefore the bone matrix has been impacted by experimental diets (Xiao, 2010). Research has documented the effect of a HFD on BMD, but the effects of HSD have not been formally studied. The decrease in BMD after a large increase in BV suggests signaling mechanisms involved with a diet high in sugar may in fact be different than mechanisms affected when consuming a high fat diet.

At the final time point, PND 14, there is uniformity between all three experimental groups. Visually the pups from each group look similar, with the exception that pups on the HSD seem to have increased spinal porosity. The visual similarities extend to limb length measurements as well as BV and BMD. There were no significant changes observed with any of the measurements. The trends between the BV and BMD for the three time points in the study can be seen in Figures 14 and 15.
Figure 16: Temporal progression of offspring bone volumes.

Figure 17: Temporal progression of offspring bone mineral densities.

These graphs show how BV, and particularly BMD, is equivalent between the three different groups.
This suggests that the catch up growth has concluded and all the pups are beginning to follow the same developmental path. However, because pups seem to grow at the same rate it does not mean that the HSD or HFD did not have negative effects on the pups. Further studies looking at the micro-architecture of the bone and following pups through adulthood may reveal additional health problems later in life for those pups whose mothers consumed a HFD or HSD.

Genetic predisposition determines up to 80% of peak bone mass in humans, whereas the remaining 20% comes from environmental factors and sex hormones during puberty (Davies, 2004). It has been well documented through epidemiological studies that there is a close link between low birth weight and adult-onset cardiovascular disease (Chen, 2010). This has later been extended to describe how “catch-up” growth can negatively affect development. Studies with children demonstrated that a greater rate of weight gain during a critical window in the first 2 weeks of birth was associated with endothelial dysfunction up to 16 years later (Singhal, 2010). This can be extended to the bone growth and development seen in this study.

At Day 7, both experimental groups increased not only limb length rapidly to catch up to the pups on the CD but they increased BV without an increase in BMD. By Day 14 limb length, BV and BMD had evened out. However, because of the initial rapid growth without an increase of BMD after 1 week of growth and the equal BMD after 2 weeks of growth in the experimental groups compared to
the CD, it can be concluded that careful balance of osteoblast and osteoclast production for bone development were not following the standard developmental patterns. Changes in these patterns may have led to changes in bone strength and signaling pathways that may affect the offspring later in life. Further studies could examine overall bone strength between the three experimental groups.

It has also been found that BMD is not the only indicator of bone strength. So while, by Day 14 both the HSD and HFD pups exhibited equivalent BMD to the pups on the CD studies in bone formation were not examined. One parameter that could be looked at for a complete look at bone health is the degree of mineral organization (Ammann, 2003). Bone strength can be increased without changes in BV or BMD simply by the quality of mineralization (Ammann, 2003).

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To further this research in the future it would be helpful to look at mesenchymal stem cells, (MSCs). MSCs can differentiate into many different cell types, they are the precursors to bone, cartilage, muscle, and stem cells while the pups are in utero (Bruder, 1994). Overall health and development depend on the ability of these cells to continue to differentiate. The numbers of these cells found at birth have been linked to better health later in life. Importantly for this study, MSCs are involved with bone growth and development.

As MSCs age they lose part of their ability to differentiate. With each division the telomeres shorten and eventually the cell will die. The number of MSC cells at birth has been found to be a measure of health later in life (Ourednick, 2005). In animal studies where mothers had been induced with gestational diabetes the pups were found to have less MSC cells found at birth (Stolzing, 2010). This was followed by an initial increase in MSC number and proliferative potential but after 12 weeks the initial increase was replaced with a loss of 50% from the number at birth (Stolzing, 2010). In this study pups in both the HFD and HSD exhibited accelerated growth compared to pups in the CD. Further research could look at MSC count for pups in each group at each time point to determine a similar pattern of accelerated growth followed by dramatic drop off can be found.

Studies have also documented the effect of consuming diets low in protein. While neither of experimental group was designed to be low in protein,
both diets contained 6-8% less protein than the CD. It has been found that offspring dams fed a low-protein diet underwent postnatal catch-up growth had accelerated telomere shortening in organs, including aorta and pancreatic islets (Chen, 2010). It is possible that rapid catch-up growth during lactation results in increased oxidative stress in tissues leading to premature cellular death (Chen, 2010).
5. CONCLUSION

This study has demonstrated decreased limb length, bone density and bone volume at birth of pups whose mothers consumed either a HFD or a HSD. Because of the similar developmental markers between mice and humans, findings from these studies can be applied to human models as well. As the pups began their growth and post-natal development, the HSD group especially exhibited accelerated “catch-up” growth.

Previous studies have demonstrated that this catch-up growth leads to deleterious health defects later in life. Links have been made directly to diabetes, osteoporosis, renal dysfunction, and cardiovascular disease. There is also documentation showing that if pups continue the HFD and HSD post-natally there is an increased likelihood of obesity.

It was reported by the Center for Disease Control (CDC) in the United States that in 2007-2008 there were 72.5 million obese adults (www.cdc.gov). Diseases associated with obesity have caused a 27% increase in healthcare costs approximately $147 billion dollars annually (www.cdc.gov). By consuming diets high in fat or high in sugar during pregnancies mothers are increasing their child’s risk of developing disease later in life.

Some research has been done into mitigating the effects of a hostile uterine environment by adding pre-natal supplements (Liang, 2009). If a simple dietary supplement can minimize the effect of a HFD or HSD, it could be implemented to help prevent the rise of diabetes, osteoporosis, obesity and
other health problems related to these. There are many studies documenting the negative effects of a hostile uterine environment. More research needs to be done on how to minimize these effects.

In U.S. households making less than $15,000 a year, 31 percent of the women are obese (www.cdc.gov). In households with more than $50,000 annually, 17 percent are obese (www.cdc.gov). Cheaper food tends to be higher in fat and sugar and be lacking in complex carbohydrates. In addition, areas of the country with a higher percentage of residents at or below the poverty line such as inner cities do not have immediate access to supermarkets and fresh healthy food (NYCEPC, 2008).

The same households without access to supermarkets and fresh healthy food do not have immediate access to healthcare. It is equally possible that there is a lack of education about the importance to pre-natal care, which in turn leads to the HFD or HSD. These diets have been shown to have deleterious health effects on the developing infant at birth, leading to health problems later in life as described above.

This research could be helpful in educating people on the effects of a HFD or a HSD on pregnancies, encouraging them make better choices for health care. Perhaps, aid could be given to women to develop a food plan that would be more affordable for their budget but also avoid a high prevalence of fat and sugar. Offering pre-natal vitamins at healthcare clinics for free or at a reduced
rate may be a method of reducing the effects of the HFD or HSD. This research is relevant to a wide audience. The health of a person begins in the womb, it is necessary that this environment provides the nutrition needed to mitigate the effects of adult onset diseases such as, diabetes, obesity, cardiovascular disease and osteoporosis later in life.
REFERENCES


SUMMARY

During fetal development, the child’s growth and maturations within the womb depends largely on the food and nutrients provided to the infant through the placenta (Liang, 2009). Recent studies have suggested strong connections between environmental conditions during fetal growth and development and lifelong effects on their growth, maturation, and long-term disease risk (Prater, 2008). The developmental origins of health and disease, or “DOHaD,” hypothesis states that the fetal environment will lay the groundwork for many health risks later in life (Hales, 2001). There is evidence that chronic adult diseases such as obesity, osteoporosis, cardiovascular disease, renal disease, and diabetes can trace their origins back to the gestational environment of a child. Neonatal size is determined primarily by the intrauterine environment (62%); parental genetic determinants are secondary (20% maternal, 18% paternal) (Davies 2005, Penrose 1952).

A fetus begins programming its metabolic and bodily functions based on its exposure to nutrients in the womb. Adaptations occurring in a stressful fetal environment, lead to negative programming of both physiology and metabolism (Barker, 1995). One stress is a high fat or high sugar diet.

Mouse models were used in this study because mice and humans have similar fetal developmental stages; mice have become a common and relevant research model for human prenatal development. This study aimed to compare
the effect of a high fat diet (HFD), high sugar diet (HSD) and a control diet (CD) on fetal growth and development at the time of birth, post-natal day 0 (PND 0), post-natal day 7 (PND 7) and post-natal day 14 (PND 14). Documentation has been presented on the effect of a negative in-utero environment but, currently there is limited research showing the effect of development after birth.

At the designated time points described above, mouse pups were harvested using CO2 inhalation. The pups were then preserved in ethanol and scanned using a high-speed in-vivo micro-computed tomography (micro-CT) system. The micro-CT created 3-D grayscale images of the fetus which were then converted to the Digital Imaging and Communication in Medicine format (DICOM).

Using DICOM images, limb length measurements were taken of each long bone, both right and left, and the jaw. These include the mandible, humerus, radius, ulna, femur, tibia and fibula. In addition to measuring limb lengths, bone volume (BV) and bone mineral density (BMD) were generated by the micro-CT. Pups were compared first to pups whose mothers consumed the same diet and then with all of the pups in the study.

When studying the limb lengths of pups harvested at the PND 0 time point, trends were observed. It was clear that pups that had developed with mothers consuming either the HFD or HSD had shorter long bones. It was also found that there was no significant statistical difference between the HFD and
HSD experimental groups. Related studies illustrate that mice on a HFD diet suffer impaired osteoblastic differentiation, contributing to delayed mineralization and lack of bone growth (Lanham, 2010). A trend was seen when looking at the mandible, another flat bone; while the HSD showed a significant difference in bone length for the CD with the mice in the HFD, the p-value was only equivalent to 0.0623. This suggested that there may be a different signaling or growth mechanism involved with long bone formation as opposed to flat bone formation.

Data from BV and BMD studies aid in understanding bone homeostasis. There was a significant difference in BV for both the HFD and HSD compared to the CD in our study. This observation must also take into account that the bones in the HFD and HSD were found to be shorter, inherently decreasing the skeletal volume. Analysis from BMD also shows a significant decrease from the CD and both experimental groups. This decrease in overall BMD suggests that by changing the diet in the two experimental groups, ossification was delayed. This study also showed that there were not only differences found between the CD and experimental groups, but also between the HFD and HSD. Pups consuming the HSD also had a significantly higher BMD than pups in the HFD diet. This observation suggests that HFD and HSD affect pup growth and development differently.
Further body developmental differences were observed with pups harvested at Day 7. It appeared that the HFD pups had caught up to the CD pups in long bone growth, although the skull bones had qualitatively noticeable delays in development.

Measurements of the shorter long bones, tibia and fibula still showed the HFD and HSD significantly shorter than the CD pups. This was similar to what was observed birth. However, measurements of the femur and ulna showed no significant difference or trend between any of the three experimental groups and when examining the radius and ulna there was a significant increase in height between the HSD and the CD. This suggests that the HFD caused pups to begin to play catch up after birth. While the HSD caused pups to catch-up at an accelerated rate and in some cases bones over developed compared to pups on the CD. In rats and humans, fetal growth impairment followed by catch-up growth is associated with reduced lifespan (Singhal, 2004; Nuyt, 2009).

Low bone mass and deterioration of the micro-architecture of bone tissue leads to bone fragility and is a symptom of osteoporosis (Goodfellow, 2010). For pups on the HFD there was not as much bone growth as was seen with pups on the HSD, additionally the BMD for the HFD pups was significantly reduced. While bone volume increased dramatically in these pups, studies have shown that even with increased bone volume there are generally shorter long bones as well as a disorganized trabecular architecture (Liang, 2009).
During skeletal growth, there is net bone formation, at peak bone mass the amount of osteoclastic bone resorption is matched by the amount of osteoblastic new bone formation (Davies, 2004). If this process is compromised and is happening at an accelerated rate, new bone formation will be unable to develop to the optimal size and material properties for daily physiological conditions (Ionova-Martin, 2010).

At the final time point, PND 14, there is uniformity between all three experimental groups. The pups from each group look similar, although the pups on the HSD seem to have increased spinal porosity. The visual similarities extend to limb length measurements as well as BV and BMD. There were no significant changes observed with any of the measurements.

This suggests that the catch up growth has concluded and all the pups are beginning to follow the same developmental path. However, because pups seem to grow at the same rate it does not mean that the HSD or HFD did not have negative effects on the pups. Further studies looking at the micro-architecture of the bone and following pups through adulthood may reveal additional health problems later in life for those pups whose mothers consumed a HFD or HSD.

Genetic predisposition determines up to 80% of peak bone mass in humans, whereas the remaining 20% comes from environmental factors and sex hormones during puberty (Davies, 2004). Documentation of epidemiological studies shows a link between low birth weight and adult-onset cardiovascular
disease (Chen, 2010). This has later been extended to describe how “catch-up”
growth can negatively affect development. Studies with children demonstrated
that a greater rate of weight gain during a critical window in the first 2 weeks of
birth was associated with endothelial dysfunction up to 16 years later (Singhal,
2010). This can be extended to the bone growth and development seen in this
study.

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uterine environment by adding pre-natal supplements (Liang, 2009). If a simple
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