Innovative Studies in Women by use of Stabilized Isotopes in Pregnancy

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Background
1. Thyroxine (T4) is both a hormone and a therapeutic drug.
2. Using conventional methods of analysis it is impossible to differentiate between the administered drug and endogenous thyroxine
Background – LT4
1. Pharmacokinetic studies are conducted to determine drug absorption, distribution, metabolism and excretion.
2. For determining the appropriate use of medicines according to patient characteristics (e.g. disease, genotype of drug-metabolizing enzymes).

3. Increased LT4 doses are often necessary early in pregnancy
4. PK studies in pregnancy are more difficult when the use of radiolabeled isotopes or ingesting high doses of the hormone are unethical.

Objectives
1. To conduct PK studies of LT4
2. To use hypothyroid patients (rendered euthyroid and at steady state)
3. To use the patient’s own prescribed dose
4. To conduct these PK studies in pregnant women, longitudinally using each woman as her own control
Challenges

1. Conducting PK studies of LT4 using traceable form of LT4
2. Measurement of very low concentrations of T4

Methods

1. Letter from FDA
2. Protocol approved by GUMC IRB
3. Studies were conducted at GUMC

A NOVEL APPROACH

$^{13}$C-LT$_4$

L-Thyroxine-[L-Tyr-ring-$^{13}$C$_6$] hydrochloride

1. Carbon-13 ($^{13}$C) is stable ($t_{1/2}>0.5$ billion years), and possesses no harmful or radiation-related effects. It is safe for use in children and pregnant women.
2. $^{13}$C-LT$_4$ derivative is highly stable and does not convert to the $^{12}$C-LT$_4$ analog.
Methods
1. Compounding pharmacy, microcellulose, capsules
2. 13C-LT4 capsules were kept at room temperature (temperature controlled and monitored hourly) in the dark, in a firmly closed dessicator until use.
3. Quality control, potency and stability testing were conducted biannually by Eagles, TX
4. GU research pharmacy

Subjects
1. 9 Women (31–42y) rendered euthyroid by LT4 replacement were recruited during pregnancy.
2. Target therapeutic TSH levels were maintained at around 1mIU/L.

Exclusion criteria
1. Baseline HCT <28.0%
2. TSH >4.5 mIU/L
3. Kidney dysfunction
4. Women taking other drugs that affect thyroidal axis interactions such as drugs that may alter TSH and thyroid hormone secretion, transport or metabolism.
### Study participants

#### Table 1. Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Patient</th>
<th>Name</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>LT4 dose 1 (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>John</td>
<td>M</td>
<td>30</td>
<td>80</td>
<td>180</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>Mary</td>
<td>F</td>
<td>25</td>
<td>60</td>
<td>165</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>Jack</td>
<td>M</td>
<td>40</td>
<td>90</td>
<td>170</td>
<td>210</td>
</tr>
</tbody>
</table>

### Dietary requirements

1. Women were requested to discontinue taking any iron containing multivitamins or pills prior to and throughout the study.
2. Fast (other than water) for 5h prior to, and 2h post ingesting the morning $^{13}$C-LT4 dose.
3. Patients did not take any other medication within 2h of $^{13}$C-LT4 dose.
4. Breakfast was based on eggs, toast, fruit, yogurt and tea.

### Study Day

1. Patients were consented and admitted to the GCRC.
2. The patients’ daily LT4 maintenance dose (75–210mcg) was replaced by a $^{13}$C-LT4 dose.
3. Only the LT4 dose on day 1 of the study was replaced by $^{13}$C-LT4.
Days 2-7

1. On each of the following days of the PK study (to 144 hours) the patient continued taking their own normal daily dose of LT4 (not $^{13}$C-LT4).
2. 6 Women returned to conduct the post-partum part of the study ≈ 3-12m following delivery.

LC/MS/MS

1. Proteins were precipitated from 100 µL serum with 150 µL methanol containing the deuterated internal standards.
2. After vortexing and centrifugation, 100 µL of supernatant were injected into the HPLC.
3. Samples were passed through a Supelco LC-18-DB (3.3 cm x 3.0 mm, 3 µm ID) analytical column and eluted with a methanol gradient.

LC/MS/MS

1. Detection by LC/MS/MS, where the ions of $^{13}$C-LT4 (m/z 782-127), d-T4 (m/z 778-127), T4 (m/z 776-127) were monitored (API-4000).
2. The standard curve had a linear dynamic range of 0.01-2.0 ng/mL for $^{13}$C-LT4 and 5-150 ng/mL for T4.
3. The limit of quantification (LOQ) is 0.002 ng/mL for $^{13}$C-LT4 and 5 ng/mL for T4.
4. Inter-day and intra-day coefficients of variation for the assay were < 5%.
**LT4 PK Profile: Single Pregnant Woman**

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>13C-LT4 (ng/mL)</th>
<th>TT4 (µg/dL)</th>
<th>TT3 (ng/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.000</td>
<td>8.8</td>
<td>155</td>
</tr>
<tr>
<td>0.5</td>
<td>0.019</td>
<td>9.9</td>
<td>134</td>
</tr>
<tr>
<td>1.0</td>
<td>0.044</td>
<td>9.9</td>
<td>152</td>
</tr>
<tr>
<td>1.5</td>
<td>0.084</td>
<td>10.3</td>
<td>179</td>
</tr>
<tr>
<td>2.0</td>
<td>0.123</td>
<td>9.1</td>
<td>119</td>
</tr>
<tr>
<td>3.0</td>
<td>0.141</td>
<td>10.0</td>
<td>176</td>
</tr>
<tr>
<td>4.0</td>
<td>0.208</td>
<td>10.4</td>
<td>126</td>
</tr>
<tr>
<td>5.0</td>
<td>0.298</td>
<td>9.7</td>
<td>135</td>
</tr>
<tr>
<td>8.0</td>
<td>0.461</td>
<td>8.7</td>
<td>136</td>
</tr>
<tr>
<td>11.0</td>
<td>0.534</td>
<td>8.3</td>
<td>130</td>
</tr>
<tr>
<td>12.0</td>
<td>0.512</td>
<td>9.8</td>
<td>136</td>
</tr>
<tr>
<td>13.0</td>
<td>0.486</td>
<td>9.9</td>
<td>133</td>
</tr>
<tr>
<td>14.0</td>
<td>0.449</td>
<td>8.9</td>
<td>125</td>
</tr>
<tr>
<td>15.0</td>
<td>0.384</td>
<td>8.9</td>
<td>127</td>
</tr>
<tr>
<td>16.0</td>
<td>0.223</td>
<td>8.6</td>
<td>113</td>
</tr>
</tbody>
</table>

**13C-LT4 PK: Non-Pregnant Woman**

Fig. 5: PK profile of a non-pregnant woman

**Data Analysis**

Data analysis was conducted using a noncompartmental approach (WinNonlin) and population PK modeling (NONMEM VI).
% change in 13C-LT4 AUC

Area under the concentration curve (AUC) relative to week of gestation
1. We successfully conducted PK studies of a single dose of LT4, at the patient’s level doses, in non-pregnant and pregnant women.
2. The AUC$_{0-\infty}$ were significantly higher during pregnancy than in the same woman ≈ 6m postpartum.
3. The increase in LT4 AUC during pregnancy could be attributed to a decrease in LT4 clearance.

These preliminary results also suggest that:
4. LT4 pharmacokinetics change significantly with gestational age.
5. There is a large variability in the PK parameters in pregnant women, and a relatively narrower range of variability in non-pregnant women.

Future research:
- More patients
- The mechanisms responsible for gestational differences in PK
- And whether these should necessitate dose schedule changes in pregnancy.
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Longitudinal Comparison of Thyroxine Pharmacokinetics
Between Pregnant and Nonpregnant Women:
A Stable Isotope Study

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Jason: Thriss, Ph.D.* and Helain A. Landy, M.D.*
Why do we need clinical Pharmacokinetic (PK) studies

1. Data obtained from such studies are useful for the design and conduct of subsequent clinical trials.
2. Necessary for appropriate analysis and evaluation of the efficacy and safety data
3. Provide useful information for the evaluation of the mechanism of action
4. For determining the appropriate use of medicines according to patient characteristics (e.g. disease, genotype of drug-metabolizing enzymes)
**Background**

LT4 has a narrow therapeutic range (critical-dose drugs)

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**13C-LT₄ PK**

Knowledge of the rates of equilibration and the distribution of thyroxine in body fluids and tissues is fundamental for an understanding of the action of the hormone on its target organs.

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**Critical dose drugs**

**Definition:**

1. Pharmaceuticals that have a narrowly defined range between risk and benefit
2. Less than a twofold difference in the minimum toxic concentration and minimum effective concentration in the blood
3. Exhibit limited or erratic absorption, formulation-dependent bioavailability, and wide intra-patient PK variability