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Examining the Effect of Maternal Obesity on Vitamin D Metabolism in the Placenta

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Abstract

In the United States and in many other developed countries, obesity is becoming an increasingly widespread problem due to several factors including poor nutrition and sedentary lifestyles. Previous studies have shown obese people to have less circulating vitamin D than lean people and this study aimed to determine whether obese mothers pass less vitamin D through their placenta to their offspring than lean mothers do and whether vitamin D deficiency resulting in fewer vitamin D receptors in the placenta can be reversed by treatment of placental cells with the active and inactive forms of vitamin D. The data collected from this study show that placentas from obese mothers have less vitamin D receptors than placentas from lean mothers and that placental cells treated with the active form of vitamin D show increased expression of vitamin D receptors. Preliminary tests of mitochondrial respiration have found that placental cells from an obese mother respond more dramatically in terms of oxygen consumption rate to treatment with both the active and inactive forms of vitamin D than placental cells from a lean mother do. Vitamin D plays an important role in the placenta, and future studies should be conducted to determine the daily dosage of vitamin D necessary to reverse vitamin D deficiency during pregnancy.

Introduction

Obesity is becoming an increasingly prevalent health problem in many developed countries. In the United States, it is estimated that over one-third of the adult population is obese, measured by having a Body Mass Index (BMI) greater than 30 (Hales et al. 2018). Obesity has been shown to be a risk factor for a number of health problems including hypertension and cardiovascular disease. These health problems can also be passed to future generations, as previous studies have shown that offspring of obese mothers are predisposed to obesity and to health problems associated with obesity. It is estimated that over 40% of women who enter pregnancy in the United States are either overweight or obese (Chu et al. 2009), showing just how widespread this problem is becoming.

Previous studies have shown obese people to have lower levels of circulating vitamin D than lean people, though the cause for this has not yet been extensively studied (Santos et al. 2015). Vitamin D is a lipid soluble vitamin that has important properties including aiding in calcium absorption, being antimicrobial, and helping to reduce inflammation. Vitamin D does not occur

naturally in many foods and is best obtained through direct sunlight exposure resulting in its synthesis or through vitamin D supplements resulting in absorption. Once vitamin D enters the bloodstream, it is converted to its inactive form [25(OH)D] in the liver and subsequently to its active form [$1,25(OH)_2D$] in the kidney (Danik and Manson 2012). Previous studies have found evidence to support that vitamin D has an important role in regulating immune responses during gestation (Liu et al. 2011) and another study found that offspring of mothers who had low circulating vitamin D concentrations during late pregnancy had reduced bone mass at 9 years of age (Javaid et al. 2006).

This study aimed at testing the hypothesis that obese mothers pass less vitamin D through their placenta to their offspring than lean mothers, based on the knowledge that obese people tend to have less circulating vitamin D than lean people. This study also aimed to observe if treatment of cytotrophoblasts (the main cell type in the placenta) with vitamin D could increase vitamin D receptor (VDR) levels in placental cells and partially reverse the effects of deficiency. Human placentas were collected for this study following Caesarean sections and Western blots were run to measure VDR levels in placentas of lean and obese mothers who had male or female offspring. Cytotrophoblasts were also isolated from collected placentas and were treated with active and inactive forms of vitamin D for 24 and 48 hours so that vitamin D receptor levels and mitochondrial respiration could be measured with treated cells to determine if deficiency could possibly be partially reversed with vitamin D treatment. Vitamin D levels were also measured in corresponding maternal serum and cord blood samples to observe whether maternal serum and cord blood vitamin D levels are positively correlated and whether maternal serum vitamin D levels were correlated with maternal BMI.

Materials and Methods

Sample Collection – Placentas were collected from Caesarean-section births at Oregon Health and Sciences University in Portland, Oregon and were classified as either coming from a lean (18.0-24.9), overweight (25.0-29.9), or obese (>30.0 BMI) mother.

Tissue Processing and Sampling – Tissue was collected from five different locations on each placenta, the samples were combined and homogenized in lysis buffer using a mini-bead beater.

Maternal serum and cord blood vitamin D content – An ELISA Vitamin D plate (abcam) was used to determine the vitamin D active form concentrations in maternal and cord blood pairs.

Western Blotting – Proteins were separated on PAGE gels and were then transferred onto nitrocellulose membranes. Membranes were blocked in 5% nonfat milk powder in TBST solution and blots were probed in VDR primary antibody in 1% nonfat milk powder in TBST solution overnight in a 4^{o} C cold room. Blots were then probed for one hour in secondary antibody in 1% nonfat milk powder in TBST at room temperature. Membranes were visualized using chemiluminescence and band intensity was measured using a G:Box.

Isolation of cytotrophoblasts cells – Cytotrophoblasts were isolated from placental samples collected after C-section births by another member in the lab. Cytotrophoblasts were then treated with active and inactive forms of vitamin D for 24 and 48 hours and VDR levels and mitochondrial respiration of cells was then measured.

Measure of mitochondrial respiration – Mitochondrial respiration of cytotrophoblasts treated with active and inactive forms of vitamin D was determined by using Seahorse equipment after cells had been plated for 72 hours.

Statistical analysis – Data was analyzed using Microsoft Excel and GraphPad to determine the p-value of data sets.

Results

Table 1 – Clinical data of study participants

Pre-pregnancy or First Trimester BMI Group	**NW**		**OB**	
Sex of Fetus	**Male n=6**	**Female n=6**	**Male n=6**	**Female n=6**
Pre-pregnancy BMI (kg/m^2)	22.3 (19.3-24.7)	22.5 (18.3-24.9)	37.1 (31.78-45.5)	37 (32.9-43.4)
Fetal Birth Weight (g)	3283.75 (3105-3520)	3764 (2975-4975)	3326 (308.8-749.9)	3505 (389-595.2)
Placenta Weight mean ± SEM, grams	526 (487.6-570)	577 (457.4-758.6)	549 (308.8-749.9)	504 (389-595.2)

Figure 1 – ELISA plate results showing the relationship between maternal BMI and maternal serum vitamin D concentration

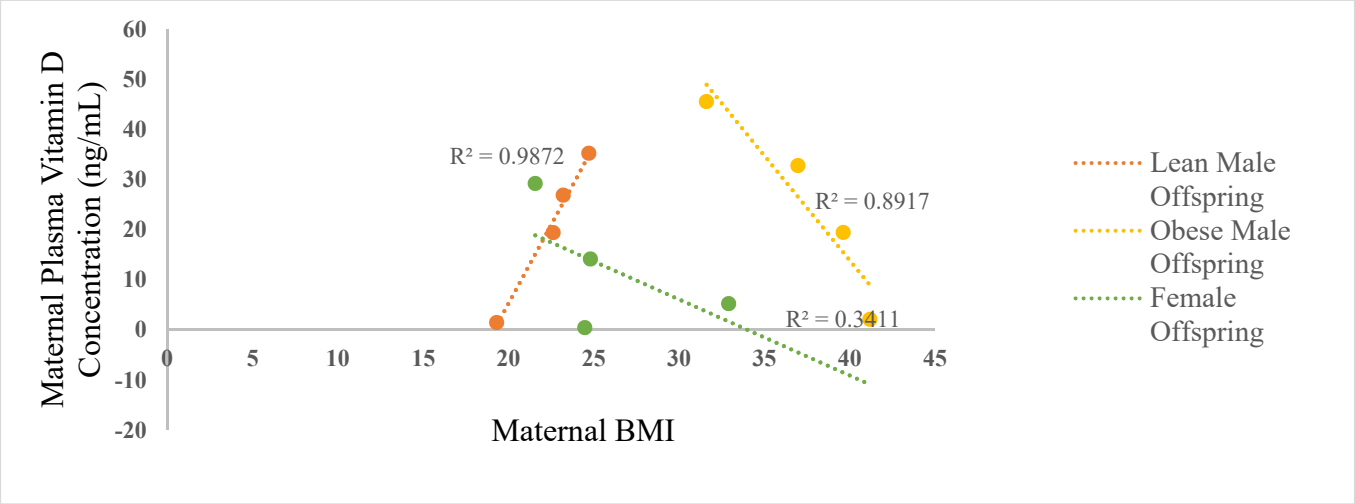


Figure 2 – ELISA plate results showing the relationship between maternal and cord blood serum vitamin D concentrations

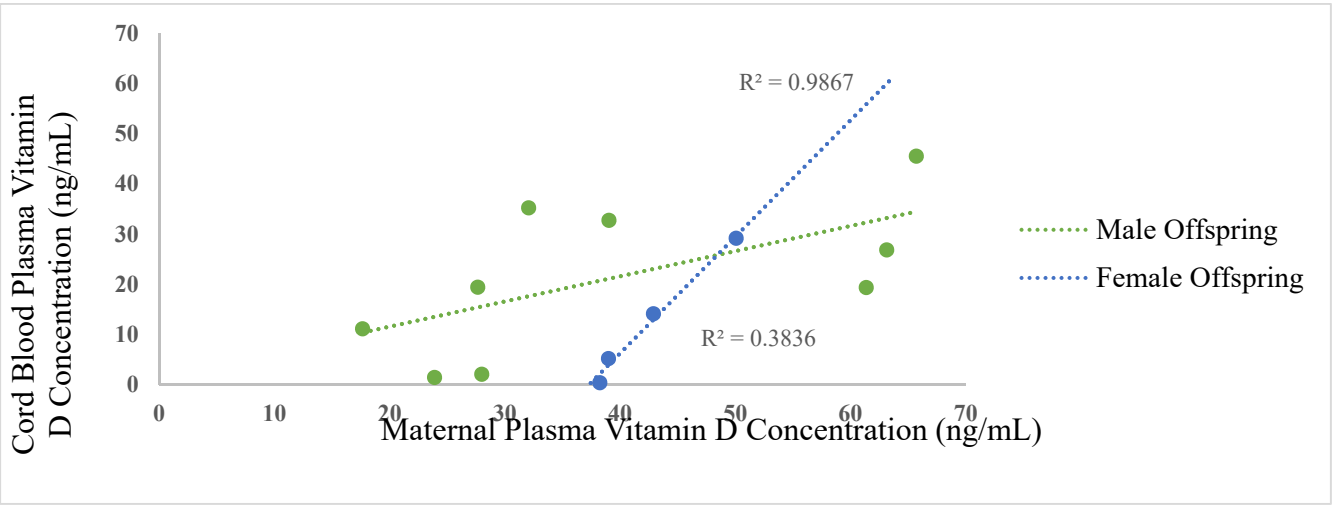


Figure 3 – Vitamin D receptors in placentas of lean and obese offspring for females (A) and males (B)

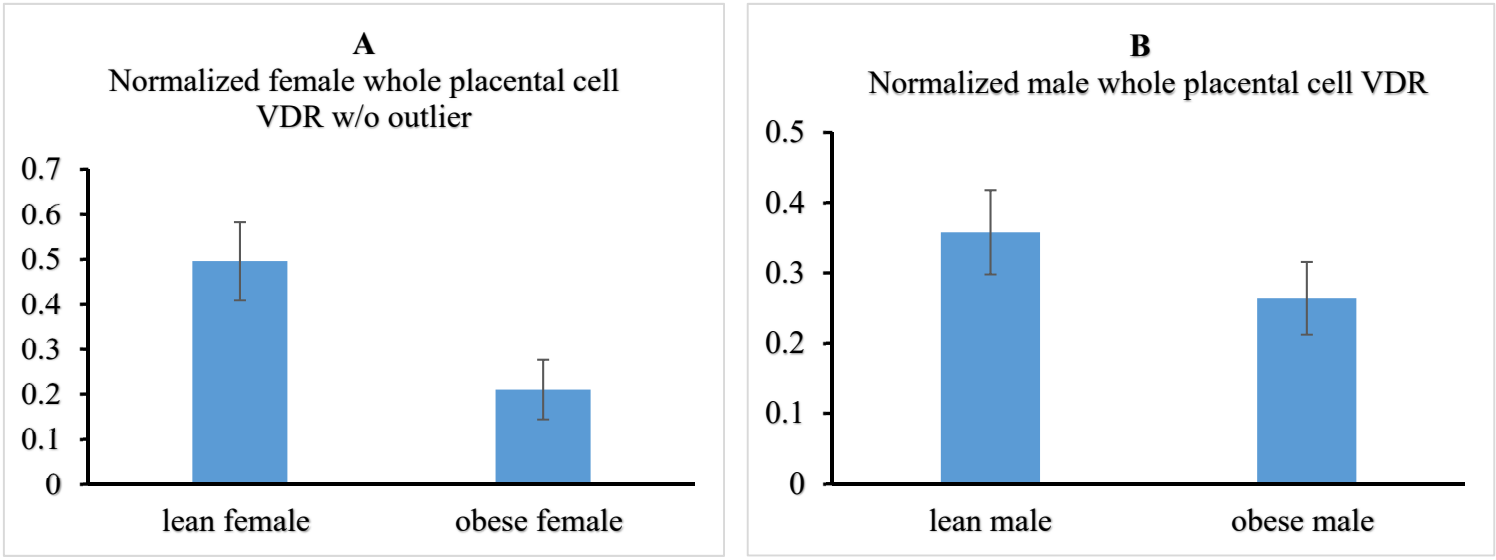


Figure 4 – Effect of active and inactive forms of vitamin D treatment on vitamin D receptor expression in isolated cytotrophoblasts

Active Form – Calcitriol

Inactive Form – Calcifediol

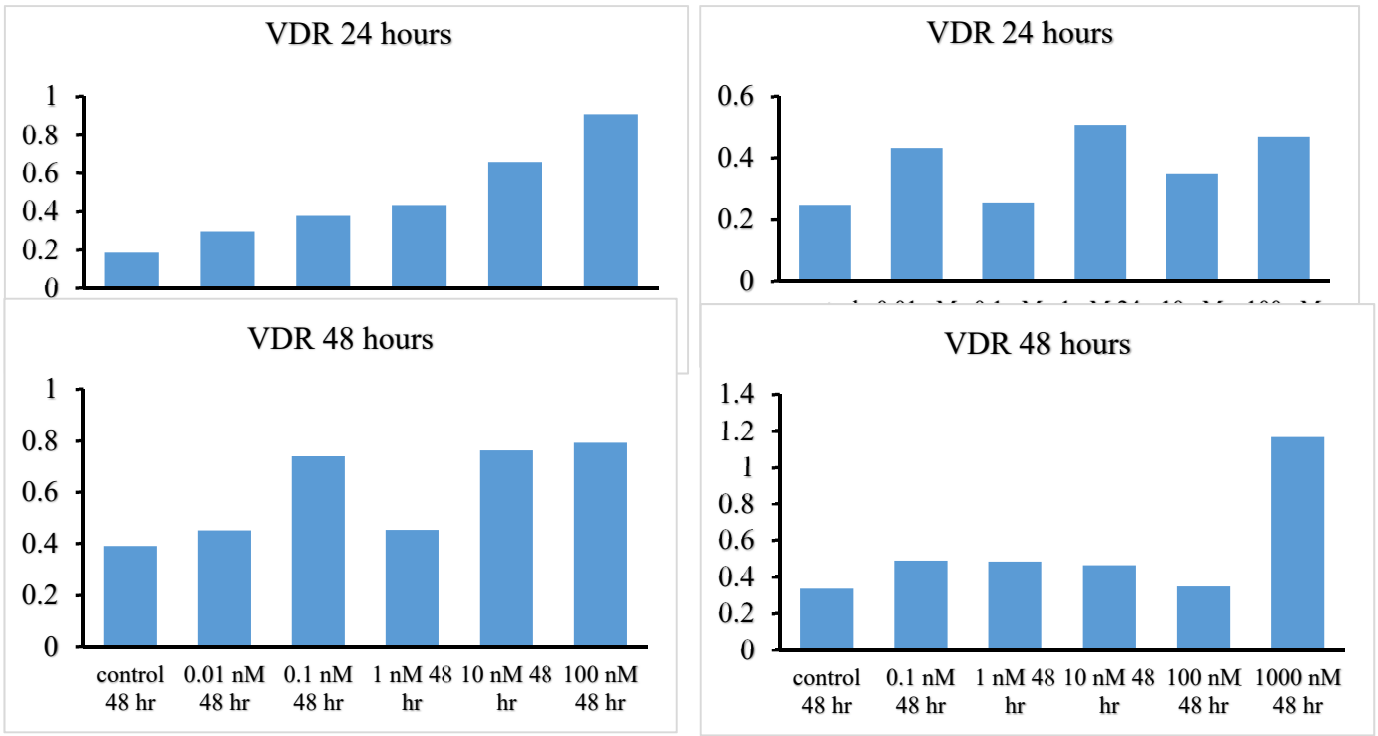
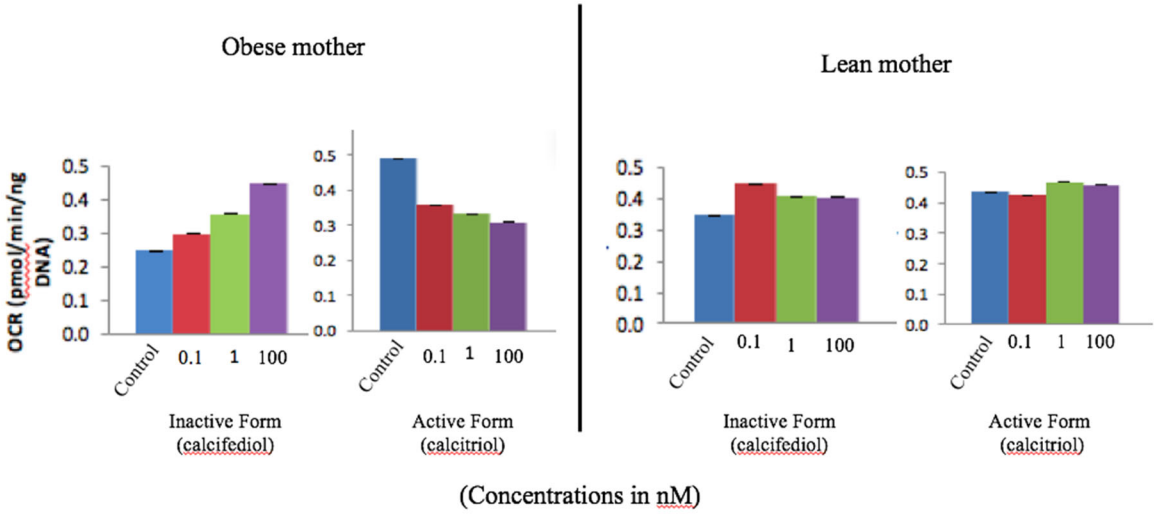


Figure 5 – Effect of vitamin D treatment on mitochondrial respiration in cytotrophoblasts cells (preliminary)



Discussion

This investigation looked at vitamin D and vitamin D receptor levels in placental and serum samples to determine the effects of maternal obesity on vitamin D concentrations in the placenta and to evaluate the importance of vitamin D in cytotrophoblasts mitochondrial respiration. Previous studies have shown that obese people have less circulating vitamin D than lean people. Vitamin D deficiency has been linked to health problems including preeclampsia, Cesarean section delivery, and gestational diabetes in pregnant women and to problems including weak bones, low birth weight, type 1 diabetes, and schizophrenia in infants and children (Shin et al. 2010).

Vitamin D Concentrations in Maternal Serum and Cord Blood Samples

A positive correlation was seen between corresponding maternal serum and cord blood vitamin D concentrations. Increasing the sample size will help determine the strength of this trend. This positive correlation indicates that the mother likely is passing vitamin D through her placenta to her offspring and that maternal serum vitamin D levels contribute to the vitamin D

levels in the cord blood of the offspring, which is likely similar to the serum vitamin D concentration of the offspring.

Vitamin D Receptor Expression in the Placenta

Vitamin D and vitamin D receptors are present in the placenta. Vitamin D is thought to play a role in reducing inflammation and infection in the placenta (Liu et al. 2009). Placentas from obese mothers who had female offspring were shown to have statistically significantly lower levels of vitamin D receptors than placentas from lean mothers who had female offspring. A similar trend was seen with obese and lean mothers of male offspring, though the data was not shown to be significant. Less vitamin D receptors on the placenta means that less active vitamin D can bind to and be utilized by the organ. Based on previous research, less vitamin D binding potentially will lead to an increased risk for infection and inflammation in the placenta.

Treatment of Cytotrophoblasts with Vitamin D

Cytotrophoblasts treated with the active form of vitamin D (calcitriol) for 24 and 48 hours were shown to have a positive dose dependent response with the concentration of vitamin D treatment correlating to the amount of vitamin D receptors in the cells after both time points. A definitive trend was not seen in cells treated with the inactive form of vitamin D (calcifediol). These results indicate that, to some extent, treatment with the active form of vitamin D can increase vitamin D receptor levels and can decrease deficiency in cytotrophoblasts cells. However, the concentration of vitamin D that deficient placental cells should be treated with the restore proper placental function is not yet known.

Mitochondrial Respiration in Cytotrophoblasts Treated with Vitamin D

We are still working to increase the sample size for this experiment, though preliminary findings show that vitamin D treatment of cytotrophoblasts affects mitochondrial respiration

much more in cytotrophoblasts from an obese mother than in cytotrophoblasts from a lean mother. This indicates that placental cells from an obese mother are likely in a greater state of stress than placental cells from a lean mother, because placental cells from an obese mother had a much more dramatic change in respiration when treated with both forms of vitamin D than was seen with placental cells from a lean mother. Once the entire data set has been normalized, more analysis can be done and conclusions can be drawn as to the significance of the results.

Vitamin D is important for its role in calcium absorption and for its anti-inflammatory and antimicrobial properties. Vitamin D is important in the placenta and an adequate amount of circulating vitamin D is important for a healthy pregnancy and for healthy fetal development. Vitamin D differences between the placentas of lean and obese mothers have been shown in this study and further studies should be conducted to determine the extent of the role vitamin D plays in the placenta and the dosage of vitamin D that pregnant women should take to reverse the effects of vitamin D deficiency.

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