Comparative Effects of D- and L-3,5,3'-Triiodothyronine in Chronic Treatment of Hypercholesteremic Dogs

By Theodore Ellison, Ph.D., Samuel M. Greenberg, Ph.D., and Tien-Hui Lin, Ph.D.

NATURALLY occurring thyroid hormones and a number of synthetic thyromimetic analogs have been shown to influence serum-lipid levels in man and in laboratory animals.1-6 Chaikoff et al.1 and Danowski and co-workers2 have observed that the serum lipids of the dog are significantly altered by the level of circulating thyroid hormone. Although the dog is less dependent on thyroid hormone for control of metabolic processes than is man,2 its serum cholesterol and other circulating lipids can be markedly altered by the administration of thyromimetic agents. This experiment was designed to measure the relative effectiveness of the chronic oral administration of D- and L-3,5,3'-triiodothyronine on the circulating-lipid patterns of intact, naturally hypercholesteremic dogs.

Methods

Naturally hypercholesteremic adult mongrel dogs (screened for elevated plasma total cholesterol) were housed individually in metabolism-type cages and received a uniform, calorie-controlled diet. The formula of the milk supplement was as follows (per cent): powdered whole milk, 87; cellulose (Alphael), 7.5; trace-salt-sucrose mix (trace salts, USP XIV [1950], 65 Gm. mixed with 1,935 Gm. sucrose), 4; vitamins A and D-sucrose mix (vitamin A, 500,000 USP units/Gm., and vitamin D, 50,000 USP units/Gm.,* 8 Gm. mixed with 1,992 Gm. sucrose), 0.5; and vitamin E acetate,† 300 Gm. mixed with 1,700 Gm. sucrose, 1.0. The final diet was prepared in batches containing the following: dry mix listed above, 360 Gm.; cottonseed oil, 25 ml., coconut oil, 25 ml.; and water, 2,400 ml. The mixture was blended for one minute in a large Waring blender.

Twenty dogs were assigned to five groups of four dogs each (two of each sex) and were administered the following \( \mu \)g./Kg. dose levels/dog for 21 weeks: placebo (group I); D-3,5,3'-triiodothyronine (D-T3), 60 (group II) and 240 (group III); and L-3,5,3'-triiodothyronine (L-T3), 4 (group IV) and 16 (group V). During the subsequent 10 weeks, daily dosages of drugs for each group were doubled. The drugs were suitably diluted with terra alba and administered in gelatin capsules in the morning and late afternoon, except on week ends when capsules were administered only in the morning. Equal numbers of placebo capsules were administered to control dogs.

Dogs were bled at intervals spaced in such a way that samples for any one test were obtained on the same day of each week. Dogs were bled from the femoral vein prior to the morning feeding. The following blood-lipid determinations were performed: plasma total cholesterol,8 plasma free and esterified cholesterol,8 plasma total lipids,9 plasma phospholipids,10 and plasma triglycerides.11 The following functional tests were performed: bromsulphalein clearance,12 alkaline phosphatase (Phosphatabst),13 plasma bilirubin,14 serum glutamic oxalaeetic transaminase (SGOT),15 protein-

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†Roche.
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bound iodine (PBI), prothrombin clotting time, glucose tolerance test, plasma urea nitrogen (Urograph*), and plasma sodium and potassium levels by flame photometry. Total blood profiles were determined at frequent intervals during the experiment.

Autopsies were performed at the end of 31 weeks of treatment. Gross pathological examination was made of the vital organs, and total lipid and cholesterol determinations were made on the livers, aortas, and adrenals.

**Results**

**Plasma Total Cholesterol**

Plasma total cholesterol of the control dogs (group I) increased only 6 per cent during the 31-week experimental period. D-T3, administered during the first 21 weeks at levels of 60 (group II) and 240 (group III) μg./Kg./day, caused plasma total-cholesterol reductions of 22 and 25 per cent, respectively. Doubling each dose of D-T3 during the last 10 weeks of the experiment (weeks 21 to 31) produced no further reduction for group II animals, but decreased plasma total cholesterol 33 per cent of the baseline value for group III animals.

L-T3, administered during the first 21 weeks at levels of 4 (group IV) and 16 (group V) μg./Kg./day, caused plasma total-cholesterol reductions of 3 and 15 per cent, respectively. Doubling each dose of L-T3 during the last 10 weeks of the experiment produced no further reduction for group IV animals, but decreased plasma total cholesterol 30 per cent of the baseline value for group V animals.

**Plasma Free and Esterified Cholesterol**

Plasma total lipids of the control dogs (group I) decreased 10 per cent during the first 21 weeks and 12 per cent of the baseline by the end of the 31-week experimental pe-

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**Table 1**

**Average Free and Esterified Plasma Cholesterol in Dogs Treated Chronically with D- or L-T3**

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug treatment (per day)</th>
<th>No. weeks treatment</th>
<th>Average free cholesterol (mg. per cent)</th>
<th>Average cholesterol esters (mg. per cent)</th>
<th>Per cent cholesterol ester (of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>None</td>
<td>1-21</td>
<td>46</td>
<td>157</td>
<td>77</td>
</tr>
<tr>
<td>II</td>
<td>None</td>
<td>22-29</td>
<td>42</td>
<td>170</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>D-T3</td>
<td>1-21</td>
<td>37</td>
<td>126</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>60 μg./Kg.</td>
<td>22-29</td>
<td>36</td>
<td>112</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>D-T3</td>
<td>120 μg./Kg.</td>
<td>35</td>
<td>198</td>
<td>75</td>
</tr>
<tr>
<td>III</td>
<td>D-T3</td>
<td>1-21</td>
<td>40</td>
<td>132</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>240 μg./Kg.</td>
<td>22-29</td>
<td>35</td>
<td>196</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>D-T3</td>
<td>480 μg./Kg.</td>
<td>48</td>
<td>147</td>
<td>75</td>
</tr>
<tr>
<td>IV</td>
<td>L-T3</td>
<td>1-21</td>
<td>44</td>
<td>142</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>4 μg./Kg.</td>
<td>22-29</td>
<td>48</td>
<td>147</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>L-T3</td>
<td>8 μg./Kg.</td>
<td>51</td>
<td>159</td>
<td>76</td>
</tr>
<tr>
<td>V</td>
<td>L-T3</td>
<td>16 μg./Kg.</td>
<td>51</td>
<td>159</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>32 μg./Kg.</td>
<td>22-29</td>
<td>38</td>
<td>120</td>
<td>76</td>
</tr>
</tbody>
</table>

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Table 2

Average Plasma Triglycerides in Dogs Treated Chronically with d- or l-T₃

<table>
<thead>
<tr>
<th>Group</th>
<th>Average plasma triglycerides (mg. per cent) at end of following weeks' treatment (per day)</th>
<th>Drug* treatment (per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>II</td>
<td>d-T₃, 60 µg./Kg.</td>
<td>d-T₃, 62 µg./Kg.</td>
</tr>
<tr>
<td>III</td>
<td>d-T₃, 240 µg./Kg.</td>
<td>d-T₃, 120 µg./Kg.</td>
</tr>
<tr>
<td>IV</td>
<td>l-T₃, 4 µg./Kg.</td>
<td>l-T₃, 480 µg./Kg.</td>
</tr>
<tr>
<td>V</td>
<td>l-T₃, 4 µg./Kg.</td>
<td>l-T₃, 8 µg./Kg.</td>
</tr>
</tbody>
</table>

Starting at end of twenty-first week.

Table 3

Average Plasma Alkaline-Phosphatase Levels in Dogs Treated Chronically with d- or l-T₃

<table>
<thead>
<tr>
<th>Group</th>
<th>Average plasma alkaline phosphatase after following weeks' treatment (Bodansky units)</th>
<th>Drug* treatment (per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>II</td>
<td>d-T₃, 60 µg./Kg.</td>
<td>d-T₃, 120 µg./Kg.</td>
</tr>
<tr>
<td>III</td>
<td>d-T₃, 240 µg./Kg.</td>
<td>d-T₃, 480 µg./Kg.</td>
</tr>
<tr>
<td>IV</td>
<td>l-T₃, 4 µg./Kg.</td>
<td>l-T₃, 8 µg./Kg.</td>
</tr>
<tr>
<td>V</td>
<td>l-T₃, 4 µg./Kg.</td>
<td>l-T₃, 32 µg./Kg.</td>
</tr>
</tbody>
</table>

Starting at end of twenty-first week.

*Three Bodansky units (Phosphatabs) considered normal; six units, borderline; 12 units, pathological range.
†Modification of method of Bodansky et al.²⁻

Results:

- In the first 21 weeks, dogs receiving L-T₃ (groups IV and V) showed no significant changes in plasma phospholipids from the values obtained in control dogs (group I).

- During the last 10 weeks of the experiment, after the doubling of the dose of L-T₃ in each group, only group V dogs had values appreciably less than those of controls. At all doses tested during the 31-week experimental period, groups receiving L-T₃ showed consistently greater depression of plasma-phospholipid levels than did the control group.

- Plasma Triglycerides

Since baseline plasma-triglyceride values were not obtained for the experimental dogs, it is necessary to compare the values for the treated dogs during the experiment with those of the control group at any test period. During the sixteenth to twenty-ninth weeks, little or no differences in plasma triglycerides from the control levels (table 2) were observed.
Physiological Tests

Bromsulphalein clearance was determined at the end of the twenty-first and twenty-ninth weeks of the experiment. Mean control values (per cent retention at one-half hour) were 4.2 and 4.0, respectively, for the two tests. The values of the thyromimetin-treated group ranged from 3.1 to 4.2 and were not significantly different from control values.

Serum alkaline-phosphatase values were determined on all dogs at four-week intervals and remained at consistent normal levels throughout the entire experiment. There was a tendency for a slight elevation in alkaline phosphatase in the dogs in groups III and V receiving the highest levels of D-T₃ and L-T₃, respectively. No single value, as determined either by the Phosphatab method or the Bodansky method, approached the "pathological range" (table 3).

Levels of protein-bound iodine in the control dogs were unchanged throughout the experimental period. The highest level of L-T₃ administered (group V) caused a significant (P < 0.05) reduction in PBI levels (table 4). No significant differences from control values were observed in any of the experimental groups tested by the following: prothrombin clotting time (at four-week intervals); glucose tolerance (twentieth week); plasma urea nitrogen (twenty-first week); plasma sodium and potassium levels (twenty and twenty-ninth weeks); and serum glutamic oxalacetic transaminase (at four-week intervals for the first 20 weeks).

Urine was collected at four-week intervals and analyzed for albumin, glucose, bilirubin, occult blood, and microscopic analysis. All tests were negative.

Total blood studies (red blood count, white blood count, hemoglobin, hematocrit, and differential) were made at 0, 17, and 29 weeks of the experimental period. No significant differences from control values were observed in any of the blood studies.

Weight and Food Consumption

All animals on this experiment gained weight. Dogs receiving the higher level of D-T₃ (group III) consistently resisted this trend to gain weight but did not lose weight. Food-consumption data indicated that D-T₃ was not an appetite depressant, since calories consumed/Kg. body weight for these dogs were similar to calories consumed by the controls and the L-T₃-treated animals.

Postmortem Evaluation

After the dogs had been chronically treated for 31 weeks with D- or L-T₃ at the prescribed dosage levels, they were examined for post-mortem evaluation. Sections of the livers, adrenals, and aortas were removed for analysis of cholesterol, total lipid, and, in the aortas, sudanophilia.

No significant differences were noted in the cholesterol content of any of the organs ana-
Table 5
Average Cholesterol Content (Including S.E.M.) of Aorta, Adrenal, and Liver, and Total Liver Lipid from Dogs Treated Chronically for Thirty-one Weeks with D- or L-T₃

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug Treatment (µg./Kg./day)</th>
<th>0 to 21 weeks Aorta (mg./Gm. wet weight)</th>
<th>0 to 21 weeks Adrenal (mg./Gm. wet weight)</th>
<th>0 to 21 weeks Liver (mg./Gm. wet weight)</th>
<th>Per cent total liver lipid (based on dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>None</td>
<td>1.49 ± 0.16</td>
<td>27.71 ± 2.83</td>
<td>2.78 ± 0.14</td>
<td>9.12 ± 1.40</td>
</tr>
<tr>
<td>II</td>
<td>D-T₃</td>
<td>1.43 ± 0.15</td>
<td>25.47 ± 2.99</td>
<td>2.58 ± 0.10</td>
<td>7.89 ± 1.47</td>
</tr>
<tr>
<td>III</td>
<td>D-T₃</td>
<td>1.45 ± 0.13</td>
<td>30.73 ± 2.17</td>
<td>2.74 ± 0.18</td>
<td>10.75 ± 2.96</td>
</tr>
<tr>
<td>IV</td>
<td>L-T₃</td>
<td>1.44 ± 0.24</td>
<td>27.75 ± 2.16</td>
<td>2.53 ± 0.16</td>
<td>8.31 ± 1.79</td>
</tr>
<tr>
<td>V</td>
<td>L-T₃</td>
<td>1.39 ± 0.14</td>
<td>23.43 ± 2.79</td>
<td>2.59 ± 0.24</td>
<td>7.47 ± 1.79</td>
</tr>
</tbody>
</table>

lyzed (table 5). Dogs that received the highest level of L-T₃ (group V) had the lowest amount of cholesterol in these organs.

Gross pathological examination of the livers of the experimental dogs revealed no evidence of necrosis or fatty infiltration. No significant differences in total liver lipids were noted (table 5).

An appropriate length of aorta was selected from each dog for examination of evidence of sudanophilia. Aortas were stained with Sudan IV and counterstained with malachite green. No visible evidence of sudanophilia was observed in any of the dog aortas.

Discussion

Both D-T₃ and L-T₃ significantly altered the blood-lipid patterns of hypercholesteremic dogs. The doses chosen were based on the equivalent effects of the two compounds in lowering blood cholesterol in rats. In the dog, the 4 µg./Kg./day level of L-T₃ had no depressing effect on plasma total-cholesterol levels. The doubling of this dose after 21 weeks still caused no cholesterol-depressing effects. However, 16 µg. of L-T₃/Kg./day was an effective level for lowering plasma total cholesterol, and doubling this dose caused still greater depressing effects. It may, therefore, be concluded that, in dogs under the conditions of this experiment, the minimum effective plasma cholesterol-depressing dose with L-T₃ lies between 8 and 16 µg./Kg./day. Since both levels of D-T₃ were effective in lowering plasma total-cholesterol values, the minimum effective dose is not so clearly discernible; however, the 60 µg./Kg./day level seemed to be fairly equivalent to the lowering effect of 16 µg. of L-T₃. Although individual-dog data have not been presented, it was noted that those dogs with the lowest initial plasma total-cholesterol levels tended to be the least responsive to either of the two test compounds.

The two compounds studied had relatively the same depressing effects on plasma total lipid as they had on plasma total cholesterol.

Significant reductions in plasma phospholipids were observed for both levels of D-T₃ and for the higher level of L-T₃ after the dosage level had been doubled at the end of the twenty-first week. Doubling the dosage levels of D-T₃ at the end of the twenty-first week produced no further reduction in plasma-phospholipid levels. Unlike the significant reduction in plasma total cholesterol after the doubling of the D-T₃ dosage levels, plasma phospholipids exhibited complete resistance to further reduction, thereby producing a more favorable cholesterol/phospholipid ratio. No pretreatment plasma-triglyceride values are available; however, average values for treated dogs were not significantly different from those of control dogs run concurrently after 21 weeks of treatment. No significant changes in triglyceride values were noted after 29 weeks of treatment when compared with the results obtained at 21 weeks (prior to doubling drug levels).

Throughout the entire experiment, all dogs remained in good health, as judged by gross appearances and actions. Dogs receiving D-T₃ gained less weight than did either the controls or the L-T₃-treated groups. The amount of food given to each dog was calculated to be slightly in excess of a maintenance
level. Since all dogs, generally throughout the experiment, ate all the food offered, the differences in the effects of the two compounds in weight gains would appear to have metabolic significance. It is not possible, from these data, to speculate on the effect the compounds might have had on weight gains if the dogs had been fed ad libitum.

These physiological and metabolic functional tests determined on the dogs at intervals during the experimental period indicated that no abnormal changes occurred during the administration of the compounds. One of two changes noted in the tests was that several alterations from average baseline values were observed in the values obtained for plasma alkaline phosphatase after the dosage levels of the compounds had been doubled, and only with the highest levels of the two compounds. The alkaline-phosphatase levels were only slightly elevated and did not approach the usual indicated pathological range. The second noticeable change in functional tests was on protein-bound-iodine levels, which were definitely depressed by both levels of L-T₃. There was a trend for D-T₃ to increase the PBI in direct proportion to the dose of the compound.

After autopsy of the dogs treated chronically with D- or L-T₃ for 31 weeks, evaluation of liver total lipids and of the cholesterol contents of aortas, adrenals, and livers revealed no significant differences between treated and control groups. The extreme resistance of dogs to the development of fatty infiltration of the aorta is attested to by the fact that all of the experimental hypercholesteremic dogs, including the controls, were negative for sudanophilia.

The results of this experiment confirm the earlier observation of Danowski et al.² that the dog is highly resistant to symptoms of thyrotoxicosis induced by high levels of exogenously administered thyromimetic agents. L-T₃ at approximately 25 times the estimated daily dose required to control human myxedema had no effect on weight gain of the dogs. Danowski concludes from his work that the dog is more tolerant to thyromimetic agents than is man because of the dog's ability to more rapidly inactivate and excrete these agents.

None of the dosage levels of thyromimetic agents used during this experiment caused a loss in body weights of the dogs from pre-treatment values. However, D-T₃ at the levels administered caused a retardation in the rate of body weight gain when compared with that of the untreated controls. A noticeable retardation in weight gain was caused by the administration of the higher level of L-T₃; however, this effect appeared to be less marked than for either of the dosage levels of D-T₃. The retardation in the rate of weight gain appears to be a result of alteration in metabolism rather than a result of depression of appetite, since isocaloric diets were administered and consumed by each dog on the basis of calculations of normal caloric requirements.³ Although there may not be a basis for a relationship between retardation of body weight gain and the effectiveness of these agents as plasma cholesterol-lowering agents, the effectiveness of D- and L-T₃ under the conditions of this experiment on these two parameters was closely parallel.

Summary

Twenty intact, naturally hypercholesteremic dogs were divided into five groups and chronically administered D-3,5,3'-triiodothyronine (D-T₃) and L-3,5,3'-triiodothyronine (L-T₃) at two levels, respectively, for 31 weeks. The administration of the drugs was divided into two periods, the first for 21 weeks, after which the dosage level for each of the four test groups was doubled for an additional 10 weeks. Plasma total cholesterol, total lipids, and phospholipids were significantly lowered by the administration at both levels of D-T₃ and the higher level of L-T₃. At the end of 21 and 29 weeks of thyromimetic administration, no differences in percentage of free or esterified cholesterol were observed for any of the treated groups when compared with the control groups. A battery of physiological and functional tests was performed at suitable intervals throughout the experimen-
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tal period with no evidence of abnormalities due to the administration of the thyromimetic agents. The only significant functional change measured was a reduction in protein-bound-iodine levels in the dogs receiving the higher level of L-T₃.

Throughout the entire experiment, all dogs remained in good health as judged by gross appearances and actions. The dogs receiving D-T₃ gained less weight than did either the controls or the L-T₃-treated groups, even though all animals ate isocaloric balanced diets. At post mortem, after 31 weeks of chronic thyromimetic administration, gross pathology was negative; chemical analyses of cholesterol content of aortas, adrenals, and livers indicated no significant differences. Analyses of livers for total lipids showed no significant differences, and no evidence of aortic sudanophilia was apparent in any of the experimental dogs.

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References

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