Thermal Inactivation of L-Thyroxin
Jacobo Wortsman,1 Dimitri C. Papadimitriou,2 Marletta Borges,3 and Charles L. Defesco3

We assessed the extent of inactivation of L-thyroxin induced by exposure to heat in the presence of two vehicles. Preparations of L-thyroxin in the dry powder form, or dispersed in the solvents propylene glycol (water-like) or ethoxylated castor oil (oil-like), were heated at temperatures ranging from 65 to 160 °C, for 5- to 15-min periods. Heating L-thyroxin to a temperature below that of cooked bovine ground meat (72 °C) produced <10% degradation. Thermal degradation was pronounced only above 90 °C, and was almost completely at 160 °C. L-Triiodothyronine was the only thermal degradation product identified after L-thyroxin was heated at 125 °C. In a separate experiment we measured the melting point of L-thyroxin, 148.81 °C. This value agrees closely with the observed thermal sensitivity. We conclude that L-thyroxin is not significantly degraded under conditions encountered during cooking of ground bovine meat for short times at moderate temperatures.

L-Thyroxin (T4), the predominant hormone produced by the thyroid gland, is widely used to treat hypothyroidism. Available in tablet form, it is readily absorbed in the gastrointestinal tract. Although the intestinal absorptive capacity for T4 has not been defined, drug overdose typically results in thyrotoxicosis factitia (1-4). A form of thyrotoxicosis factitia not caused by drug intake may occur when ground bovine meat contaminated with thyroid tissue is eaten (5). We have investigated the effect of cooking on T4. Specifically, we evaluated the thermal degradation of T4, both in dry powder form and when dispersed in vehicles simulating meat-like environments.

Materials and Methods

Materials

Sodium T4, meeting raw-material U.S.P. specifications, was provided by Boots-Flint, Inc., Lincolnshire, IL. Possible metabolites and degradation products of T4 consisting of reagent-grade 3-monoiodo-L-tyrosine, 4-iodo-D-phenylalanine, 3,5-diiodo-L-thyronine, and 3,3',5-triiodo-L-thyronine were from Aldrich Chemical Co., Milwaukee, WI. Propylene glycol was from Fisher Scientific Co., Fair Lawn, NJ. Ethoxylated castor oil (Emulphor®) was from GAF, Wayne, NJ.

Procedures

Heating of T4: Samples of T4 were dispersed in propylene glycol and ethoxylated castor oil, then heated in an oil bath at pre-specified conditions. Samples were then dissolved in a 3:7 (by vol) mixture of methanol and 10 mmol/L NaOH and assayed by liquid chromatography.

High-pressure liquid chromatography (HPLC): We used a Model 1090 liquid chromatograph interfaced to a computer, series HP 300, both from Hewlett-Packard, Richardson, TX 75083. The 150 × 4.6 mm (i.d.) column was packed with a solid phase of 5-μm-diameter Spherisorb cyanide particles. The isocratic mobile phase consisted of a 70:30 (by vol) mixture of phosphoric acid solution (3 g/L) and acetonitrile. The flow rate was 1 mL/min. We monitored the effluent at 225 nm.

Differential scanning calorimetry: Thermograms of T4 heated at temperatures increasing at a rate of 20 °C/min within the range 50–250 °C were recorded with a Model DSC4 differential scanning colorimeter (Perkin-Elmer, Richardson, TX 75080). T4 samples (3 mg) were loaded on aluminum pans, sealed, and heated while being purged continuously with nitrogen.

Thermometric studies in meat: Two raw 113-g bovine ground-meat patties were placed on a heating source at 177 °C for 4 min (final weight 85 g per patty), then, with temperature probes placed in the center and on the surface of the patties, left standing at room temperature or under a heat lamp (6). Recordings were made every 2 min for 30 min.

Results

The chromatographic system well resolved T4 from related compounds (Figure 1). Heating T4 to temperatures >90 °C resulted in a marked decrease in its concentration (Table 1), an effect more pronounced for T4 in either of the liquid matrices than for T4 in dry powder form. At higher temperatures (160 °C for 15 min), T4 soon disappeared almost totally (Table 1). L-Triiodothyronine was the only degradation product of T4 we could identify (Figure 2, middle).

We also evaluated the thermal stability of T4 by differential scanning calorimetry, which measures the thermal energy absorbed or released during phase transitions upon heating matter. The melting point is determined by a
characteristic pattern of the thermogram. T₄ began to absorb heat at 126 °C, reaching the endotherm peak (melting point) at 148.81 °C; this was then followed by a marked heat release at higher temperatures, signifying rapid degradation (Figure 3). The temperature of the cooked patties was 66 °C on the surface and 72 °C in the center at the time they were removed from the heat source.

Discussion

From these observations, we conclude that any T₄ contaminating this type of food product would remain bioavailable after broiling. This is consistent with the increase in T₄ in serum when meat contaminated with thyroid tissue is eaten (5). The results of the two independent analytical procedures we used agree that T₄ degradation occurs only at temperatures above 120 °C, with the disappearance of thyrotoxic potential. T₄ is stable in powder form up to 80 °C (7). The thermal studies indicated that T₄ in powder form remains relatively stable up to 120 °C and any degradation below this temperature is attributable to effects of the matrix incorporating the drug.

The chromatographic system allowed us to determine the stability of T₄ as a pharmaceutical product (7–10). Testing over a period of 48 h at 25 °C indicated that, in a liquid state, T₄ is stable in water or saline but degrades in dextrose. In solid state, T₄ resists degradation; therefore, tablets at 40 °C show only 12% degradation after six months, or 50% if heated at 80 °C for 168 h (7; and unpublished data). T₄ as a dry powder is not affected at these conditions. In general, heat above 120 °C can degrade T₄, and specific matrices affect the rate and temperature at which degradation becomes apparent.

The present experiments were designed to simulate conditions of cooking. Thermal degradation of T₄ was enhanced in both fluid matrices. Despite the thermal degradation, however, the thyromimetic activity of T₄ may have been increased because thermal generation of l-triiodothyronine follows short exposure of T₄ to high temperatures.

The melting point of T₄ is 148.81 °C, followed by rapid degradation. Although this temperature is slightly lower than that of cooking (177 °C), inactivation is prevented by the limited exposure to heat (5 min), which precludes thermal equilibration in the meat. Upon completion of cooking, the actual temperature of the meat, 72 °C, is too low to produce any significant T₄ degradation (<10%).

In conclusion, we have established the physical and chemical basis for the thyrotoxic potential of food products contaminated with thyroid tissue.

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References

Table 1. Stability of T₄ during Heating

<table>
<thead>
<tr>
<th>Condition</th>
<th>Dry powder</th>
<th>In propylene glycol</th>
<th>In ethoxylated</th>
<th>castor oil</th>
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<tr>
<td>Unheated</td>
<td>100</td>
<td>101.3</td>
<td>98.1</td>
<td></td>
</tr>
<tr>
<td>Heated 65 °C (15 min)</td>
<td>95.7</td>
<td>92.7</td>
<td>94.3</td>
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<tr>
<td>Heated 90 °C (15 min)</td>
<td>95.4</td>
<td>82.0</td>
<td>78.1</td>
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<td>Heated 125 °C (15 min)</td>
<td>66.2</td>
<td>33.0</td>
<td>41.6</td>
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<tr>
<td>Heated 160 °C (5 min)</td>
<td>69.5</td>
<td>25.7</td>
<td>13.0</td>
<td></td>
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<tr>
<td>Heated 160 °C (15 min)</td>
<td>12.3</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

*As percent of unheated T₄ in powder form.

Fig. 2. Chromatograms of unheated T₄ dry powder (top); T₄ dispersed in a water-like solvent (propylene glycol; PEG) and heated for 5 min at 180 °C (middle); and T₄ in PEG heated for 15 min at 180 °C (lower). Thermal exposure results in progressive disappearance of T₄, with generation of small amounts of l-triiodothyronine (middle) after short exposure to high temperature.

Fig. 3. Differential scanning calorimetry of T₄
A sample of T₄ was heated from 50 °C to 250 °C. Thermal energy absorbed is represented on the ordinate. Heat absorption associated with melting (shaded area) starts at 126 °C and reaches maximum (melting point) at 148.81 °C. The subsequent release of heat (~180–200 °C) is due to molecular degradation.