Maternal Exposure to Iodine Excess Throughout Pregnancy and Lactation Induces Hypothyroidism in Adult Male Rat Offspring

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Team Yeung
Bonnie Yeung / Amanda McCormack / Chloe Kashiwagi
Iodine

- A trace element (naturally present in some foods)
- An essential component of the thyroid hormones, thyroxine T4 and triiodothyronine T3 synthesis.

- Functions of thyroid hormone:
  
  
  Fetal development and maturation of central nervous system (Zoeller and Rovet. J Neuroendocrinol. 2004).

*DV = Daily Value
(Pennington et al 1995; Teas et al 2004; Dasgupta et al 2009)
Thyroid hormone regulation
hypothalamus-pituitary-thyroid axis

- Hypothalamus releases TRH
- Pituitary secretes TSH (stimulates iodine uptake by the thyroid)
- Thyroid hormones (T3, T4) are produced by the thyroid gland

- T4 metabolism to active T3 is catalyzed by D1 and D2.
- D1 is highly expressed in human liver and kidney.
- D2 is expressed in skeletal and cardiac muscles, CNS and pituitary gland.

D1, type 1 deiodinase; D2, type 2 deiodinase; SRIH, somatotrophin (somatostatin)-release-inhibiting hormone; T3, tri-iodothyronine; T4, tetraiodothyronine; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone. CNS, central nervous system.
Iodine status

- Iodine deficiency (ID) are found in Angola, Mauritania as well as in UK (2012).
  - low levels of iodine in soil
  - refined foods that lose their iodine

- Iodine excess (IE)
  - salt ingestion, excessive use of seaweed supplements

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Recommended Daily Amount of Iodine

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Iodine Requirement</th>
</tr>
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<tbody>
<tr>
<td>Birth to 6 months</td>
<td>100 mcg</td>
</tr>
<tr>
<td>7-12 months</td>
<td>130 mcg</td>
</tr>
<tr>
<td>1-8 years</td>
<td>90 mcg</td>
</tr>
<tr>
<td>9-13 years</td>
<td>120 mcg</td>
</tr>
<tr>
<td>14 years and older</td>
<td>150 mcg</td>
</tr>
<tr>
<td>Pregnancy women</td>
<td>220 mcg</td>
</tr>
<tr>
<td>Breastfeeding women</td>
<td>290 mcg</td>
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</table>

*mcg = micrograms

The Food and Nutrition Board of the Institute of Medicine. 2001
Hypothyroidism

• Caused by iodine deficiency or iodine excess

  - Underactive thyroid, leading to normally low thyroid hormone production.

  - Can cause a number of health problems, such as obesity, joint pain, infertility and heart disease

  - Causes goiter (a swelling of the neck resulting from enlargement of the thyroid gland)
Consumption of iodine during pregnancy and/or lactation

Iodine deficiency


Iodine excess consumption


Aims:

- To investigate the consequences of rat dams’ exposure to IE throughout pregnancy and lactation on the hypothalamus-pituitary-thyroid axis function of their male offspring in adult life.

- To elucidate whether maternal IE exposure induces epigenetic changes in the thyroid of the male offspring.

IE: iodine excess
Experimental design

Wistar rats
Mating: 8 weeks-old with 2:1 ratio
Individual cages after confirmed pregnancy

Treatments: Control (water), 5-HI (0.6mg/L NaI) from GD1 to PND21
^5HI: 5x normal daily consumption of iodine by rat

PND1: 8 pups per dam
PND21: 8 males per cage
PND90: Harvest (blood, hypothalamus, pituitary, thyroid gland, heart, kidney, liver)
Table 1. Body weight, dry heart weight, dry heart to body weight ratio, T3, T4, TSH serum concentrations of adult male offspring

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<tr>
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<tr>
<td>BW (g)</td>
<td>354 ± 11.5</td>
<td>336 ± 6.4</td>
</tr>
<tr>
<td>DHW (g)</td>
<td>0.27 ± 0.01</td>
<td>0.22 ± 0.01**</td>
</tr>
<tr>
<td>DHW/BW (g)</td>
<td>0.76 ± 0.02</td>
<td>0.66 ± 0.01**</td>
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Results are expressed by means ±SEM, n=6-10 per group

^ Values multiplied by 1000.

*P<0.05, **P<0.01, ***P<0.001 vs Control.

BW: body weight
DHW: dry heart weight
DHW/BW: dry heart to body weight ratio
TSH, thyroid-stimulating hormone.
Measurement of T3, T4 and TSH
-MILLIPLEX MAP Rat Thyroid Magnetic Bead Panel - Endocrine Multiplex Assay

- Samples capture by: the fluorescent-coded magnetic beads
- Detection antibody: A biotinylated antibody
- Reporter: Streptavidin-PE conjugate
- Signal detection: a CCD-based instrument Luminex

Each individual bead is identified and the result of its bioassay is quantified based on fluorescent reporter signals.

TSH, thyroid-stimulating hormone.
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<td>DHW/BW (g)^A</td>
<td>0.76 ± 0.02</td>
<td>0.66 ± 0.01**</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; (ng/dL)</td>
<td>25.0 ± 1.1</td>
<td>18.4 ± 0.7**</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; (μg/dL)</td>
<td>7.06 ± 0.2</td>
<td>6.31 ± 0.2*</td>
</tr>
<tr>
<td>TSH (ng/mL)</td>
<td>1.2 ± 0.1</td>
<td>3.3 ± 0.5***</td>
</tr>
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^ Values multiplied by 1000.

*P<0.05, **P<0.01, ***P<0.001 vs Control.

5-HI caused maternal hypothyroid condition

BW: body weight
DHW: dry heart weight
DHW/BW: dry heart to body weight ratio
TSH, thyroid-stimulating hormone.
Figure 1: Gene Expression

A. Hypothalamus

B. Pituitary

Trh: thyrotropin releasing hormone
Dio2: type 2 iodothyronine deiodinase
Trhr: thyrotropin releasing hormone receptor
Gh: growth hormone
Tsha: alpha subunit of thyroid stimulating hormone
Tshb: beta subunit of thyroid stimulating hormone
Figure 1: Protein Expression

Tsha: alpha subunit of thyroid stimulating hormone
Tshb: beta subunit of thyroid stimulating hormone
Figure 2: Thyroid Morphology

Control 5-HI

Mag 100X

Mag 400X
Figure 3: Gene/Protein Expression

A. Thyroid

B. Thyroid

Tshr: thyroid stimulating hormone receptor
Slc5a5: solute carrier family 5, member 5
Tpo: thyroperoxidase
Tg: thyroglobulin
Mct8: monocarboxylate transporter 8
Nis: sodium-iodide symporter
Figure 4: Gene/Protein Expression

A.

Thyroid

B.

Pax8: paired box 8
Nkx2.1: Nk2 homeobox 1
Protein Carbonylation Detection

• OxyBlot Protein Oxidation Detection Kit
  • Collect and lyse cells
  • Immunoblot detection of carbonyl groups
• Primary antibody
  • Detect carbonyl groups
• Secondary antibody
  • Peroxidase-conjugated

Figure 4: Protein Carbonylation

Thyroid
Determining D1 Activity

D1 responsible for activation/inactivation of thyroid hormones

• Liver and kidney homogenates incubated with radiolabeled iodine tracer
• Measured liberated radiolabeled iodine to determine D1 enzyme activity
Figure 5: Dio1/D1 Activity

A. Bar graph showing Dio1/Rp19 (fold change) in liver and kidney for control and 5-HI treatment groups.

B. Bar graph showing D1 activity (pmol/min/mg) in liver and kidney for control and 5-HI treatment groups.
# Epigenetic Modifications:

<table>
<thead>
<tr>
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<th>Sample Enzyme(s)</th>
<th>Mode of Action</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA methylation</td>
<td>Dnmt1 and Dnmt3</td>
<td>Adds a methyl group to CpG site in DNA</td>
<td>Repression</td>
</tr>
<tr>
<td>Histone methylation</td>
<td>Polycomb Repressive Complex 2 (PRC2)</td>
<td>Adds one or more methyl groups to a lysine residue in histones</td>
<td>Repression (generally)</td>
</tr>
<tr>
<td>Histone acetylation</td>
<td>Histone acetyltransferase (HAT)</td>
<td>Adds an acetyl group to histone lysine residues</td>
<td>Activation (generally)</td>
</tr>
<tr>
<td>Histone deacetylation</td>
<td>Histone Deacetylase (HDAC)</td>
<td>Removes an acetyl group from histone lysine residues</td>
<td>Repression (generally)</td>
</tr>
</tbody>
</table>
**FIGURE 6: DNA METHYLATION**

**A.**

*Dnmt1* and *Dnmt3* mRNA content via RT-PCR; normalized to *Rpl19*

**B.**

*Dnmt1* and *Dnmt3* protein content via Western Blot; Gapdh as loading control

Dnmt mRNA and protein content were elevated in the thyroid of the treated group as compared to the control group.
Figure 6C: Global DNA Methylation

Imprint Methylated DNA Quantification Kit (Sigma Aldrich)

- Measures absorbance to compare relative methylation of sample to control

Both H3K9 and H3K27 were found to have increased trimethylation, as compared to the control group, in the thyroid tissue of the treatment group.
• Methylation of H3K9 and H3K27 are critical to turning off developmental genes and silencing the “extra” X chromosome

• H3K27 methylation may have a role in silencing BRCA1 gene

• H3K27 methylation may have a role in Fetal Alcohol Spectrum Disorder

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0154836
https://epigenie.com/key-epigenetic-players/histone-proteins-and-modifications/histone-h3k27/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3226033/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC419884/
There was increased acetylation found on both H3 and H4 in the thyroid tissue of the treatment group as compared to the control group.
Acetylation of Histones is seen in genes actively being transcribed

Compared to the control group, thyroid tissue in the treatment group had reduced Hat mRNA and reduced HAT activity; and elevated Hdac mRNA with increased HDAC activity.
Authors’ Conclusion:

“The results presented here demonstrate that the exposure of rat dams to IE during pregnancy and lactation induces primary hypothyroidism in their male offspring in adulthood. Additionally, the data strongly suggest that several epigenetic mechanisms are involved in the repression of thyroid gene expression observed in the IE-exposed animals.”
Further studies:

- Prenatal vs Postnatal IE exposure
- IE exposure continuous through PND 90
- Considerations for outcome of female offspring
Discussion:

Team Freeman: Why do you think they looked at overall epigenetic marks within the thyroid rather than gene-specific patterns? What do you think they would find if they looked at the overall epigenetic marks outside of the thyroid?

Team Illingworth: Would you expect to see a difference in the treatment group if the excess iodine exposure lasted until PND 90 instead of PND 21?

Team Pulczinski: How would you determine the window of exposure that would lead to lasting effects in the hypothalamus-pituitary-thyroid axis from iodine excess in the offspring?

Team Casin: How does this apply to public health? Do you agree with their conclusion?