Selective thyroid hormone receptor-β activation: A strategy for reduction of weight, cholesterol, and lipoprotein (a) with reduced cardiovascular liability

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Few treatments for obesity exist and, whereas efficacious therapeutics for hyperlipidemia are available, further improvements are desirable. Thyroid hormone receptors (TRs) regulate both body weight and cholesterol levels. However, thyroid hormones also have deleterious effects, particularly on the heart. The TRβ subtype is involved in cholesterol lowering and possibly elevating metabolic rate, whereas TRα appears to be more important for control of heart rate (HR). In the current studies, we examined the effect of TRβ activation on metabolic rate and HR with either *TR*$\alpha_1^{-/-}$ mice or the selective TRβ agonist KB-141 in mice, rats, and monkeys. 3,5,3′-triiodi-L-thyronine (T_3) had a greater effect on increasing HR in WT than in *TR*$\alpha^{-/-}$ mice (ED_{15} values of 34 and 469 nmol/kg/day, respectively). T_3 increased metabolic rate [whole body oxygen consumption (MV_{O_2})] in both WT and *TR*$\alpha^{-/-}$ mice, but the effect in the *TR*$\alpha_1^{-/-}$ mice at the highest dose was half that of the WT mice. Thus, stimulation of MV_{O_2} is likely due to both TRα and -β. T_3 had equivalent potency for cholesterol reduction in WT and *TR*$\alpha^{-/-}$ mice. KB-141 increased MV_{O_2} with selectivities of 16.5- and 11.2-fold vs. HR in WT and *TR*$\alpha_1^{-/-}$ mice, respectively. KB-141 also increased MV_{O_2} with a 10-fold selectivity and lowered cholesterol with a 27-fold selectivity vs. HR in rats. In primates, KB-141 caused significant cholesterol, lipoprotein (a), and body-weight reduction (up to 7% after 1 wk) with no effect on HR. TRβ-selective agonists may constitute a previously uncharacterized class of drugs to treat obesity, hypercholesterolemia, and elevated lipoprotein (a).

Obesity and atherosclerosis are important medical problems with major impact on morbidity and mortality. Current treatments for obesity have shown limited efficacy and safety; therefore, there is a need for improved therapies (1). A major risk factor for atherosclerosis is low-density lipoprotein (LDL) cholesterol. Although there are excellent treatments for elevated LDL cholesterol, therapeutic goals are commonly not met. As targets for lowering of cholesterol become more aggressive, there is a need for more modalities to meet these goals. Lipoprotein (a) [Lp(a)] is an important risk factor, elevated in many patients with premature atherosclerosis, and few therapies lower Lp(a) (2).

Thyroid hormones reduce body weight, LDL cholesterol, and Lp(a); thus, exploitation of these properties may be useful for therapy (3–6). Unfortunately, endogenous thyroid hormones are nonselective and produce undesirable side effects, particularly cardiac stimulation (7, 8). Development of thyromimetics devoid of cardiac effects could have therapeutic potential as antiobesity and lipid-lowering agents.

Thyroid hormone receptors (TRs) are divided into two primary subtypes (TRα and -β), which are the products of two genes of the superfamily of nuclear hormone receptors (4, 7). TRs mediate distinct physiologic effects due to differences in tissue abundance or receptor-specific activity (9). Studies in patients with the syndrome of resistance to thyroid hormone, in which there are abnormal TRβ, and with *TR*$\alpha_1^{-/-}$ mice suggest that TRα is the major TR regulating heart rate (HR) (4, 9–12). TRβ is critical in controlling hepatic cholesterol metabolism and thyroid-stimulating hormone (TSH) suppression, which may be due to high expression of TRβ in liver (70–80% of total TR) and pituitary (10, 12, 13). The TRβ-selective agonist GC-1 reduces plasma cholesterol levels with minimal cardiac effects in mice and rats; however, GC-1 also exhibits relatively decreased uptake into the heart compared with liver, and these differences, rather than TRβ selectivity (14), might account for its selective pharmacology. The effect of GC-1 on metabolic rate has not been reported; therefore, the potential of GC-1 or other TRβ-selective agonists as antiobesity compounds is unclear. Clinically useful TRβ agonists would require high selectivity for cholesterol reduction with modest metabolic rate increases (5–10%) without tachycardia over a wide therapeutic dose range to be safe and efficacious.

The role of TRβ in modulating metabolic rate is currently unclear. GC-1 up-regulates uncoupling protein 1 in brown adipose tissue (15), whereas a study using *TR*$\beta^{-/-}$ mice suggests no effect of TRβ on body weight (16). No studies have measured whole body metabolic rates by using either *TR*$\alpha_1^{-/-}$ mice or TRβ-selective agents.

KB-141 is a TR agonist that binds to the human (h)TRβ with a 14-fold higher affinity than to hTRα (17). In the current studies, we used KB-141 to examine effects of selective activation of TRβ in control and TR$\alpha^{-/-}$ mice, cholesterol-fed rats, and cynomolgus monkeys. The latter more closely resemble humans in terms of lipoprotein metabolism and regulation of body weight (18, 19). 3,5,3′-Triiodi-L-thyronine (T_3), the major active form of thyroid hormone, increased the metabolic rate in both WT and TR$\alpha^{-/-}$ mice, but the increase for the WT animals was greater than with the TR$\alpha^{-/-}$ mice. These data imply that both TRα and -β regulate the metabolic rate. As compared with T_3, KB-141 reduced plasma cholesterol levels selectively vs. increasing HR in all three models. Relative to T_3, KB-141 also increased whole body oxygen consumption (MV_{O_2}) in both mice and rats more than HR. In monkeys, KB-141 decreased body weight after 1 wk of treatment by up to 7% and Lp(a) by up to 56% without tachycardia. These studies suggest that selective stimulation of the TRβ might be exploited as a therapeutically effective means to lower weight, plasma cholesterol, and Lp(a) without eliciting deleterious cardiac effects.

Abbreviations: TR, thyroid hormone receptors; hTR, human TR; HR, heart rate; LDL, low-density lipoprotein; TSH, thyroid-stimulating hormone; MV_{O_2}, whole body oxygen consumption; Lp(a), lipoprotein (a); T_3, 3,5,3′-triiodi-L-thyronine; TRAF, thyroid hormone alkaline phosphatase.

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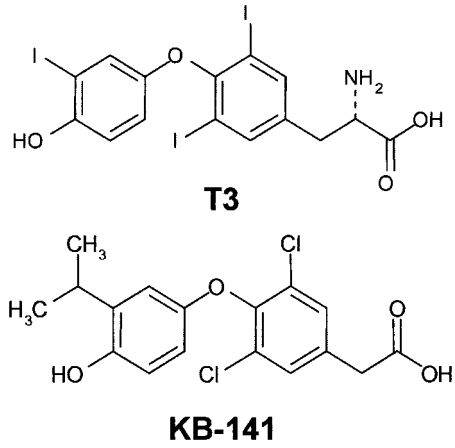

Fig. 1. Chemical structures of T_3 (3,5,3′-triiodo-L-thyronine) and KB-141.

Methods

Studies in *TR*$\alpha_1^{-/-}$ Mice. Development of *TR*$\alpha_1^{-/-}$ mice has been described (9–11). After 2 wk of cholesterol feeding (1.5% cholesterol, 0.5% cholic acid), WT or *TR*$\alpha_1^{-/-}$ mice were treated orally with vehicle (10% *m*-pyrol, 5% ethanol, 5% cremaphor, and 80% water for all studies) or 1.54–462 nmol/kg T_3 per day for 7 days ($n = 10$ per group). Before and after 7 days of treatment, MV_{O_2} was determined in conscious mice by using Oxymax chambers (Columbus Instruments, Columbus, OH) (18, 19). The mice were then anesthetized with i.p. sodium pentobarbital (30 mg/kg), and HRs were measured by using lead II ECG. Afterward, blood samples were removed from the vena cava and analyzed for plasma HDL and LDL cholesterol and blood chemistries (liver enzymes, electrolytes, blood urea nitrogen, etc.), as described (14). TSH could not be measured in mice because no reliable assay existed at the time these studies were performed. An identical study was done by using KB-141 (154–2,920 nmol/kg per day), a TRβ-selective agonist that will be described below.

Previous studies on *TR*$\alpha^{-/-}$ mice from our laboratories were done in mice instrumented for conscious HR and body temperature measurements with telemetry (10). To reproduce these conditions, animals were implanted with ECG and temperature leads and after recovery, the *TR*$\alpha_1^{-/-}$ and WT mice were treated orally (after 2 wk of cholesterol feeding) with either vehicle or 154 nmol/kg T_3 per day for 7 days ($n = 5$ per group). Body temperatures and HR were monitored throughout the study with telemetry (Datasciences, St. Paul, MN) and at end of the study, MV_{O_2} was determined.

Studies with KB-141. ***TR-binding affinity studies and reporter cell assay.*** TR-binding affinities were measured as described (20, 21). Briefly, KB-141 (Fig. 1) was incubated with [^{125}I]T_3 (200 pM) and recombinant hTRα_1 or -β_1 (20 pM) until equilibrium, and unbound ligand was separated from receptor-bound ligand. IC_{50} values denote the concentration of KB-141 inhibiting 50% of the binding of [^{125}I]T_3. The K_d for [^{125}I]T_3 is lower for hTRα_1 (58 ± 5 pM) than for hTRβ_1 (112 ± 8 pM); therefore, the IC_{50} for an unlabeled compound with equal affinity (K_i) for the two subtypes is lower for hTRβ_1 than for hTRα_1. The Cheng–Prusoff relationship for a competitive inhibitor was used to obtain the affinity (K_i) for binding to the hTRs (20–22). Normalized hTR selectivity was calculated as IC_{50} [(hTRα_1)/IC_{50} (hTRβ_1) × 1.7].

The KB-141 agonist profile was determined by using Chinese hamster ovary K1 cells stably transfected with expression vectors for hTRα_1 or -β_1 and subsequently with a reporter vector encoding a secreted form of alkaline phosphatase (23, 24) and containing a thyroid hormone response element. The cell lines thyroid hormone alkaline phosphatase (TRAF)-α and -β were exposed to serial dilutions of agonists and incubated for 48 h. Alkaline phosphatase in the cell culture media was then measured by using a chemiluminescent assay (24, 25).

Cholesterol-fed rat studies. Sprague–Dawley rats (250–300 g) were cholesterol-fed for 2 wk and were then dosed once daily with KB-141, T_3 (Sigma), or vehicle ($n = 5$–6 per group) by oral gavage for 7 days. MV_{O_2} was then measured in conscious rats by using Oxymax chambers. Rats were then anesthetized with i.p. sodium pentobarbital (30 mg/kg), and HR was measured by using lead II ECG. Blood was collected and analyzed for cholesterol, TSH, and blood chemistries as described (14). The percent of total cholesterol composed of LDL was >90% after cholesterol feeding.

The relative tissue uptake of T_3 and KB-141 was determined. Rats were anesthetized with 30 mg/kg pentobarbital, i.p., and either T_3 or KB-141 (10 μmol/kg) was injected into the right jugular vein ($n = 3$ per group). Plasma and tissue samples were collected 1 h after treatment. Samples were weighed and homogenized with three volumes of deionized water, and tissue concentrations were determined by using liquid chromatography/MS, as described (14).

A separate group of rats was used for isolated heart studies to examine cardiac contractile function and hypertrophy (heart weight/body weight). After 7 days of treatment with vehicle, T_3, or KB-141 (1.54–2,920 mol/kg per day, $n = 5$ per group), hearts were removed and retrogradely perfused with Krebs–Henseleit buffer, and the left ventricles were fitted with a balloon for measurement of developed pressure and HR as described (26).

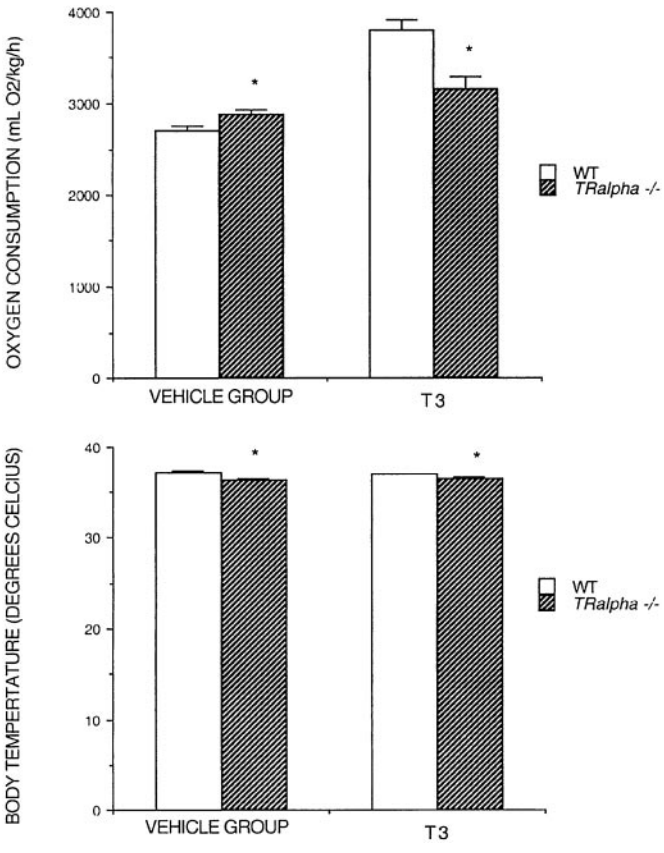

Fig. 2. Oxygen consumption and body temperatures in WT and *TR*$\alpha^{-/-}$ mice before and after 154 nmol/kg T_3 per day (7-day treatment).

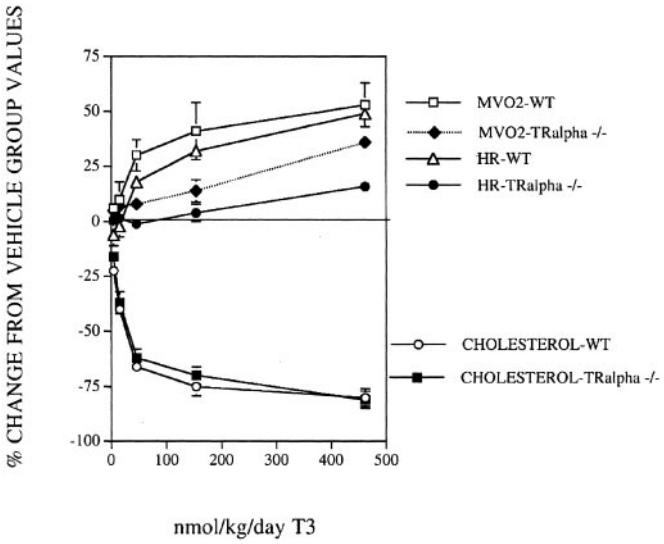

Fig. 3. Effect of T_3 on HR, plasma cholesterol, and MV_{O_2} in $TR\alpha_1^{-/-}$ mice and WT mice after 7 days of treatment.

Studies in Cynomolgus Monkeys. Cynomolgus monkeys were dosed with KB-141 (154, 462, and 924 nmol/kg per day) or T_3 (46.2 and 154 nmol/kg per day) ($n = 5$ per group) orally once daily for 7 days. At the end of this period, animals were anesthetized with ketamine (3–5 mg), and blood pressure and HR were determined by using a pressure cuff at the base of the tail. Blood samples were taken for analysis of cholesterol and blood chemistries.

Results

Effect of T_3 in Cholesterol-Fed $TR\alpha_1^{-/-}$ Mice. We tested the response of conscious $TR\alpha_1^{-/-}$ and WT mice (telemetry) to 7 days of 154 nmol/kg T_3 per day. Confirming previous results (10, 11), baseline HR was significantly ($P < 0.05$) lower in $TR\alpha_1^{-/-}$ mice, as was body temperature (488 ± 12 vs. 530 ± 4 beats per min and 36.4 ± 0.1 vs. 37.2 ± 0.2°C for $TR\alpha^{-/-}$ and WT, respectively; Fig. 2). Despite a lower body temperature, baseline MV_{O_2} was slightly, but significantly ($P < 0.05$), higher in the untreated $TR\alpha_1^{-/-}$ mice (2,885 ± 51 vs. 2,697 ± 53 ml/kg O_2 per hour for vehicle-treated $TR\alpha_1^{-/-}$ and WT, respectively; Fig. 2). T_3 increased MV_{O_2} and HR to significantly ($P < 0.05$) higher levels in WT compared with $TR\alpha_1^{-/-}$ mice (632 ± 15 and 564 ± 18 beats per min; 3,801 ± 121 and 3,168 ± 118 ml/kg O_2 per hour, respectively), although the increase in MV_{O_2} for $TR\alpha_1^{-/-}$ mice was significant ($P < 0.05$). Body temperature at predrug values was maintained in both groups. These observations indicate that T_3 acting through TRβ can increase both MV_{O_2} and HR, and that body temperature may not predict changes in MV_{O_2}.

Dose–response studies with T_3 in $TR\alpha_1^{-/-}$ and WT mice are shown in Fig. 3. T_3 had reduced potency on HR in $TR\alpha_1^{-/-}$ mice relative to WT ($P < 0.05$). MV_{O_2} was increased by T_3 in a dose-dependent manner in $TR\alpha_1^{-/-}$ mice, but the increase was less than in WT mice ($P < 0.05$); however, the increase in MV_{O_2} was greater than that of HR (Fig. 3). We assigned potency values as ED_{50} for cholesterol (dose reducing cholesterol 50%), ED_{15} for HR (dose increasing HR 15%, viewed as the highest acceptable increase), and ED_5 for MV_{O_2} (dose increasing MV_{O_2} 5%, viewed as lower end of efficacious range). Potency ratios were then calculated for HR vs. MV_{O_2} and vs. cholesterol lowering, showing selectivity (Table 1). MV_{O_2} was increased by 5% with a 4.7-fold selectivity over a 15% increase in HR in WT mice and with a 45.5-fold selectivity vs. a 15% increase in HR in $TR\alpha_1^{-/-}$ mice. Thus, the selectivity for a 5% increase in MV_{O_2} vs. HR in $TR\alpha_1^{-/-}$ mice normalized for WT mice was 9.7-fold (Table 1). The effects of T_3 on cholesterol are similar in $TR\alpha_1^{-/-}$ animals relative to WT. This lowering is likely due to a decrease in LDL cholesterol, because >90% of the total plasma cholesterol in these animals is the LDL form (27–32). Thus, under these conditions, cholesterol lowering is primarily regulated by TRβ, MV_{O_2} by both TRβ and -α, and HR mostly by TRα.

Table 2. Binding affinities calculated from displacement of T_3 from full length hTRα and -β

Compound	TRα_1 IC_{50}, nM	TRβ_1 IC_{50}, nM	Normalized TRα/β selectivity
T_3	0.4	0.3	0.8
KB-141	23.9	1.1	13.1

Studies with KB-141. ***Binding affinities and transactivation*** **in vitro.** The ability of KB-141 to compete with $[^{125}I]T_3$ for binding to human TRα_1 and -β_1 is shown in Tables 2–4. Binding affinity of KB-141 to hTRα_1 is 1.7% that of T_3 (IC_{50}s for T_3 and KB-141 of 0.4 and 23.9, respectively), whereas the binding affinity to hTRβ_1 is 27% that of T_3 (IC_{50}s for T_3 and KB-141 of 0.3 and 1.1, respectively). Thus, the normalized hTRβ_1 binding selectivity for KB −141 is 10-fold.

KB-141 is a full agonist in both the TRα and -β reporter cell systems (*Methods* and Table 3). KB-141 is equipotent to T_3 in TRAF-β cells (EC_{50}s of 3.5 and 3.4 nM for T_3 and KB-141, respectively), but KB-141 has 12% the potency of T_3 in TRAF-α cells (EC_{50}s of 1.3 and 11.2 nM for T_3 and KB-141, respectively). Thus, the TRβ/-α selectivity KB-141 normalized to that of T_3 is 8.3 (Table 3).

Effect of KB-141 in $TR\alpha_1^{-/-}$ and WT Mice. KB-141 increased MV_{O_2} with selectivities of 16.5- and 11.2-fold vs. HR in WT and $TR\alpha_1^{-/-}$ mice (Table 1). Similar selectivities of 14.2- and 13.6-fold, respectively, were observed for cholesterol lowering vs. HR in both groups of animals (Table 1). KB-141 was less potent for all parameters compared with T_3, which is consistent with its lower affinity for TRβ than T_3.

Effect of KB-141 in cholesterol-fed rats. Dose–response effects of T_3 and KB-141 on HR, MV_{O_2}, and cholesterol lowering in cholesterol-fed rats are shown in Fig. 4 and Table 4. Cholesterol was reduced in a dose-dependent manner by T_3 and KB-141 (primarily LDL-cholesterol reduction), with KB-141 being ≈30% as potent as T_3 (Table 4), consistent with the lower TRβ-binding affinity for KB-141. HR was increased by T_3 and KB-141,

Table 1. Potencies and potency ratios for T_3 and KB-141 in WT and $TR\alpha_1^{-/-}$ mice

Compound	Mice	HR ED_{15}	MVO_2 ED_5	Cholesterol ED_{50}	HR/MVO_2	HR/Cholesterol
T_3	WT	33.6	7.2	28.9	4.7	1.4
	$TR\alpha_1^{-/-}$	469	10.3	35.8	45.5	38.2
KB-141	WT	2,838	172	201	16.5	14.2
	$TR\ \alpha_1^{-/-}$	2,556	228	187	11.2	13.6

All values are mean ± SE and are shown as nmol/kg per day. 45.5/4.7 = 9.5 is selectivity for MV_{O_2} vs. HR normalized for T_3 response in WT mice. 38.2/1.2 = 27.2 is selectivity for cholesterol vs. HR normalized for T_3 response in WT mice.

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Table 3. Transactivation in TRAF-α and -β cells

Compound	TRAF-α EC_{50}, nM	TRAF-β EC_{50}, nM
T_3	1.3 (100% agonism)	3.5 (100% agonism)
KB-141	11.2 (101% agonism)	3.4 (105% agonism)

although KB-141 was significantly less potent (Table 4). Thus, KB-141 is ≈27-fold more selective for cholesterol lowering vs. tachycardia compared with T_3. TSH was reduced by T_3 and KB-141 in parallel with cholesterol reduction, consistent with TRβ-dependent actions (Table 4).

MV_{O_2} was increased in a dose-dependent manner by T_3 and KB-141 (Fig. 4, Table 4), although the slope of the curve was significantly ($P < 0.05$) shallower for KB-141 based on the log curve fit. KB-141 has a 9-fold greater selectivity for MV_{O_2} changes compared with HR (potency ratio for ED_{15} HR/ED_5 MV_{O_2}, Table 4).

Levels of T_3 and KB-141 were determined in samples from a separate group of rats. The tissue/plasma ratios for heart were 1.0 and 0.7; for liver, 7.5 and 5.6; for adipose tissue, 0.2 and 0.3; and for skeletal muscle, 0.6 and 0.4 for T_3 and KB-141, respectively. T_3 and KB-141 appear to be similar in their abilities to penetrate these tissues, suggesting that the selectivity for cholesterol lowering (and possibly MV_{O_2}) is due to TRβ selectivity.

Isolated Rat Heart Studies. Cholesterol-fed rats were treated with T_3 or KB-141 for 7 days, after which the hearts were removed and cardiac function *ex vivo* was determined. There were no differences in body weights among groups. HR changes in isolated perfused hearts mirrored drug effects *in vivo* such that KB-141 had a lower potency for producing tachycardia (ED_{15} = 31 vs. 2,893 nmol/kg per day for T_3 and KB-141, respectively). KB-141 had no effect on inotropy (contractility) or lusitropy (rate of relaxation) nor did it cause cardiac hypertrophy, except at very high doses (shown as ratio of heart weight to body weight in Fig. 5). By contrast, T_3 caused a dose-dependent increase in heart weight/body weight ratio (ED_{15} = 49 nmol/kg per day), suggesting increased cardiac work. T_3 caused a significant and dose-dependent increase in inotropy (e.g., left ventricular developed pressure = 121 ± 4 mm Hg for vehicle and 153 ± 4 mm Hg at 462 nmol/kg T_3 per day). These results are consistent with the large increase in MV_{O_2} and direct cardiac effects of T_3. Because these effects are not observed with KB-141, it is likely that the T_3 effects on heart weight and inotropy are mediated through TRα.

Primate Studies. KB-141 was administered for 7 days to cynomolgus monkeys to determine effects in a model more closely resembling humans. Plasma cholesterol (Fig. 6) was reduced in a dose-dependent manner by KB-141 with a reduction of ≈35% at the highest dose, and most of this reduction was due to reduction in LDL cholesterol. Baseline LDL cholesterol was 49 ± 2 mg/dl, and HDL cholesterol was 43 ± 2 mg/dl. No tachycardia was observed. Despite the short duration of treatment, body weight was also significantly reduced by KB-141 by 5% at the 462 nmol/kg per day dose and 7% at the 924 nmol/kg per day dose compared with paired predrug weights (Fig. 5). The animals were fed ad libitum; food consumption and blood chemistries were normal (blood urea nitrogen, creatinine, electrolytes, liver enzymes, creatine kinase, etc.), and the animals quickly regained their weight after withdrawal of treatment, suggesting that the animals were healthy. No effect of KB-141 on blood pressure was noted. T_3 at 46.2 and 154 nmol/kg per day produced cholesterol lowering equivalent to 924 nmol/kg per day KB-141 but with significant tachycardia. KB-141 significantly reduced Lp(a) by ≈50% at the 462- and 924-nmol/kg per day doses (Fig. 6), as did T_3 (data not shown).

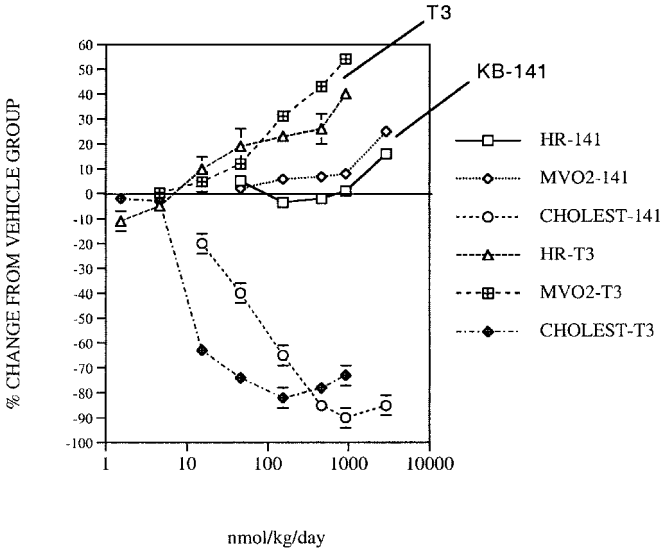

Fig. 4. Effect of T_3 or KB-141 (KB) on HR, plasma cholesterol, and MV_{O_2} consumption in cholesterol-fed rats after 7 days of treatment.

Discussion

We addressed the potential for selectively stimulating TRβ vs. -α for inducing weight loss and lowering plasma cholesterol and Lp(a) levels without excessively stimulating HR, by comparing T_3 with the TRβ-selective KB-141. We confirmed that KB-141 was TRβ-selective in both TR-binding and cell-based assays. We used three *in vivo* model systems: WT and $TR\alpha^{-/-}$ mice, cholesterol-fed rats, and cynomolgus monkeys. Each system offers distinct advantages, and results with the three systems were complementary. For mice and rats, we measured MV_{O_2} as an index of metabolic rate, which contributes to thyroid hormone-induced weight loss (4), and in primates we measured weight.

T_3 lowered cholesterol to a similar degree in WT and $TR\alpha_1^{-/-}$ mice but showed differences in effects on MV_{O_2} and HR. Whereas T_3 induced a large increase in HR in WT mice, the increase was much less for the $TR\alpha_1^{-/-}$ mice. T_3 increased significantly MV_{O_2} in both the WT and $TR\alpha_1^{-/-}$ mice, although the maximal stimulation in the $TR\alpha_1^{-/-}$ mice was ≈60% that observed in the WT mice. These data confirm previous results

Table 4. Potency ratios for T_3 and KB-141 *in vivo*

Compound	ED_{15} HR/ED_{50} cholesterol	ED_{15}HR/ED_5 MV_{O_2}	ED_{30} TSH/ED_{50} cholesterol
T_3	30.8/20.6 = 1.5	30.8/21.5 = 1.4	6.7/21.6 = 0.3
KB-141	2,904/72.3 = 40.3 (27-fold vs. T_3)	2,904/232 = 12.5 (9-fold vs. T_3)	31.8/72.3 = 0.4 (1.3-fold vs. T_3)

All *in vivo* ED values are shown as nmol/kg per day. ED_{15} HR is the dose causing 15% increase in HR; ED_{50} cholesterol is the dose causing 50% reduction in cholesterol; ED_5 is the dose causing 5% increase in metabolic rate. ED_5 was chosen because it represents the potential therapeutic increase for antiobesity effects. ED_{30} TSH is the dose causing a 30% reduction in TSH.

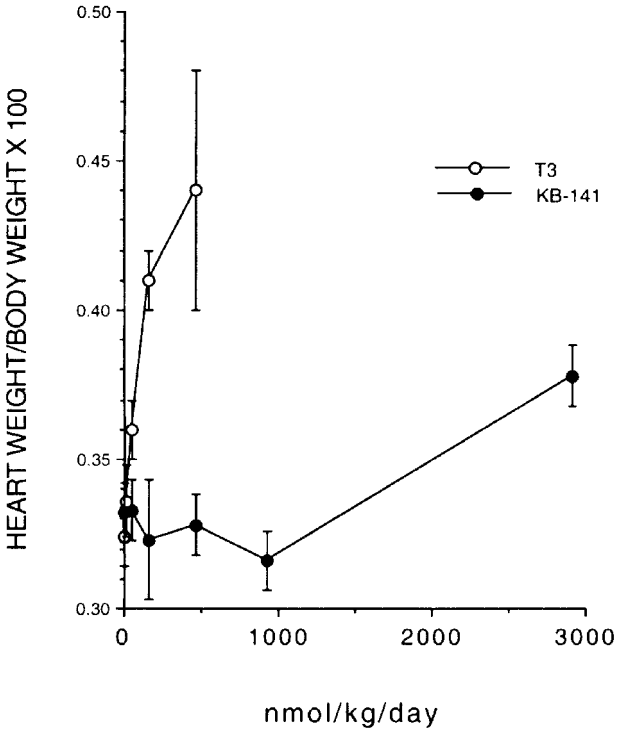

Fig. 5. Effect of KB-141 or T_3 on heart weight/body weight ratio in rats treated for 7 days.

that TRβ primarily regulates plasma cholesterol levels and TRα primarily regulates HR (9–11,14). They also suggest that both TRα and -β can regulate MV_{O_2}. In support of the notion that TRβ-selective stimulation might increase MV_{O_2} without increasing HR was the observation that in $TR\alpha^{-/-}$ mice, there is a 10-fold window in which MV_{O_2} could be therapeutically increased by T_3 without tachycardia, whereas T_3 showed no selectivity in WT mice (Fig. 3).

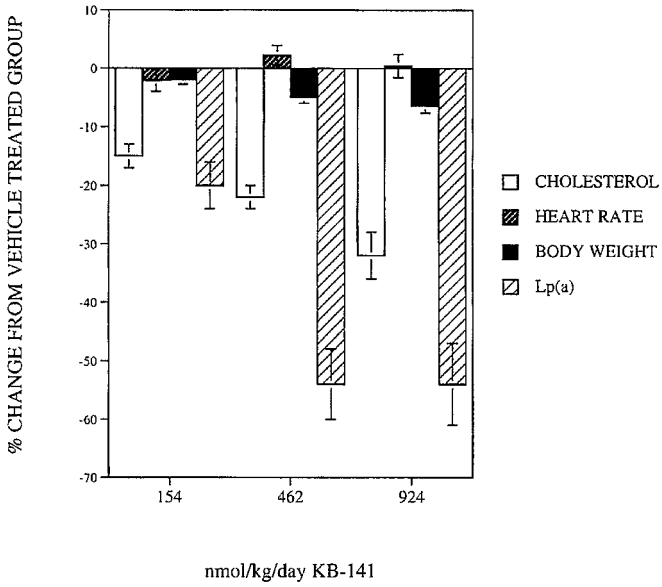

Fig. 6. Effect of KB-141 on HR, cholesterol body weight, and Lp(a) in cynomolgus monkeys after 7 days of treatment (46.2, 154 nmol/kg T_3 per day = 25%, 24% incr. HR, respectively).

In comparing the WT and $TR\alpha_1^{-/-}$ mice, we surprisingly found that body temperature can be dissociated from MV_{O_2}. As shown previously (10, 11), $TR\alpha_1^{-/-}$ mice display lower body temperatures than WT mice, but these $TR\alpha_1^{-/-}$ mice paradoxically have slightly but significantly higher metabolic rates than WT (Fig. 2). Further, whereas T_3 treatment increased MV_{O_2} in both WT and $TR\alpha_1^{-/-}$ mice, it did not change body temperature. We do not understand why this dissociation occurs; it is conceivable that the set point for body temperature is altered in $TR\alpha_1^{-/-}$ mice. As expected, KB-141 showed similar selectivities for cholesterol and MV_{O_2} in both WT and $TR\alpha_1^{-/-}$ mice.

Compared with T_3, KB-141 exhibited selective stimulation of MV_{O_2} and reduction of plasma cholesterol levels relative to HR in cholesterol-fed rats. KB-141 was 27-fold more selective for cholesterol lowering (ED_{50}) vs. tachycardia than T_3 and 9-fold more selective for the MV_{O_2} response. The greater selectivity of KB-141 for cholesterol lowering vs. effects on MV_{O_2} suggests that both TRα and -β mediate the latter effect.

We also examined effects of T_3 and KB-141 on hearts isolated from control and treated animals. Rodent ventricles contain TRβ receptors (33, 34) and therefore might respond to a TRβ agonist. However, KB-141 had no effect on contractility or rate of relaxation. Whereas T_3 induced cardiac hypertrophy, this was not observed for KB-141. Therefore modest degrees of metabolic rate increases can be seen without cardiac side effects.

TSH was suppressed by KB-141 in rats and this mirrored cholesterol-lowering. These observations are consistent with TRβ-selective activation being critical for both effects (4). They also imply that in a therapeutic setting, use of a compound like KB-141 would suppress plasma and tissue levels of T_3 and T_4. Whereas the administered ligand in this example would compensate for the endogenous hormones in terms of TRβ stimulation, it may not appreciably activate TRα, which could therefore cause a relative TRα hypothyroidism; future work may address this.

Actions of one compound previously reported to cause selective TR modulation might be explained in part by selective tissue uptake. GC-1 has selective effects on cholesterol vs. HR in rodents but is not as readily taken up by the rat heart relative to the liver as compared with T_3 (14). The thyromimetic, CGS-23425, reduces cholesterol with no thermogenic or HR effects, although the mechanism for this separation is unclear (23). The selectivity for cholesterol lowering vs. tachycardia for SKF-94901 can be partially explained by selective tissue uptake (14, 35). The distribution of KB-141 relative to plasma was equivalent to T_3 in heart, liver, muscle, and adipose tissue. Therefore the selectivity for lowering of cholesterol and MV_{O_2} is likely due to the TRβ selectivity of KB-141.

KB-141 was also examined in cynomolgus monkeys that have a lipoprotein profile resembling humans (primarily LDL cholesterol) more closely than rodents. Plasma cholesterol was reduced in a dose-dependent manner by KB-141 with a reduction of $\approx$35% at the highest dose. No tachycardia was observed, showing selectivity for cholesterol lowering for KB-141, unlike T_3. In the monkeys, KB-141 also lowered Lp(a) levels by 50%. Lp(a) has been suggested previously as a target for nonselective thyroid agonists (6). Thus these atherogenic particles may be regulated by TRβ, and this class of thyromimetics may deserve further exploration as a regulator of Lp(a), for which current therapies have limited efficacy.

Monkeys are also a better model than rodents for examining effects on weight loss, because the animals are not growing. Body weight was significantly reduced by KB-141 by 5% at the 462 nmol/kg per day dose and 7% at the 924 nmol/kg per day dose compared with paired predrug weights, most likely due to increased MV_{O_2}. Muscle loss and frank myopathy can be observed in hyperthyroidism (4), but the KB-141-treated animals appeared healthy and maintained their pretreatment level of

food consumption, and body chemistries (electrolytes, renal function indices, blood urea nitrogen, and liver functions) remained normal. Thus, it is unlikely that the weight loss was due to a toxic effect of KB-141. Interestingly, little TRβ mRNA is reported in skeletal muscle in humans (4).

In summary, our results in three species show that a separation between potential beneficial vs. deleterious effects of TR stimulation is possible. These effects include reductions of plasma cholesterol and Lp(a) and weight. It is likely in all three cases that the separation is due to the ability of KB-141 to selectively activate TRβ vs. -α. Thus, selective thyromimetics may offer several types of therapeutic potential and are candidates for further exploration.

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