Vitamin D: 10 things your mother never told you

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Things our mothers told us about food:

1. Veggies will make you tall/strong
2. Liver is good for you
3. Milk is good for you
4. Mushrooms are good for you
5. Soda will rot your teeth
6. Coffee will stunt your growth
7. Eat all of your food because there are children starving who would love to have it.

But…as a child did your mother ever tell you about vitamin D?
Ten things to know about vitamin D

1. What is it?
2. Where can I get some?
3. What does the body do with it? How is it used?
4. How much do I need? Am I deficient?
5. How much is too much?
6. What may be keeping me from getting enough?
7. How common is deficiency?
8. What happens if I don’t have enough (deficient)?
9. What happens if I don’t have enough during pregnancy?
10. Are there long term consequences of deficiency?
Ten things to know about vitamin D

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1. What is vitamin D?

Vitamin D is known as an essential “nutrient”
- At least 10 different forms of vitamin D

Inactive form
- 7-dehydrocholesterol
- cholecalciferol (vitamin D3)
- ergocalciferol (vitamin D2)

Inactive form
- 25-hydroxyvitamin D

Active form
- Calcitriol
- 1, 25-dihydroxyvitamin D₂
2. Where can I get some?

### Diet & supplements

<table>
<thead>
<tr>
<th>Vitamin D2 (ergocalciferol):</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-irradiated mushrooms</td>
</tr>
<tr>
<td>Oral supplements</td>
</tr>
<tr>
<td>Artificially fortified foods (e.g. milk, cereals, bread, margarine)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vitamin D3 (cholecalciferol):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultraviolet B light (290-315 nm): the major source of vitamin D3</td>
</tr>
<tr>
<td>Fatty fish:</td>
</tr>
<tr>
<td>Salmon</td>
</tr>
<tr>
<td>Mackerel</td>
</tr>
<tr>
<td>Tuna</td>
</tr>
<tr>
<td>Sardines</td>
</tr>
<tr>
<td>Eel</td>
</tr>
<tr>
<td>Cod liver oil</td>
</tr>
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*Kitson & Roberts 2012, Journal of hepatology*
2. Where can I get some?

- Is vitamin D a vitamin? Is it an essential nutrient?
2. Where can I get some?

- Sun exposure

- Dietary intake
  - Fortified foods, supplements (D2 or D3), fish oils (D3)
  - ergocalciferol (vitamin D2)
  - Egg yolk
  - Liver

- Other sources:
  - 7-dehydrocholesterol
  - cholecalciferol (vitamin D3)
  - 25-hydroxyvitamin D
  - Calcitriol
  - 1, 25-dihydroxyvitamin D₂
2. Where can I get some?

- Synthesis of vitamin D in the skin (epidermis)
- Is vitamin D a vitamin? Is it an essential nutrient?
2. Where can I get some?

- What about nocturnal animals?
- Species differ in how much they need

A. *Rousettus aegyptiacus*:
  - caves, tombs, and buildings

B. *Pteropus hypomelanus*:
  - trees

*Southworth L. et al 2013*
3. What does the body do with it?

- Vertebrates and invertebrates have conserved VitD pathways
- Most get requirements from sunlight

*Reschly E et al 2007*
3. What does the body do with it?

- Converts it to stable but inactive metabolites

- UVB 
  - Skin 
  - Liver 
  - Dietary intake 
  - Fortified foods, supplements (D2 or D3), fish oils (D3) 
  - Egg yolk 
  - Liver
3. What does the body do with it?

- Converts it to instable but active metabolites

- Skin: UVB irradiation leads to 7-dehydrocholesterol to cholecalciferol (vitamin D3)

- Liver: Cyp2r1 converts 25-hydroxyvitamin D to calcitriol (active form) via Cyp27B1

- Kidney: Cyp24A1 converts calcitriol to 1,24,25 trihydroxy vitamin D

- Stored: Fat, Muscle

- Excreted: 24,25 DHD

Dietary intake:
- Fortified foods, supplements (D2 or D3), fish oils (D3)
- Egg yolk, Liver

VitD-binding protein in kidney facilitates the transport of calcitriol.
3. What does the body do with it?

- Active vitamin D (1, 25-dihydroxyvitamin D) is steroid hormone
- Circulating signaling molecule
3. What does the body do with it?

- Active vitamin D (1, 25-dihydroxyvitamin D) is steroid hormone
- Circulating signaling molecule

Affected tissues
- Brain
- Gut (intestines)
- Heart
- Muscle
- Liver
- Pancreas
- etc..

Calcium absorption
Phosphate absorption
Insulin secretion

Cell growth
Immune function
etc…
4. How much do I need?

- Still under investigation – Guidelines vary
- IOM recommends adequate levels are:
  - $\geq 50\text{nmol/L or } \geq 20\text{ng/ml}$

Vitamin D 25(OH)D range guidelines from various organizations:

<table>
<thead>
<tr>
<th></th>
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<th>Endocrine Society</th>
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<tr>
<td>Deficient</td>
<td>0-30 ng/ml</td>
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<td>0-31 ng/ml</td>
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<tr>
<td>Insufficient</td>
<td>31-39 ng/ml</td>
<td>21-29 ng/ml</td>
<td>12-20 ng/ml</td>
<td></td>
</tr>
<tr>
<td>Sufficient</td>
<td>40-80 ng/ml</td>
<td>30-100 ng/ml</td>
<td>&gt;20 ng/ml</td>
<td>32-100 ng/ml</td>
</tr>
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www.vitamindcouncil.org
5. How much is too much?

- Still under investigation
  - Toxic levels are >150ng/ml -> Hypercalcemia
  - Do not take >10,000 IU/day > 3 months

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[www.vitamindcouncil.org](http://www.vitamindcouncil.org)
6. What keeps me from getting enough?

- Insufficient diet

Dietary intake:
- Fortified foods, supplements (D2 or D3), fish oils (D3)
- Ergocalciferol (vitamin D2)
- Egg yolk
- Liver

Skin:
- UVB
- 7-dehydrocholesterol
- Cholecalciferol (vitamin D3)

Liver:
- Cyp2r1
- 25-hydroxyvitamin D

Kidney:
- Cyp27B1
- Calcitriol
- 1,25-dihydroxyvitamin D2

Stored:
- Fat
- Muscle

Inactive form:
- 24,25 DHD

Inactive form:
- 1,24,25 trihydroxy vitD

Excreted form:
- Cyp24A1

VitD-binding protein
6. What keeps me from getting enough?

- Insufficient sun exposure

![Diagram of vitamin D metabolism]

Stored
- Fat
- Muscle

Inactive form
- 24,25 DHD

Active form

Dietary intake
- Fortified foods, supplements (D2 or D3), fish oils (D3)
- ergocalciferol (vitamin D2)
- Egg yolk
- Liver

Excreted form
- Cyp24A1

Inactive form
- 1,24,25 trihydroxy vitD

VitD-binding protein
- Kidney

Active form
- Calcitriol

Cyp27B1
- Kidney

Cyp2r1
- Liver

UVB
- Skin

7-dehydrocholesterol
- cholecalciferol (vitamin D3)

25-hydroxyvitamin D
- Liver
6. What keeps me from getting enough?

- Genetic differences at transport/metabolism genes

![Diagram showing the conversion of vitamin D from UVB exposure to active form and storage]

- UVB exposure leads to 7-dehydrocholesterol in the skin, which is converted to cholecalciferol by UVB.
- Cholecalciferol enters the liver where it is converted to 25-hydroxyvitamin D (25(OH)D) by the enzyme Cyp2r1.
- In the kidney, 25(OH)D is converted to 1,25-dihydroxyvitamin D (1,25(OH)2D) by Cyp27B1, which is the active form.
- 1,25(OH)2D can be stored in fat and muscle.
- Excretion occurs through inactive forms such as 24,25 DHD and 1,24,25 trihydroxy vitD.

Dietary intake of fortified foods, supplements, and foods like egg yolk and liver provides vitamin D.

- VitD-binding protein transports vitamin D from the liver to the kidney for metabolism.

Key processes:
- **Active form**: 1,25(OH)2D
- **Inactive form**: 24,25 DHD, 1,24,25 trihydroxy vitD
- **Excreted form**: Cyp24A1
6. What keeps me from getting enough?

- Outside at wrong time of day

Southworth L. et al 2013
6. What keeps me from getting enough?

- Seasonal differences in exposure to sunlight

![Graph showing 25(OH)-D levels over the year with intervention points highlighted.]

On VitD supplement (500IU)
No VitD supplement

*Nutrition in the prevention and treatment of disease*
6. What may be keeping me from getting enough?

- Skin pigment - melanin: competes with > 7-dehydrocholesterol
- Prolonged UVB exposure: converts previtamin D3 into inactive compounds (tachysterol and luminiseterol).
- Clothing
- Window Glass
- Sun Screens that block UVB
- Too much time indoors not enough time outdoors
- Clouds
- Smog & other air pollution
- Season (Winter)
- Distance from equator
- Adiposity/obesity
- Intestinal malabsorption:
  - Disease (crohn’s disease) & Pharmaceuticals
- Age
- Ethnicity (eg. genetic differences in vitamin D metabolism genes)
7. How common is “deficiency”?  

- Age & gender dependent

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age group (yrs)</th>
<th>Avg nmol/l</th>
<th>%&lt;50nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1-8</td>
<td>~70</td>
<td>10</td>
</tr>
<tr>
<td>Male</td>
<td>9-13</td>
<td>~60</td>
<td>21</td>
</tr>
<tr>
<td>Male</td>
<td>14+</td>
<td>~60</td>
<td>30-36</td>
</tr>
<tr>
<td>Female</td>
<td>1-8</td>
<td>~70</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
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<td>27</td>
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<td>Female</td>
<td>14+</td>
<td>~60</td>
<td>34-38</td>
</tr>
</tbody>
</table>

NHANES 2001-2006
7. How common is “deficiency”? 

- Ethnicity dependent (genetic & cultural differences)

Modified Forrest K 2010, Nutr Res.
7. How common is “deficiency”? 

- Geographical location dependent

**Global populations**

- No global standard of screening pregnant women
- Supplementation is widespread

**US (NHANES)**
- Females 12-44yrs

*Looker et al. 2011*

*Saraf et al 2015*
4. How much do I need?

- Are all of these people really “deficient”? Should we all be on supplements?

Saraf et al 2015

Vitamin D deficiency prevalence by ethnicity and gender
4. How much do I need?

- Still under investigation
- Growing evidence that needs may differ by genetic differences
8. What happens if I don’t have enough?

- Increased risk for disease/disorders:

Bone health
  • Rickets: bone, muscle, respiratory, impaired growth
  • Osteoporosis: low bone mineral density
  • Osteomalacia: muscle atrophy, bone pain and fatigue

Immune health
  • Infection
  • Autoimmune disorders (MS, autism)

Cardiometabolic health
  • Cardiovascular disease
  • Diabetes

Neurological health
  • Parkinsons, Alzheimer’s, epilepsy etc.

Cancer
  • Colon, breast, lung, prostate

Reproductive health
  • Male and female fertility

Fetal development?
9. What happens if I don’t have enough during pregnancy?

- Still under investigation
9. What happens if I don’t have enough during pregnancy?

- High percent of mothers are at risk of inadequacy

![Graph showing maternal deficiency prevalence across regions.](Saraf et al 2015)

- Low vitamin D during pregnancy = increased risk for:
  - Gestational diabetes
  - Small for gestational age (low birthsize)
  - Low birth weight
  - Preterm birth

HOW?
4. What is “enough” during pregnancy?

- Still under investigation
- Growing evidence that needs may differ between individuals

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**TABLE 2** Association between maternal vitamin D status and the risk of SGA by race/ethnicity

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>SGA</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR(^1) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>White women</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum 25(OH)D at &lt;22 wk(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;37.5 nmol/L</td>
<td>3 (1.5)</td>
<td>8 (10.4)</td>
<td>10.6 (2.6, 42.5)</td>
<td>7.5 (1.8, 31.9)</td>
</tr>
<tr>
<td>37.5–75 nmol/L</td>
<td>107 (54.6)</td>
<td>27 (35.1)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>&gt;75 nmol/L</td>
<td>86 (43.9)</td>
<td>42 (54.5)</td>
<td>1.9 (1.1, 3.4)</td>
<td>2.1 (1.2, 3.8)</td>
</tr>
<tr>
<td>Quartile 1 [21.0–58.0 nmol/L]</td>
<td>49 (25.0)</td>
<td>25 (32.5)</td>
<td>3.1 (1.3, 7.6)</td>
<td>2.7 (1.1, 6.8)</td>
</tr>
<tr>
<td>Quartile 2 [58.1–71.4 nmol/L]</td>
<td>49 (25.0)</td>
<td>8 (10.4)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Quartile 3 [71.5–90.6 nmol/L]</td>
<td>49 (25.0)</td>
<td>15 (19.5)</td>
<td>1.9 (0.7, 4.8)</td>
<td>1.9 (0.7, 5.1)</td>
</tr>
<tr>
<td>Quartile 4 [90.7–245.0 nmol/L]</td>
<td>49 (25.0)</td>
<td>29 (37.6)</td>
<td>3.6 (1.5, 8.7)</td>
<td>3.9 (1.6, 9.7)</td>
</tr>
<tr>
<td><strong>Black women</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum 25(OH)D at &lt;22 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;37.5 nmol/L</td>
<td>48 (45.7)</td>
<td>17 (50.0)</td>
<td>1.4 (0.6, 3.1)</td>
<td>1.5 (0.6, 3.5)</td>
</tr>
<tr>
<td>37.5–75 nmol/L</td>
<td>50 (47.6)</td>
<td>13 (38.2)</td>
<td>1.0 (ref)</td>
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</tr>
<tr>
<td>&gt;75 nmol/L</td>
<td>7 (6.7)</td>
<td>4 (11.8)</td>
<td>2.2 (0.6, 8.7)</td>
<td>2.2 (0.5, 9.0)</td>
</tr>
<tr>
<td>Quartile 1 [13.8–30.0 nmol/L]</td>
<td>27 (25.7)</td>
<td>11 (32.4)</td>
<td>1.8 (0.6, 5.5)</td>
<td>1.7 (0.5, 5.5)</td>
</tr>
<tr>
<td>Quartile 2 [30.1–38.8 nmol/L]</td>
<td>26 (24.8)</td>
<td>6 (17.7)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Quartile 3 [40.4–49.3 nmol/L]</td>
<td>26 (24.8)</td>
<td>5 (14.7)</td>
<td>0.8 (0.2, 3.1)</td>
<td>0.8 (0.2, 3.2)</td>
</tr>
<tr>
<td>Quartile 4 [49.4–137.2 nmol/L]</td>
<td>26 (24.8)</td>
<td>12 (35.3)</td>
<td>2.0 (0.7, 6.1)</td>
<td>1.8 (0.5, 5.8)</td>
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</table>

\(^1\) Adjusted for prepregnancy BMI, smoking during pregnancy, and SES. Additional adjustment for season, maternal age, gestational age at blood sampling, marital status, insurance status, smoking in the year before pregnancy, periconceptional multivitamin use, or preconceptional physical activity had no meaningful impact on the results.

\(^2\) Distribution differs by case status, \(P < 0.0001\) (Pearson chi-squared test).

*Bodnar LM et al 2010*
10. Are there long term consequences?

- Still under investigation
Strong evidence that diet during pregnancy affects adult health

The Dutch famine birth cohort study
• Children from pregnancies during the famine have increased disease risk
• Timing during development was important

http://www.dutchfamine.nl
Gestational timing of undernutrition matters

Increase in coronary artery disease linked to malnutrition during early gestation only

<table>
<thead>
<tr>
<th>Born before famine</th>
<th>Time of exposure to famine</th>
<th>Conceived after famine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Late gestation</td>
<td>Midgestation</td>
</tr>
<tr>
<td>General</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of subjects</td>
<td>289</td>
<td>160</td>
</tr>
<tr>
<td>Men (%)</td>
<td>48</td>
<td>44</td>
</tr>
<tr>
<td>Maternal characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age (y)</td>
<td>29</td>
<td>31^1</td>
</tr>
<tr>
<td>Weight at the end of gestation (kg)</td>
<td>67</td>
<td>62^1</td>
</tr>
<tr>
<td>Weight gain in the last trimester (kg)</td>
<td>3.2</td>
<td>0.0^1</td>
</tr>
<tr>
<td>Occupation of head of family, manual (%)</td>
<td>83</td>
<td>71</td>
</tr>
<tr>
<td>Primiparous (%)</td>
<td>35</td>
<td>24^1</td>
</tr>
<tr>
<td>Birth characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3396</td>
<td>3183^3</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>32.8</td>
<td>32.4^3</td>
</tr>
<tr>
<td>Ponderal index (kg/m^3)</td>
<td>26.2</td>
<td>26.0^3</td>
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| Coronary artery disease |          |             |                |                  |
| No. of cases           | 24        | 12          | 11             | 11               | 25          |
| Cumulative incidence (%) |         |             |                | 13^d             | 8           |
| Age at onset (y)       | 51        | 50          | 50             | 47^d             | 49          |

*Painter et al 2006, Am J Clin Nutr*
What about before and after pregnancy?

- Multiple stages may be important

Preconception

Mom

Dad

Oocyte

“Egg”

Sperm

Gestation

Single cell

Lactation

Multicellular

Multi-tissue/organ

Rapid and widespread changes in cell number, function and location
What about my grandchildren?

- Still under investigation

- Focus of Ideraabdullah lab research
Ideraabdullah lab studies the genome & epigenome

- Genome = DNA = code that determines how cells function

**Human genome**
46 chromosomes (23 each)
20,000+ protein coding genes

http://www.differencebetween.info
Ideraabdullah lab studies the genome & epigenome

- Epigenome = Factors that regulate how the genome is interpreted

Epigenetic modifications

- DNA methylation: Feature regulates gene activity

Methylated DNA sequence:

- A-C-G-T-T-C-G-G
- T-G-C-A-A-G-C-C

Gene activity: ON

Unmethylated DNA sequence:

- A-C-G-G-T-G-C-G
- T-G-C-C-A-C-G-C

Gene activity: OFF

Regulatory sequence
Epigenetic modifications

- Diet-induced changes in DNA methylation and changes gene activity

Regulatory sequence

methylated

CH₃

A-C-G-T-T-C-G-G

T-G-C-A-A-G-C-C

Gene A OFF

Gene activity

unmethylated

A-C-G-G-T-G-C-G

T-G-C-C-A-C-G-C

Gene A ON

Regulatory sequence
Epigenome changes & disease

During development:

Epigenome  \rightarrow  Dysregulation/disruption

Change  \rightarrow  Gene activity

Abnormal  \rightarrow  Development of different cells/tissues/organs

Disease/ Susceptibility
The genome and epigenome are heritable

Heritable

*Capable of being passed from one generation to the next.*
Does vitamin D deficiency during pregnancy affect pups?

Treatments in mouse

- Deficient Mother
- G1 Adult males
- Vitamin D depleted (LVD)
  Or
- Vitamin D sufficient (CON)
- Vitamin D sufficient
Reduction in plasma 25(OH)D levels is strain dependent

- Diet reduced level of vitamin D in blood

Xue J et al, Clinical Epigenetics, 2016
Reduction in plasma 25(OH)D levels is strain dependent

- Deficient diet reduced level of vitamin D in blood
- Genetic differences in strain A & B = differences in status

Xue J et al, Clinical Epigenetics, 2016
Increase in body weight and fat mass in adult pups

- Deficient diet = increased pup bodyweight & fat mass

*Xue J et al, Clinical Epigenetics, 2016*
Increase in body weight and fat mass in adult pups

- Deficient diet = increased pup bodyweight & fat mass
- Genetic differences in strain A & B = differences in growth & adiposity

**Xue J et al, Clinical Epigenetics, 2016**
Decrease in DNA methylation

- Some but not all genes were changed

\[ \text{SnrpnICR} \quad \text{Grb10DMR} \]

Adult Liver

A B A B

[Graph showing DNA methylation levels for different conditions.]
Decrease in DNA methylation

- Some but not all genes were changed
- Genetic differences in strain A & B = differences in methylation
Are there multigenerational consequences?

Treatments in mouse

Deficient Mother

G₁
Adult males
Liver
sperm

G₂
Neonates
liver
sperm

G₂
Adult males

Unexposed dam

Vitamin D depleted (LVD)

Or

Vitamin D sufficient (CON)

Modified from Macpherson et al 2017
Grandmaternal deficiency alters neonatal bodyweight

- Bodyweight changes
- Genetic strain dependent

Xue J et al, Clinical Epigenetics, 2016
Grandmaternal deficiency alters neonatal & adult methylation

- Bodyweight changed
- Genetic strain differences

Xue J et al, Clinical Epigenetics, 2016
Ongoing studies

- Other genes in the genome are changed (all chromosomes)
Ongoing studies

- Changed genes are related to vitamin D deficiency diseases
  - Development
  - Cancer (prostate, colorectal, breast, lung, thyroid, ovarian..)
  - Reproduction
  - Embryo development
  - Dermatological disorders (ichthyosis type 1, hyperpigmentation)

### Top Diseases and Bio Functions

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### Physiological System Development and Function

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Conclusions

- Maternal vitamin D deficiency during pregnancy
  1. Differs between mice with genetic differences
  2. Changes body weight of offspring
  3. Changes fat mass of offspring
  4. Changes epigenome of offspring
  5. Can affect body weight and epigenome of multiple generations

6. Effects of maternal vitamin D deficiency on offspring differs between individuals with genetic differences
   - Genetic differences = vitamin D status = birth outcomes
Acknowledgements

Lab members
Jing Xue
Anandita Pal
Eddie Pietryk
Marwa Elnagheeb
Laetitia Meyrieu

Collaborators
CC mice – UNC-CH
Lisa Tarantino
Will Valdar
Fernando Pardo-Manuel de Villena
Terry Furey

Bioinformatics - UNCC
Corey Brouwer
- Raad Gharaibeh

Funding & Support
K22ES023849
#550KR141618
UNC center for environmental health and susceptibility (CEHS)
Human studies: diet and heredity

Sex-specific, male-line transgenerational responses in humans

Marcus E Pembrey, Lars Olov Bygren, Gunnar Kaati, Sören Edvinsson, Kate Northstone, Michael Sjöström, Jean Golding and The ALSPAC Study Team.

Effects transmitted through paternal lineage:

- Paternal grandfather food intake linked to grandson’s mortality risk
- Paternal grandmother food intake linked to granddaughters mortality risk
- Paternal smoking <11yrs linked to increased BMI in sons.
Human studies: diet and heredity

Paternal obesity is associated with IGF2 hypomethylation in newborns: results from a Newborn Epigenetics Study (NEST) cohort


Effects transmitted through paternal lineage:

- Paternal BMI linked to decreased DNA methylation at IGF2 in cord blood
How is vitamin D metabolized in the body?

SKIN

DHCR7: A vital enzyme switch between cholesterol and vitamin D production

Anika V. Prabhu, Winnie Luu, Dianfan Li, Laura J. Sharpe, Andrew J. Brown

School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, NSW, Australia

National Center for Protein Sciences, State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Received 18 July 2016, Revised 29 September 2016, Accepted 29 September 2016, Available online 30 September 2016
Challenges of human studies

- Limited access to multiple generations
- Cell/tissue sample collection
- Developmental timing
- Phenotypic variability
- Multiple/aggregate exposures
- Associations ≠ causal?
Benefits of mouse models

- Fast generation time (18-21 days)
- Inbred strains: genetically identical or genetically diverse
  10X genetic differences vs. humans (Ideraabdullah et al 2004)
- Access to any tissue/cell type
- Access to any developmental window
- Controlled exposure
- Many conserved genes
- Inducible system: test causality
Evidence for vitamin D role in epigenetic mechanisms

- Positive correlation between vitamin D status and global DNA methylation
- Targets histone demethylases (JmjC & LSD families)
- Proposed to regulate recruitment of HATs, HDACs, HMTs, and chromatin remodelers

Reviewed in Fetahu 2014 frontiers in physiology
Genetic reference population

Collaborative Cross - recombinant inbred lines
- Highly genetically divergent

http://csbio.unc.edu/CCstatus/index.py
Strain dependent differences in extent of depletion

<table>
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<tr>
<th>GeneticLine</th>
<th>1</th>
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<th>5</th>
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<td>D</td>
<td>AB</td>
<td>D</td>
<td>ABC</td>
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% change in 25(OH)D

-80%
-60%
-40%
-20%
0%
**Liver, 164 mapped genes**

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<td>1.05E-02 - 4.61E-07</td>
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<td>1.68E-09 - 1.68E-09</td>
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**Sperm, 199 mapped genes**

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Vitamin D levels influenced by genetic background

Maternal Plasma 25 (OH) D levels after 11+ weeks on diet

- Differences in extent of depletion
Vitamin D dependent differentially methylated regions

- Most of methylation changes >20% and enriched on Chr 17

Cross 1 (AxB)

Cross 2 (BxA)

Liver

Sperm
Vitamin D dependent differentially methylated regions

- Perturbation is cell type and cross dependent and mostly LOM
  - \( q < 0.01 \)

Cross 1 (AxB)
- 312 DMRs
  - 75% hypo-methyl.

Cross 2 (BxA)
- 69 DMRs
  - 71% hypo-methyl.

Liver
- 3,362 DMRs
  - 72% hypo-methyl.

Sperm
- 247 DMRs
  - 67% hypo-methyl.
25(OH)D levels borderline significant correlations
Standard least squares – all in model

Mean(Vit D ng/ml) & N(Vit D ng/ml) vs. Cyp24A1_rs27602985_genotype & 2 more

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<tr>
<td></td>
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<td>P=0.07</td>
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Cyp24A1_rs27602985_genotype
Cyp2r1_rs31478658_genotype
CG_rs29512564_genotype
Cyp24A1_rs27602985_genotype
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CG_rs29512564_genotype