

THE EFFECT OF VITAMIN D
ON PLACENTAL
DEVELOPMENT AND
PREGNANCY SUCCESS

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III. Abstract

Vitamin D₃ deficiency is prevalent around the world, with 30-60% of Australians having 25(OH)D₃ levels below 50 nmol/L. There has been a resurgence of interest in vitamin D₃ in the last decade as its deficiency has been shown to be associated with an increasing number of diseases, including pregnancy complications. The role of vitamin D₃ in pregnancy is unclear, although vitamin D₃ metabolism genes are expressed in the placenta and circulating active vitamin D₃ increases 3-fold during pregnancy. As many pregnancy complications are associated with poor placental development, we hypothesise that vitamin D₃ deficiency may impair placental development and thereby contribute to the pathogenesis of pregnancy complications.

To determine the effect of dietary vitamin D₃ and calcium deficiency on the placenta we used a mouse model. Mice were fed diets deficient in vitamin D₃ and/or calcium, mated with normal males and killed at d18.5 *post-coitus*. Pregnant and non-pregnant mice had altered vitamin D₃ metabolism, namely alterations in biochemistry and kidney gene expression. During pregnancy serum 1,25(OH)₂D₃ levels are elevated. The level of expression of the gene for the enzyme responsible for activation of vitamin D, vitamin D₃ 25-hydroxylase (*CYP27B1*), was highest in kidneys of pregnant mice compared to levels in non-pregnant mice as well as to levels in the placenta of pregnant mice, indicating that increased renal 1,25(OH)₂D₃ production is a feature of pregnancy. Pregnant mice consuming diets deficient in both vitamin D₃ and calcium resulted in higher incidence of preterm birth (PTB) as defined by a delivery before d18.5 of gestation. While pregnant mice had comparable placental weights regardless of vitamin D and calcium deficient diets, the placental morphometry was altered such that there was increased

capacity for feto-maternal exchange. Consistent with this, the average fetal weight was significantly greater in those dams consuming a low calcium diet regardless of the dietary vitamin D level, suggesting that placental adaptations allowed greater fetal growth.

We next examined the human placental expression profile of vitamin D₃ metabolism and the interacting insulin-like growth factor (IGF) pathway genes across gestation. We found that vitamin D₃ pathway genes (*VDR*, *CYP2R1*) increase while IGF genes (*IGF1R*, *IGF2*) decrease from early to late gestation, reflecting the most important timeframes in gestation for the action of these pathways. Correlations in gene expression were found between *IGF2* and *VDR*, as well as *IGF1R* and *VDR*, suggesting new interactions between the pathways. Immunohistochemistry revealed reducing *CYP27B1*, *VDR* and *IGF2* protein across gestation, while *CYP24A1* was not altered. As *VDR* protein is reduced, with high mRNA levels, this indicates a high turnover of *VDR* protein in term placentas.

Human placental gene expression from pregnancies with medical complications was also compared to placentas from normal term-delivered controls. The mRNA level for *VDR* was reduced in placentas of pregnancies with preeclampsia and spontaneous preterm deliveries. In addition, *CYP24A1* mRNA levels were reduced in placentas from pregnancy cases with gestational diabetes and fetuses that were considered small for gestational age. This suggests that placental vitamin D₃ metabolism is altered both across gestation and in pregnancy complications. Despite these changes in gene expression for vitamin D related genes, there was only one altered IGF-family gene, with *IGF1* increased in placentas from pregnancies with preterm deliveries. As *IGF1* interacts with the vitamin D pathway, this could be an interacting pathway in pathology of preterm birth.

In conclusion, vitamin D₃ and calcium metabolism are altered by pregnancy, with deficiency of both vitamin D₃ and calcium resulting in PTB in mice. Dietary vitamin D and calcium deficiency increased fetal weight which was associated with changes in both placental morphometry and gene expression. In human placenta, the expression of vitamin D₃ metabolic pathway components is altered both across gestation and between complicated and uncomplicated pregnancies. These studies together strongly indicate a role for vitamin D₃ action in normal and complicated pregnancy.

IV. Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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V. Abstracts arising from this thesis

1. Laurence JA, Bianco-Miotto T, Anderson P, Roberts CT, Placental expression of the vitamin D₃ and insulin-like growth factor families across gestation, Fetal and Neonatal Workshop, 2014, Yanchep, W.A, Australia (Oral)
2. Laurence JA, Anderson P, Roberts CT, Dietary vitamin D₃ and calcium restriction and its effects on murine placental morphometry, Health Sciences Postgraduate Research Conference, The University of Adelaide, 2013, Adelaide, S.A., Australia (Poster)
3. Laurence JA, Anderson P, Bianco-Miotto T, Roberts CT, Murine placental and kidney gene expression is altered by dietary vitamin D₃ and calcium deficiency, Australian Society for Medical Research SA Annual Scientific Meeting, 2013, Adelaide, S.A., Australia (Oral)
4. Laurence JA, Anderson P, Bianco-Miotto T, Roberts CT, Dietary vitamin D₃ and calcium restriction and its effects on murine placental morphometry, Fetal and Neonatal Workshop, 2013, Barossa Valley, S.A., Australia (Oral)
5. Laurence JA, Anderson P, Bianco-Miotto T, Roberts CT, Effect of dietary calcium on vitamin D₃ related genes in mouse kidney and placenta, Australian Health and Medical Research Council Annual Meeting, 2012, Adelaide, S.A., Australia (Poster)
6. Laurence JA, Anderson P, Roberts CT, Pregnancy alters body composition, vitamin D₃ and calcium levels in mice, The Annual Scientific Meeting of the Endocrine Society of Australia and the Society for Reproductive Biology, 2012, Gold Coast, QLD, Australia (Oral)
7. Laurence JA, Bianco-Miotto T, Andraweera P, Anderson P, Dekker GA, Roberts CT, Placental gene expression of vitamin D₃ and insulin-like growth

- factor (IGF) genes in pregnancy complications, Australian Society for Medical Research SA Annual Scientific Meeting, 2012, Adelaide, S.A., Australia (Oral)
8. Laurence JA, Anderson P, Furness D, Roberts CT, Effect of dietary vitamin D₃ and calcium deficiency on pregnancy success in C57Bl6 mice, Annual Meeting of the Society for Reproductive Biology, 2011, Cairns, QLD, Australia (Oral)
 9. Laurence JA, Anderson P, Furness D, Roberts CT, Effect of dietary vitamin D₃ and calcium deficiency on pregnancy success in C57Bl6 mice, Faculty of Health Sciences Post Graduate Research Conference, 2011, Adelaide, S.A., Australia (Poster)
 10. Laurence JA, Anderson P, Furness D, Roberts CT, Effect of dietary vitamin D₃ and calcium deficiency on pregnancy success in C57Bl6 mice, Robinson Institute Research Centre for Reproductive Health Research Day, 2011, Adelaide, S.A., Australia (Poster)
 11. Laurence JA, Anderson P, Furness D, Roberts CT, Effect of dietary vitamin D₃ and calcium deficiency on pregnancy success in C57Bl6 mice, ANZSCDB Adelaide Cell and Developmental Biology Meeting, 2011, Adelaide, S.A., Australia (Poster)

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VII. Abbreviations

1,25(OH)₂D₃ - 1α,25-dihydroxy vitamin D₃

25(OH)D₃ - 25-hydroxy vitamin D₃

AGA - appropriate for gestational age

ANOVA - analysis of variance

ANCOVA - analysis of covariance

CYP - cytochrome P450 enzyme

CYP2R1 - vitamin D₃ 25-hydroxylase

CYP27B1 - 25 hydroxy vitamin D₃ 1α-hydroxylase

CYP24A1 - 1,25 dihydroxy vitamin D₃ 24-hydroxylase

DNA - deoxyribonucleic acid

GDM - gestational diabetes mellitus

GH - gestational hypertension

IGF1 - insulin-like growth factor 1

IGF1R - insulin-like growth factor receptor 1

IGF2 - insulin-like growth factor 2

IGF2R - insulin-like growth factor receptor 2

IHC - immunohistochemistry

IQR - interquartile range

IUGR - intrauterine growth restriction

LBW - low birth weight

OR - odds ratio

PBS - phosphate buffered saline

PE - preeclampsia

PTB - preterm birth

PTH - parathyroid hormone
PTHrP - parathyroid hormone related protein
qPCR - quantitative real time PCR
RNA - ribonucleic acid
RR - relative risk
RQI - RNA Quality Index
SD - standard deviation
SEM - standard error of the mean
SGA - small for gestational age
sPTB - spontaneous preterm birth
VDR - vitamin D receptor
Vdr null - VDR knockout mice
VDRE - vitamin D responsive element
WT - wild type