Thyroid Hormone Reduces Cholesterol via a Non-LDL Receptor-Mediated Pathway

Ira J. Goldberg, Li-Shin Huang, Lesley A. Huggins, Shuiqing Yu, Prabhakara R. Nagareddy, Thomas S. Scanlan, and Joel R. Ehrenkranz

Department of Medicine (I.J.G., L.-S.H., L.A.H., S.Y., P.R.N.), Columbia University, New York, New York 10032; Departments of Physiology and Pharmacology (T.S.C.), Oregon Health and Science University, Portland, Oregon 97239; and Department of Medicine (J.R.E.), Intermountain Healthcare and University of Utah School of Medicine, Salt Lake City, Utah 84132

Although studies in vitro and in hypothyroid animals show that thyroid hormone can, under some circumstances, modulate the actions of low-density lipoprotein (LDL) receptors, the mechanisms responsible for thyroid hormone’s lipid-lowering effects are not completely understood. We tested whether LDL receptor (LDLR) expression was required for cholesterol reduction by treating control and LDLR-knockout mice with two forms of thyroid hormone T3 and 3,5-diiodo-L-thyronine. High doses of both 3,5-diiodo-L-thyronine and T3 dramatically reduced circulating total and very low-density lipoprotein/LDL cholesterol (~70%) and were associated with reduced plasma T4 level. The cholesterol reduction was especially evident in the LDLR-knockout mice. Circulating levels of both apolipoprotein B (apo)B48 and apoB100 were decreased. Surprisingly, this reduction was not associated with increased protein or mRNA expression of the hepatic lipoprotein receptors LDLR-related protein 1 or scavenger receptor-B1. Liver production of apoB was markedly reduced, whereas triglyceride production was increased. Thus, thyroid hormones reduce apoB lipoproteins via a non-LDLR pathway that leads to decreased liver apoB production. This suggests that drugs that operate in a similar manner could be a new therapy for patients with genetic defects in the LDLR. (Endocrinology 153: 5143–5149, 2012)
(11, 12). Up-regulation of SR-B1 would explain the reduction in high-density lipoprotein (HDL) sometimes seen with thyroid hormone administration and could lead to increased reverse cholesterol transport (12). One study recently reported that the thyromimetic T-0681 does not alter expression of the LDLR in wild-type (WT) mice, an effect that implies a mechanism of action other than via LDLR. Surprisingly, T-0681 treatment failed to reduce cholesterol in LDLR-knockout (Ldlr<sup>−/−</sup>) mice (12), suggesting that LDLR were essential for the cholesterol-decreasing actions of this drug.

The goal of the following study was to determine whether thyroid hormone affects cholesterol levels exclusively of effects on the LDLR.

**Materials and Methods**

**Mice and thyroid hormone treatments**

All studies were approved by the Columbia University Institutional Animal Care and Use Committee. WT C57BL/6 and Ldlr<sup>−/−</sup> mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Male mice (3- to 4 months of age) were maintained on chow diets and allowed to eat and drink ad libitum. At the initiation of the study they were switched to a Western-type diet (WTD) containing 42% fat, 42.7% carbohydrate, 15.2% protein, 0.15% cholesterol; total 4.5kcal/g (Harlan Teklad, Madison, WI). After 1 wk, the animals were divided into groups receiving vehicle (58.5% saline/1.5% sodium bicarbonate; total 4.5kcal/g) (EMD Chemicals, Philadelphia, PA) or T<sub>3</sub> (1.25 mg/kg) or T<sub>2</sub> (1.25 mg/kg or 12.5 mg/kg) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) via daily gavage for another week. Mass spectrometry and nuclear magnetic resonance profiles showed no T<sub>3</sub> or T<sub>4</sub> contamination in the T<sub>2</sub>. Mice were killed or used for kinetic studies at the end of thyroid hormone treatment. All blood samples were collected in 4-h fasted mice. Blood glucose was obtained from a tail prick using a glucometer. For other assays, blood samples were drawn via retro-orbital plexus in anesthetized mice.

**Plasma lipid, T<sub>4</sub>, and apolipoprotein B (apoB) measurements**

Plasma levels of triglyceride, free fatty acids, and cholesterol were measured using the following kits: Infinity Triglyceride Reagent (Fisher Scientific, Pittsburg, PA), NEFA-HR (WAKO Chemicals USA, Inc., Richmond, VA). Because total plasma cholesterol in WT mice is predominantly derived from HDL, plasma lipoproteins were isolated by ultracentrifugation (TLA100 rotor, Beckman Instruments, Fullerton, CA). Cholesterol and triglyceride in very low density lipoprotein (VLDL) (d < 1.006 g/ml), intermediate density lipoprotein (IDL)/LDL (d = 1.006–1.063 g/ml), and HDL (d = 1.063–1.21 g/ml) fractions were measured as described above. Circulating T<sub>4</sub> was measured using Mouse/Rat T<sub>4</sub> Total kit (Calbiotech, San Diego, CA). Plasma apoB100 and B48 in the plasma (1 μl) were separated in 4% SDS-PAGE, stained with Coomassie blue, and quantitated by densitometry.

**Liver expression of lipoprotein receptors and other metabolic genes**

At the conclusion of each study, liver was collected. Livers were homogenized in a RIPA buffer from Pierce Chemical Co. (Rockford, IL) (30 μl Tris-HCl, 150 μl NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) and protease inhibitors. Homogenates (20 μg/sample) were used for Western blot analysis using the following antibodies against mouse proteins: anti-LDLR and anti-LDLR-related protein (LRP) 1 (Abcam, Cambridge, MA), anti-SR-BI (Novus Biologicals, Littleton, CO), and anti-glyceroldehyde 3-phosphate dehydrogenase (Cell Signaling Technology, Danvers, MA).

Hepatic mRNA levels were assessed by real time PCR. Primers for each gene are included in Supplemental Table 1 published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org.

**Triglyceride and apoB production**

Lever production of lipoproteins was assessed in 4-h fasted mice injected with [35S]methionine (200 μCi/mouse; PerkinElmer, Wellesley, MA) and Triton WR 1339 (500 mg/kg; Sigma Chemical Co., St. Louis, MO), as described previously (13). Labeled apoB was assessed directly from plasma by 4% SDS-PAGE and quantified by densitometric scanning of x-ray film and normalized to trichoroacetic acid-precipitable counts as previously described (14).

**Statistical analysis**

Comparisons between two treatment groups were performed using Student’s t test. Comparisons among three or more groups were performed using one-way ANOVA. Data are given as mean ± SEM.

**Results**

**Plasma cholesterol reduction in Ldlr<sup>−/−</sup> mice treated with T<sub>3</sub> and T<sub>2</sub>**

T<sub>3</sub> and T<sub>2</sub> treatments caused a marked reduction in total cholesterol (Table 1). Feeding control mice with a WTD led to a marked increase in cholesterol from 160 to 1336 mg/dl. Treatment with T<sub>3</sub> (0.75 mg/kg) had a dramatic effect on circulating cholesterol, which was reduced approximately 70% to 406 mg/dl. Triglyceride levels, in contrast, were similar in control and T<sub>3</sub>-treated animals. Circulating glucose was substantially reduced from 160 to 77 mg/dl. Body weight was not affected by this short-term T<sub>3</sub> treatment. Heart weights were increased, whereas adipose, muscle, and liver weights were not significantly altered (Supplemental Table 2). We also performed a dose-response curve in Ldlr<sup>−/−</sup> mice using decreasing doses of T<sub>2</sub>. Even at 7.5 μg/kg the mice had a marked reduction in cholesterol (Supplemental Table 3).

We then assessed the effects of T<sub>2</sub> on these mice. The dose of T<sub>2</sub> used in these experiments was extrapolated from par-
duced cholesterol levels (by heart weight (Supplemental Table 2).

WT C57BL/6 mice (Table 1). Both T3 and T2 (12.5 mg/kg dose) led to a similar cholesterol reduction of approximately 70% and reduced glucose.

To determine whether T2 at these doses worked by a mechanism that did not alter pituitary function, we measured circulating T4. Both T3 and T2 (12.5 mg/kg dose) treatments led to a marked suppression of circulating T4 levels, indicating suppression of the pituitary axis.

Changes in lipoprotein fractions

Lipoproteins are shown in Table 2. In all mice the reductions in cholesterol were due to decreases in VLDL and

TABLE 1. Blood biochemistry in Ldlr<sup>−/−</sup> and WT C57BL/6 mice treated with thyroid hormones

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Ldlr&lt;sup&gt;−/−&lt;/sup&gt; Chow (baseline)</th>
<th>WT C57BL/6 Chow (baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T&lt;sub&gt;3&lt;/sub&gt; (7) (0.75 mg/kg)</td>
<td>T&lt;sub&gt;2&lt;/sub&gt; (7) (1.25 mg/kg)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Con (15)</td>
<td>T&lt;sub&gt;3&lt;/sub&gt; (7) (0.75 mg/kg)</td>
</tr>
<tr>
<td>Triglyceride</td>
<td></td>
<td>T&lt;sub&gt;3&lt;/sub&gt; (7) (12.5 mg/kg)</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td>T&lt;sub&gt;3&lt;/sub&gt; (5) (12.5 mg/kg)</td>
</tr>
<tr>
<td>Body Weight</td>
<td></td>
<td>T&lt;sub&gt;3&lt;/sub&gt; (5) (12.5 mg/kg)</td>
</tr>
<tr>
<td>HDL</td>
<td></td>
<td>T&lt;sub&gt;3&lt;/sub&gt; (5) (12.5 mg/kg)</td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td>T&lt;sub&gt;3&lt;/sub&gt; (5) (12.5 mg/kg)</td>
</tr>
<tr>
<td>IDL</td>
<td></td>
<td>T&lt;sub&gt;3&lt;/sub&gt; (5) (12.5 mg/kg)</td>
</tr>
<tr>
<td>VLDL</td>
<td></td>
<td>T&lt;sub&gt;3&lt;/sub&gt; (5) (12.5 mg/kg)</td>
</tr>
</tbody>
</table>

Age-matched male Ldlr<sup>−/−</sup> or WT C57BL/6 mice on a Chow diet were used for baseline blood biochemistry. Mice were switched to a WTD for 1 wk and then bled for blood biochemistry (i.e. 1 wk WTD). These mice were continued on WTD with an addition of daily oral gavage of thyroid hormone (TH) at indicated doses for T<sub>3</sub> or T<sub>2</sub>, or of vehicle control (Con) for another week (i.e. 1 wk TH treatment). P values (vs. Con):<sup>a</sup> P < 0.05; <sup>b</sup> P < 0.01; <sup>c</sup> P < 0.001. Number of mice in each group is given in parenthesis.

TABLE 2. Plasma lipoprotein levels in Ldlr<sup>−/−</sup> and WT C57BL/6 mice treated with thyroid hormones

A. Ldlr<sup>−/−</sup> Lipoproteins

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Con (0.75 mg/kg)</th>
<th>T&lt;sub&gt;3&lt;/sub&gt; (0.75 mg/kg)</th>
<th>1.25 mg/kg</th>
<th>12.5 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>981 ± 311</td>
<td>276 ± 49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>919 ± 84</td>
<td>348 ± 43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IDL+LDL</td>
<td>673 ± 100</td>
<td>124 ± 32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>449 ± 70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>232 ± 120&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL</td>
<td>79 ± 14</td>
<td>59 ± 12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96 ± 11</td>
<td>83 ± 13</td>
</tr>
<tr>
<td>LDL</td>
<td>38 ± 7</td>
<td>12 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38 ± 7</td>
<td>18 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IDL</td>
<td>35 ± 9</td>
<td>5 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28 ± 5</td>
<td>15 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL</td>
<td>104 ± 30</td>
<td>41 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86 ± 18</td>
<td>48 ± 22&lt;sup&gt;a&lt;/sup&gt;</td>
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B. C57BL/6 Lipoproteins

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Con (0.75 mg/kg)</th>
<th>T&lt;sub&gt;3&lt;/sub&gt; (0.75 mg/kg)</th>
<th>1.25 mg/kg</th>
<th>12.5 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>37 ± 13</td>
<td>12 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38 ± 7</td>
<td>18 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IDL+LDL</td>
<td>35 ± 9</td>
<td>5 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28 ± 5</td>
<td>15 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL</td>
<td>104 ± 30</td>
<td>41 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86 ± 18</td>
<td>48 ± 22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Con, Control. P values (vs. Con):<sup>a</sup> P < 0.001; <sup>b</sup> P < 0.01; <sup>c</sup> P < 0.05.
Liver lipoprotein production

We next assessed whether T₃ alters lipoprotein production. Both apoB100 and apoB48 secretion into plasma were markedly reduced by T₃ (Fig. 1B). Surprisingly, triglyceride secretion was almost doubled by T₃ treatment (Fig. 1C). Thus, the liver produces fewer, but more triglyceride-rich, lipoproteins.

T₃-mediated changes in liver gene and protein expression

We first assessed whether in WT mice, T₃ regulated LDLR. It did not; liver expression of LDLR is shown in Fig. 1D.

T₃ increased mRNA levels for the gene (Me1) coding for malic enzyme 1 (Fig. 2A), a gene known to be induced by activation of hepatic TR (7). Although T₂ also tended to increase this mRNA the changes were not statically significant. Me2 was not significantly altered by either T₃ or T₂ treatment (Fig. 2B). Thyroid treatment did not alter mRNA levels of LDLR-related protein 1 (Lrp1), proteoglycan syndecan 1 (Sdc1), or its sulfation enzyme N-deacetylase/N-sulfotransferase 1 (Ndst1). Apob, or microsomal triglyceride transfer protein (Mttp) (Fig. 2, C–E, G, and H). Scavenger receptor B-1 (Srb1) mRNA levels were reduced by thyroid, although HDL levels were not increased (Fig. 2F). Western blot analysis also showed that hepatic LRP1 and SR-B1 proteins were not increased by T₃ or T₂ (Fig. 2I).

Taken together, the reduction in lipoprotein production (Fig. 1) with no changes in liver lipoprotein receptors (Fig. 2I) is likely to explain the reduced levels of circulating cholesterol in thyroid hormone-treated Ldlr⁻/⁻ mice.

Assessment of plasma levels of apoB48 and apoB100

One possible action of thyroid hormones is to increase apoB RNA editing, which should decrease apoB100 and increase apoB48 (16, 17). Figure 1A shows that T₃ and T₂ reduced both apoB100 and apoB48 in Ldlr⁻/⁻ mice to a similar extent. These data suggest that apoB RNA editing is not likely to be involved in T₃- and T₂-induced cholesterol reduction.

Discussion

Despite the long history of research into the relationship of thyroid hormones and lipid metabolism, the precise molecular pathways by which thyroid hormones affect cholesterol are, surprisingly, more complicated than the literature suggests. Although it has been reported that thyroid hormones modulate the LDLR and apoB editing, our studies show that these processes are not responsible...
for thyroid hormone reductions in circulating cholesterol, at least with the very high doses used in mice on a Western diet. Rather, we found that both T2 and T3 reduce circulating cholesterol exclusive of the LDLR and, unlike the reported effects of thyromimetics, also without increases in SR-B1. Even in WT mice, we were unable to show an increase in LDLR with T3 treatment. Finally, our kinetic data show that T3 increased liver production of triglycerides while at the same time it dramatically reduced apoB secretion.

Our studies were not meant to investigate the use of thyroid hormone as a therapeutic agent (10), but to define...
a non-LDLR pathway that could be an important target for cholesterol reduction therapies. For this reason, we chose a T₃ dose that was likely to reduce cholesterol levels. The low-dose T₂ (1.25 mg/kg) given orally, 5-fold greater than ip T₃ dose used by prior investigators (18), had only a small effect on plasma cholesterol levels. Therefore, we employed a 1 order of magnitude higher dose of T₂, similar to the d-T₂ dose used in humans (4), to obtain a cholesterol-lowering effect similar to that of T₃. It should be noted that a similar dose of d-T₂ reduced cholesterol, but was associated with cardiac toxicity in humans. We suspect that the dose that we used in mice would have had a similar toxicity because the T₃-treated mice had heart enlargement and reductions in circulating levels of T₄, indicative of pituitary suppression of the thyroid.

Our studies are the first to probe the effects of thyroid hormones in WTD-fed mice lacking the LDLR. These studies reveal that the cholesterol-lowering effect of T₂ and T₃ in vivo does not require the LDLR. Two recent studies on the effects of thyromimetics on lipid metabolism showed an induction of SR-B1 with no changes in LDLR (11, 12). Van Berkel and associates (19, 20) have published two papers in which they hypothesized that SR-B1 is a remnant receptor, and induction of this receptor, as reported by others using thyromimetics, could have been a reason for the efficacy of T₃ and T₂ in our mice. However, we found no increase in SR-B1. Rat LDLR promoter contains thyroid-responsive element responsive to thyroid treatment (21). Evidence for a role of the LDLR in the cholesterol-reducing actions of thyroid hormones come from a recent report that thyromimetic, T-0681, reduced cholesterol in Apoe⁻/⁻ but not in chow-fed Ldlr⁻/⁻ mice (12). These data strikingly contrast with those we have obtained using Ldlr⁻/⁻ mice and suggest that either T-0681 differs in action from T₂ and T₃ or that the dietary conditions altered responses to these hormones.

As a first step toward uncovering a novel pathway that could increase plasma clearance of apoB-containing lipoproteins via thyroid hormone treatment, we assessed liver lipoprotein production. The dramatic reductions in liver production of apoB clearly implicate reduced production as a primary mechanism for cholesterol reduction. The results of our experiments can be compared with those of Davidson et al. (16) in chow-fed rats. Like these investigators, we observed decreased apoB100 secretion. In contrast, we found reduced apoB48 and also increased triacylglyceride secretion rates, whereas they reported no change in apoB48 or triacylglyceride secretion. The reduction in both forms of apoB means that production and not primarily editing was involved. Experimental thyroid deficiency is reported to not change lipoprotein production (22).

Therefore, excess thyroid and hypothyroidism may affect lipoproteins via different mechanisms. Another difference between our studies and several in the literature is our use of a more physiologically relevant WTD because dietary absorption of cholesterol and triglyceride drives hepatic apoB production, especially in Ldlr⁻/⁻ mice.

What could reduce apoB production and at the same time increase triglyceride production? Our findings reported here are consistent with observations that thyroid increases triglycerides in some human subjects (23). This is thought to result from a peripheral action of thyroid hormone to enhance lipolysis, which in turn leads to increased free fatty acid release from adipose tissue. Under some conditions triglyceride removal may be balanced due to increases in lipoprotein lipase and hepatic lipase (24), and this results in a lack of changes in circulating triglyceride levels. Another process that might account for increased hepatic triglyceride production is reduced activation of the transcription factor farnesoid X receptor (25). In addition, studies have also shown that thyroid positively regulates the expression of the genes encoding CYP7A1 (26), the rate-limiting enzyme for the biosynthesis of bile acids. Hepatic overexpression of CYP7A1 significantly reduces plasma LDL cholesterol in Ldlr⁻/⁻ mice (27). The possible role of bile acid homeostasis in the thyroid effect on plasma cholesterol levels is currently under investigation.

Our findings have potential clinical significance. Newer methods to reduce plasma cholesterol may be of clinical relevance in several situations: 1) Non-LDLR-mediated therapies could be used for treatment of homozygous familial hypercholesterolemia. Because statins induce sterol-regulatory element binding protein 2 and LDLR expression, leading to more rapid clearance of LDL from the bloodstream, they are relatively ineffective in patients with homozygous hypercholesterolemia (28). The current treatment for these patients is often liver transplantation (29). Thus, there is a need for other cholesterol-lowering therapies that do not require a functional LDLR. 2) Statin medications for cholesterol have significant side effects that occur in up to 10% of patients (30). 3) Statins are not sufficient for all patients. Our studies illustrate a method for LDL cholesterol reduction via reducing apoB production and offer the hope of finding therapies that work via a similar mechanism but without peripheral organ toxicity.

Acknowledgements

Address all correspondence and requests for reprints to: Ira J. Goldberg, Division of Preventive Medicine and Nutrition, De-
partment of Medicine, Columbia University, 630 West 168th Street, PH110–305, New York, New York 10032. E-mail: igj3@columbia.edu.

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